

14

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June 18, 1993

Ms. Lynn A. Kszos, Aquatic Toxicologist
Environmental Sciences Division
Oak Ridge National Laboratory
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Oak Ridge, Tennessee 37831-6351

Dear Lynn:

Enclosed is a copy of the report presenting results of TVA copper sulfate reference toxicant testing using juvenile freshwater mussels (Anodonta imbecillis) following the liquid media protocol. The test was conducted from May 12-21 and resulted in a 9-day LC₅₀ of 1.12 mg Cu/L. Attachments to the report include Toxicity Test Bench Sheets and Statistical Analyses (I), Copper Analysis Request and Results (II), and Personnel Training Documentation (III). This test did not include side by side testing of feeding regimes due to a misinterpretation of the Statement of Work (SOW). The test will be repeated as specified in the SOW in time to provide the necessary food quality information prior to initiation of sediment testing in July.

Please call Don Wade (205-386-2068) or me (205-729-3340) if you have questions or comments regarding the report or conductance of the study.

Sincerely,

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Jennifer Moses, Unit Supervisor
Aquatic Research Laboratory

JM:DTH
Enclosures

cc: R. J. Pryor, EB 1A-K
D. C. Wade, OSA 1B-M (w/enclosures)
Files, WR, HB 2C-C (w/enclosures)

Prepared by Jennifer Moses

PLARC501-1530

MASTER

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STANDARD REPORT FORM

STATIC RENEWAL TESTS USING ANODONTA IMBECILLIS
(FRESHWATER MUSSELS)

Test Title: Anodonta imbecillis Copper Sulfate Reference Toxicant Test,
Clinch River - Environmental Restoration Program (CR-ERP)

Principle Investigator: Damien J. Simbeck

Starting Date: May 12, 1993

Ending Date: May 21, 1993

1.0 EXECUTIVE SUMMARY

Reference toxicant testing using juvenile freshwater mussels was conducted as part of the CR-ERP biomonitoring study of Clinch River sediments to assess the sensitivity of test organisms and the overall performance of the test. [1] Tests were conducted using moderately hard synthetic water spiked with known concentrations of copper as copper sulfate.

Toxicity testing of copper sulfate reference toxicant was conducted from May 12-21, 1993. The organisms used for testing were juvenile fresh-water mussels (Anodonta imbecillis). Results from this test showed an LC₅₀ value of 1.12 mg Cu/L which is lower than the value of 2.02 mg Cu/L obtained in a previous test. Too few tests have been conducted with copper as the toxicant to determine a normal range of values.

2.0 SAMPLE COLLECTION/TREATMENTS

- 2.1 Test Sample Identification (Chemical/Effluent/Elutriate, etc.):
The samples used for biomonitoring were serial dilutions of preserved copper stock solutions diluted in moderately hard synthetic water. Stock solutions were prepared on May 6, 1993, from EPA Reference Toxicant copper sulfate (50 mg Cu/mL), lot #188, and were used daily during the test.
- 2.2 Control and/or Dilution Water: Moderately hard synthetic water.
- 2.3 Sample Dates and Times: Not applicable.
- 2.4 Sampling Method: Not applicable

2.5 Sample Storage/Handling: All samples were renewed daily. Moderately hard synthetic water was prepared as needed and stored in 20-L carboys under aeration until used. Copper stock solutions were preserved using 1:1 HNO₃ following procedures for preservation of metals samples. [2]

2.6 Sample Transport: Not applicable

2.7 Sample Pretreatment: Sample temperature was raised to 24°C in a warm water bath. Food (phytoplankton) and non-toxic, filtered (100 µm Nytex® screen) sediment were added daily prior to initial chemical measurements.

2.8 Test Treatments: Nominal test concentrations were Control (0 mg Cu/L), 0.31 mg Cu/L, 0.63 mg Cu/L, 1.25 mg Cu/L, 2.5 mg Cu/L, and 5.0 mg Cu/L. Actual copper concentrations verified by laboratory analysis of the prepared test series were Control (0 mg Cu/L), 0.34 mg Cu/L, 0.67 mg Cu/L, 1.30 mg Cu/L, 2.60 mg Cu/L, 5.70 mg Cu/L

3.0 TEST ORGANISMS/CULTURING CONDITIONS

3.1 Species: Anodonta imbecillis, freshwater mussel

3.2 Source: In vitro culture, April 27-May 4, 1991, TVA Aquatic Research Laboratory. The gravid adult from which glochidia were extracted was obtained from Taylor's Catfish ponds, Town Creek, Alabama, on April 13, 1993. Adults were maintained in a 40-L aquarium with approximately 5 L non-toxic sediment from Taylor's ponds and 20 L Tennessee River water. Water was changed at least once per week with bloomed phytoplankton water and sediment was changed monthly.

3.3 Culture medium: Mussel culture medium used to transform larvae (glochidia) into juveniles consisted of a 2:1 mixture of cell culture medium (MEM) and 0.22 µm filtered catfish plasma. Antibiotics and antimycotics were added in small concentrations to prevent bacterial and fungal contamination. [3]

3.4 Temperature of Culture: 24°C ± 1°C

3.5 Culture water: After transformation of larval mussels, the free-living juveniles were placed in 100 µm filtered TR water with bloomed indigenous algae. Non-toxic sediment (100 µm filtered) were added to provide additional food and substrate for healthy growth of the juvenile mussels. [4]

3.6 Temperature of Culture: 24°C ± 1°C

- 3.7 General Maintenance: Cultures were maintained in 1000-mL Nalgene® trays in 24-h dark incubators for 6-10 days after transformation. Cultures were fed bloomed phytoplankton daily. Water and sediment were changed a minimum of once every two days. Health and survival of the culture were checked by microscopic examination of animals when culture water was changed.
- 3.8 Phytoplankton preparation: Phytoplankton was bloomed in 20-L glass aquaria 4-7 days (until dark green). Blooms were initiated by adding concentrated solids from ARL channel water to filtered (100 µm) TR water. Algal nutrients used for Selenastrum cultures were added (1 mL/L) to boost algal blooms. [5] Blooms were centrifuged to concentrate the algal cells into a dark green suspension, obtaining about 0.5 L per aquarium. Prepared phytoplankton concentrate was refrigerated until used.
- 3.9 Sediment preparation: Whole, non-toxic sediment from Taylor's Catfish ponds, Town Creek, Alabama, was filtered through a 100-µm nylon mesh filter. Filtered sediment was stored at 4°C until used.

4.0 TEST METHODS

- 4.1 Mussels, Anodonta imbecillis, Survival Test, TVA Test Method, SOP-22, liquid media protocol. [5]
- 4.2 Modification/Deviations to SOP-22: None
- 4.3 Date/Time Test Initiated: May 12, 1993/0935 CDT
- 4.4 Date/Time Test Terminated: May 21, 1993/0900 CDT
- 4.5 Age of test organisms: 8 days old
- 4.6 Test Chamber: 50 mm-diameter glass cylinder (75 mm tall) with 100-µm nylon mesh bottom, placed in 250-mL crystallizing dish
- 4.7 Volume per Chamber: 150 mL
- 4.8 Number of organisms per replicate: 10
- 4.9 Number of Replicates per Treatment: 4
- 4.10 Dilution Water: Moderately hard synthetic water
- 4.11 Test Control: Moderately hard synthetic water spiked with pH adjusted reagent water to exclude pH as a variable since copper stock solutions were acid preserved.
- 4.12 Test Temperature: 24°C ± 1
- 4.13 Photoperiod: 24-h dark

- 4.14 Renewal Period: 24-h
- 4.15 Physical and Chemical Parameters Measured: Parameters measured daily ("initial") on fresh test solutions and control waters (following addition of silt and algae) were temperature (temperature adjusted to equal "final" temperature before renewal), DO, pH, and conductivity. Alkalinity, and hardness were measured daily on the control and highest concentration.

"Final" measurements of temperature, DO, and pH were measured daily in one replicate per treatment before renewal. Conductivity was measured in a combination of two or more replicates per treatment following renewal. Mean values and ranges are reported in Section 6.3.2. Final alkalinity and hardness were measured in the control and 5.7 mg Cu/L treatment following renewal on Days 1 and 2. Both parameters were found to increase only slightly from initial measurements, so no final titrations were performed during the remainder of the test. One day during the study samples were collected for copper determination by combining test water from the replicates for each treatment (following renewal) in 500 mL bottles and adding 1:1 HNO₃ for preservation. The test copper series was sent to TVA's Environmental Chemistry Laboratory in Chattanooga, Tennessee, for analysis. (Note: The sample of nominal 5.0 mg Cu/L test solution was mixed and preserved immediately following addition of silt rather than after 24-hours of exposure in the test. One hundred percent mortality had already occurred in this treatment before samples were collected for copper analysis.)

- 4.16 Statistics: Revised statistical procedures contained in the fourth edition of EPA's acute toxicity methods require a decision process for testing statistical assumptions before selecting a specific program to determine LC₅₀ values. [6] Decision processes followed for testing mussel survival response are shown in Section 6.2.3. The program used for LC₅₀ determination for this test was the Probit Analysis.

5.0 QUALITY ASSURANCE

- 5.1 Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage; glassware preparation; test organism culturing/acquisition and acclimation; test organism handling during test; and maintaining appropriate test conditions were conducted according to the protocol as described in this report, the ARL Quality Assurance Plan and SOP Manual, and EPA/600/4-89/001. [5][6] Any known deviations were noted during the study and are reported herein.

5.2 Physical and Chemical Methods

- 5.2.1 Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable). All ARL chemicals are recorded in a bound Laboratory Chemical Logbook and specific chemicals used were documented on a chemical record sheet contained in the study notebook.
- 5.2.2 Instruments: All identification, service, and calibration information pertaining to ARL laboratory instruments is contained in bound Laboratory Instrument Logbooks and specific instruments used were documented on an instrument record sheet, along with daily calibration record sheets, contained in the study notebook.
- 5.2.3 Temperature was measured using mercury thermometers. The instrument was standardized and inspected with readings made according to TVA procedure DS-42.11. [2]
- 5.2.4 Dissolved oxygen was measured using a YSI Model 57 oxygen meter. The instrument was standardized (using the Winkler method) and readings were taken according to TVA procedures DS-43.6 and DS-42.4, respectively. [2]
- 5.2.5 The pH was measured using an Orion Model SA250 meter equipped with an Orion Ross combination electrode. The instrument was standardized and readings were made according to TVA procedures DS-43.7 and DS-42.8, respectively. [2]
- 5.2.6 Conductance was measured using a YSI Model 32 SCT meter. The instrument was standardized and readings were taken according to TVA procedures DS-43.3 and DS-42.3, respectively. [2]
- 5.2.7 Alkalinity was measured by titration of 100 mL samples with 0.02 N H_2SO_4 to an end point of 4.5 according to TVA procedure DS-42.1. [2]
- 5.2.8 Hardness was determined by titration of 50 mL samples with EDTA to a colorimetric endpoint using an indicator (Instructions provided by Reagent Manufacturer [Calgon]), Schwarzenbach Method.
- 5.2.9 Total residual chlorine was determined using the DPD Titrimetric Method according to TVA procedure DS-42.9, Rev. 0. [2]

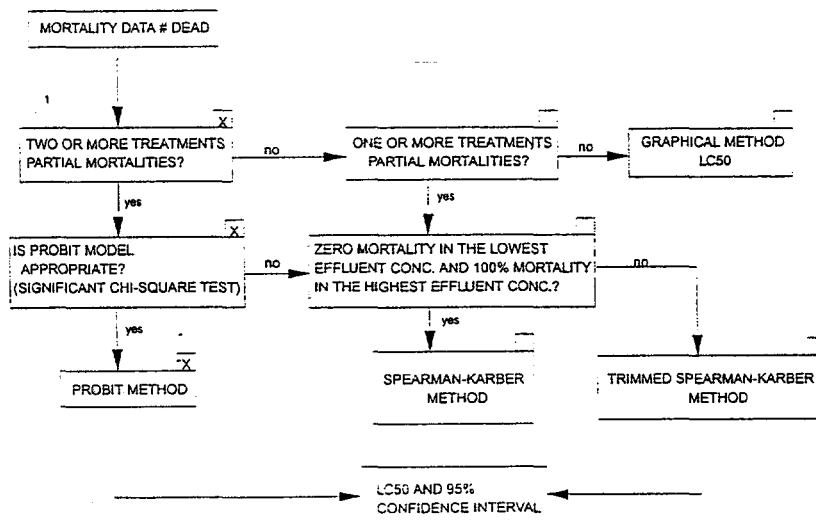
6.0 RESULTS

- 6.1 Summary of Results: Nine day exposure of juvenile freshwater mussels, Anodonta imbecillis, to copper reference toxicant resulted in an LC_{50} value of 1.12 mg Cu/L, based on the measured copper concentrations.

6.2 Results, Survival Data:

6.2.1 Statistical Decision Process for Determining Toxicity Endpoints for 9-Day Exposure of the Juvenile Mussel, Anodonta imbecillis, to Copper, May 12-21, 1993.

DETERMINATION OF THE LC50 FROM A MULTI-EFFLUENT/ CONCENTRATION ACUTE TOXICITY TEST



6.2.2 Daily Percent Survival Summary for Anodonta imbecillis, Copper Sulfate Reference Toxicant Study, CR-ERP, May 12-21, 1993.

Treatment	Total Daily % Survival								
	1	2	3	4	5	6	7	8	9
Control	100	100	100	100	100	100	100	100	100
0.34 mg Cu/L	100	100	100	100	100	100	100	100	100
0.67 mg Cu/L	100	100	100	100	100	100	98	98	85
1.30 mg Cu/L	100	100	98	90	85	80	78	45	40
2.60 mg Cu/L	75	55	50	40	25	5	5	3	3
5.70 mg Cu/L	45	18	3	0	0	0	0	0	0

6.2.3 Nine-day Percent Survival Summary for Anodonta imbecillis, Copper Sulfate Reference Toxicant Study, CR-ERP, May 12-21, 1993.

Mussel Survival Data (% Survival)											
Treatment	Replicate										Mean
	1	2	3	4	5	6	7	8	9	10	
Control	100	100	100	100							100
0.34 mg Cu/L	100	100	100	100							100
0.67 mg Cu/L	70	100	80	90							85
1.30 mg Cu/L	40	40	50	30							40
2.60 mg Cu/L	0	0	0	10							3
5.70 mg Cu/L	0	0	0	0							0

6.2.4 Test Endpoint: 9-day LC₅₀ = 1.12 (0.98-1.28, 95% C L) mg Cu/L.

6.3 Results: Water chemistry summary for Anodonta imbecillis, Copper Sulfate Reference Toxicant Study, CR-ERP, May 12-May 21, 1993.

6.3.1 Test Temperature: 24.2°C (23.3°-24.7°C)

6.3.2 See: Appendix A Water Chemistry Mean Values and Ranges for Anodonta imbecillis, Copper Sulfate Reference Toxicant Study, CR-ERP, May 12-21, 1993.

7.0 CONCLUSION

Toxicity testing of copper sulfate reference toxicant was conducted from May 12-21, 1993. The organisms used for testing were juvenile fresh-water mussels (Anodonta imbecillis). Results from this test showed an LC₅₀ value of 1.12 mg Cu/L which is lower than the value of 2.02 mg Cu/L obtained in a previous test. Too few tests have been conducted with copper as the toxicant to determine a normal range of values.

8.0 REFERENCES

1. Phipps, T. L., and L. A. Kszos, Statement of Work to Provide Assistance to Staff of the Clinch River-Environmental Restoration Program (CR-ERP) and to Evaluate Toxicity Tests of Water and Sediment Conducted by CR-ERP Staff, Environmental Sciences Division, Biomonitoring Group, Oak Ridge National Laboratory (April 1993).
2. Field Operations Natural Resource Engineering Procedures Manual, Vol. 1, Division of Natural Resource Operations, Tennessee Valley Authority.
3. Isom, B. G., and R. G. Hudson, "In Vitro Culture of Parasitic Freshwater Mussel Glochidia," The Nautilus, Vol. 96, No. 4, 1982, pp. 147-151.
4. Hudson, R. G., and B. G. Isom, "Rearing Juveniles of the Freshwater Mussels (Unionidae) in a Laboratory Setting," The Nautilus, Vol. 98, No. 4, 1984, pp. 129-135.
5. Aquatic Research Laboratory Quality Assurance Program and Standard Operating Procedures Manual, Division of Water Resources, Tennessee Valley Authority (October 1992).
6. Weber, C. I. (ed.), Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms, EPA/600/4-90/027 (September 1991).

PLARC501-1596

Appendix A

Water Chemistry Mean Values and Ranges for Anadonta imbecillis,
Copper Sulfate Reference Toxicant Study, CR-ERP, May 12-21, 1993

Source	Temperature		Dissolved Oxygen		pH		Conductivity		Alk		Hardness	
	Final	(°C)	Initial	(mg/L)	Initial	(S.U.)	Final	(µmhos)	Initial	(µmhos)	Initial	(mg/L)
CONTROL	24.2	(24.0-24.4)	8.0	7.9	6.9	7.8	348	363	34	363	89.7	(87.2-90.6)
0.312 mg/L	24.2	(23.8-24.4)	8.0	7.9	6.7	7.8	350	364	-	364	-	-
0.625 mg/L	24.3	(24.1-24.7)	8.0	7.9	6.6	7.8	350	365	-	365	-	-
1.250 mg/L	24.2	(24.0-24.6)	8.1	7.9	6.7	7.8	349	363	-	363	-	-
2.500 mg/L	24.1	(23.3-24.5)	8.1	7.9	6.5	7.8	353	368	33	368	92.3	(88.9-94.1)
5.000 mg/L	24.1	(24.0-24.3)	8.1	8.0	6.5	7.7	356	368	28	368	89.6	(87.2-92.3)

* mg/L as CaCO₃

PLARC501-1597

ATTACHMENT I

TOXICITY TEST BENCH SHEETS AND STATISTICAL ANALYSES

ARL ACUTE TOXICITY TEST (MUSSEL) MASTER CHECKLIST Study: Mussel Rel Tox May '93

Date Issued: 5-7-93 To: DJS From: JM

1. Test organism availability: T.L.* DJS C.U.† Date 5-6-93
2. Sample Collection Coordination: Date NA By NA With NA
3. Glassware availability: T.L. DJS Tech Date 5-10-93
4. Silt/Phytoplankton availability: Silt- By DJS/JT Date 4/30/93
Phytoplankton- By DJS Date 5-6-93
5. Glassware prep. complete: Date 5-10-93 By DJS
6. Data Sheet prep. complete: Date 5-10-93 By DJS
7. Test organism availability verification: T.L. DJS
8. Test Initiation: Date/Time 5/12/93 0935

9. Test Renewal:	Day#:	0	1	2	3	4	5	6	7	8	9
Meter Calib.	T.L.	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>JPP</u>	<u>JPP</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>
	T.M.*	-	-	-	-	-	-	-	<u>TS</u>	<u>TS</u>	-
Log Samples	T.L.	<u>NA</u>	<u>NA</u>	<u>NA</u>	<u>NA</u>	<u>NA</u>	<u>NA</u>	<u>NA</u>	<u>NA</u>	<u>NA</u>	<u>N/A</u>
	T.M.	<u>NA</u>	<u>NA</u>	<u>NA</u>	<u>NA</u>	<u>NA</u>	<u>NA</u>	<u>NA</u>	<u>NA</u>	<u>NA</u>	<u>N/A</u>
Initial Chem.	T.L.	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>N/A</u>
	T.M.	<u>JPP</u>	<u>JPP</u>	<u>JPP</u>	<u>JPP</u>	<u>JPP</u>	<u>TS/SP</u>	<u>JPP</u>	<u>TS</u>	<u>TS</u>	<u>N/A</u>
Renewal/Counts	T.L.	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>
	T.M.	<u>JPP</u>	<u>JPP</u>	<u>JPP</u>	<u>JPP</u>	<u>JPP</u>	<u>TS/SP</u>	<u>JPP</u>	<u>TS</u>	<u>TS</u>	-
Final Chem.	T.L.	<u>N/A</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>
	T.M.	<u>N/A</u>	<u>JPP</u>	<u>JPP</u>	<u>JPP</u>	<u>JPP</u>	<u>TS/SP</u>	<u>JPP</u>	<u>TS</u>	<u>TS</u>	-
Meter Recal.	T.L.	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>JPP</u>	<u>DJS</u>	<u>TS</u>	<u>DJS</u>	<u>NA</u>	<u>NA</u>	<u>NA</u>
	T.M.	-	<u>JPP</u>	<u>JPP</u>	<u>JPP</u>	<u>JPP</u>	<u>TS</u>	-	<u>NA</u>	<u>NA</u>	<u>NA</u>
Paperwork reviewed	T.L.	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>JPP</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>	<u>DJS</u>
	T.M.	-	<u>JPP</u>	<u>JPP</u>	<u>JPP</u>	<u>JPP</u>	-	<u>DJS</u>	-	-	-

10. Test Termination: Date/Time 5-21-93/0900 Nominal:
LC50 = 1.07
12. Run Stats: Date 5-21-93 By DJS EC50 = 0.80 (seen or death)
13. Report Prep.: Date 6/9-6/16 By DJS/JM Actual
LC50 = 1.12
14. Report Final: Date 6-18-93 By JM Approved: DOW

*Team Leader

†Culture Unit

*Team Member

Date: 05-06-93

Copper stocks prepared as follows by D. Simbeck using EPA Copper Sulfate reference toxicant, Lot # 184, Ampules # 2025, Ampules # 2026, 50 mg/mL Cu:

1. Combine contents of 2 ampules (7.0 mL) in 100 mL beaker.
2. Add 5 mL CuSO_4 to a 100 mL vol. flask and fill to line to obtain Stock #I = 2,500 mg/L. Pour into 100 mL beaker to mix.
3. Prepare diluted stock series:

<u>Cu Conc. Stock</u>	<u>mL Stock #I</u>	<u>mL 50:50 HNO_3</u>	<u>Dilute to 100 mL</u>
Control	0.00	1	100
46.75 mg/L	1.87	1	100
93.75 mg/L	3.75	1	100
187.50 mg/L	7.50	1	100
375.00 mg/L	15.00	1	100
750.00 mg/L	30.00	1	100

4. Prepare test solutions:

<u>Cu Conc. Stock</u>	<u>mL</u>	<u>Dilute to 1000 mL</u>	<u>Cu Conc. Test</u>
Control	6.67	993.33	0.000
46.75 mg/L	6.67	993.33	0.312
93.75 mg/L	6.67	993.33	0.625
187.50 mg/L	6.67	993.33	1.250
375.00 mg/L	6.67	993.33	2.500
750.00 mg/L	6.67	993.33	5.000

Damien J. Simbeck

MUSSEL TEST RECORD SHEET
Anodonta imbecillis

Project Mussel Ref Tox May 1993

Beginning Ending
Date/Time 5-12-93 10935 Date/Time 5-21-93 10900

Personnel Simbeck Posey Sesler Adrian M. Gonzalez (ORNL)

Mussels placed in test vessels: Date 5-11-93 Time 1200 1245, 1400 1530

Mussels per test vessel: 10 in Med #1330

Test Treatments:

1	Control	11	
2	0.312 mg/L Cu	12	
3	0.625 mg/L Cu	13	
4	1.250 mg/L Cu	14	
5	2.500 mg/L Cu	15	
6	5.000 mg/L Cu	16	
7		17	
8		18	
9		19	
10		20	

Control/Dilution Water: F-Med #'s 1330, 1332, 1335, 1336, 1338

Source ARL prepared Date Collected/Prepared 5/10, 5/10, 5/14, 5/14
5/14

CULTURE HISTORY:

Culture ID	Date Started	Date Transformed
<u>93-A</u>	<u>4-27-93</u>	<u>5-4-93</u>

FOOD/SUBSTRATE PREPARATION:

Phytoplankton Started	Phytoplankton Concentrated
<u>5-24-93</u>	<u>5-6-93</u>

Silt Collected: Date/Location 4/30/93 1 Beck Pond @ Taylor's

Silt Filtered (Date) 4/30/93 Silt Weighed (Date) 5/10/93

Volume Weighed 5 mL

	Wt. of pan	Wt. of pan and silt	Wt. of silt
Replicate 1	<u>1,037.44 mg</u>	<u>2,216.49 mg</u>	<u>1,179.05 mg</u>
Replicate 2	<u>1,026.36 mg</u>	<u>2,202.16 mg</u>	<u>1,175.80 mg</u>
Replicate 3	<u>1,065.16 mg</u>	<u>2,247.45 mg</u>	<u>1,182.29 mg</u>

Average dry weight 1,179.05/5 mg/mL PLARC501-130(5)
235.81 mg/mL 3mL 1. grid = 707.43mg dry silt

TREATMENT: Control

DAY:	0	1	2	3	4	5	6	7	8	9
DATE:	5/12	5/13	5/14	5/15	5/16	5/17	5/18	5/19	5/20	5/21
Rep 1: # Alive	10	10	10	10	10	10	10	10	10	10
# Stressed		0	0	0	0	0	0	6	0	0
# Dead		0	0	0	0	0	0	0	0	0
Rep 2: # Alive	10	10	10	10	10	10	10	10	10	10
# Stressed		0	0	0	0	0	0	0	0	0
# Dead		0	0	0	0	0	0	0	0	0
Rep 3: # Alive	10	10	10	10	10	10	10	10	10	10
# Stressed		0	0	0	0	0	0	0	0	0
# Dead		0	0	0	0	0	0	0	0	0
Rep 4: # Alive	10	10	10	10	10	10	10	10	10	10
# Stressed		0	0	0	0	0	0	0	0	0
# Dead		0	0	0	0	0	0	0	0	0
	JPP	JPP	JPS	JPP	JPP	JPP	JPP	JPS	JPS	JPS

TREATMENT: 0.312 mg/L Cu

DAY:	0	1	2	3	4	5	6	7	8	9
DATE:	5/12	5/13	5/14	5/15	5/16	5/17	5/18	5/19	5/20	5/21
Rep 1: # Alive	10	10	10	10	10	10	10	10	10	10*
# Stressed		0	0	0	0	0	0	0	0	0
# Dead		0	0	0	0	0	0	0	0	0
Rep 2: # Alive	10	10	10	10	10	10	10	10	10	10*
# Stressed		0	0	0	0	0	0	0	0	0
# Dead		0	0	0	0	0	0	0	0	0
Rep 3: # Alive	10	10	10	10	10	10	10	10	10	9*
# Stressed		0	0	0	0	0	0	0	0	1
# Dead		0	0	0	0	0	0	0	0	0
Rep 4: # Alive	10	10	10	10	10	10	10	10	10	10*
# Stressed		0	0	0	0	0	0	0	0	0
# Dead		0	0	0	0	0	0	0	0	0
	JPS/Time	JPP	JPS	JPP	JPP	JPS	JPP	JPS	JPS	JPS

*slows but active. Very little growth. Definitely smaller than controls

TREATMENT: 0.625 mg/L Cu

DAY:	0	1	2	3	4	5	6	7	8	9
DATE:	5/12	5/13	5/14	5/15	5/16	5/17	5/18	5/19	5/20	5/21
Rep 1: # Alive	10	10	10	10	10	10	10*	10*	6*	4*
# Stressed		0	0	0	0	0	0	0	4	3
# Dead		0	0	0	0	0	0	0	0	3
Rep 2: # Alive	10	10	10	10	10	10	10*	10*	10*	10
# Stressed		10	0	0	0	0	0	0	0	0
# Dead		0	0	0	0	0	0	0	0	0
Rep 3: # Alive	10	10	10	10	10	10	10*	9*	8*	7*
# Stressed		0	0	0	0	0	0	0	1	1
# Dead		0	0	0	0	0	0	1	1	2
Rep 4: # Alive	10	10	10	10	10	10	10*	10*	9*	6
# Stressed		0	0	0	0	0	0	0	1	3
# Dead		0	0	0	0	0	0	0	0	1

JPP JPP JPP JPP JPP JPP JPP JPP JPP JPP JPP

0.625 very slow by day 2

* All closed, inactive by day 6
some with very minimal valve - full movement.

TREATMENT: 1.250 mg/L Cu2

DAY:	0	1	2	3	4	5	6	7	8	9
DATE:	5/12	5/13	5/14	5/15	5/16	5/17	5/18	5/19	5/20	5/21
Rep 1: # Alive	10	10*	10*	10*	28*	4	5	4	4	0
# Stressed		0	0	0	0	12	0	1	0	4
# Dead		0	0	0	2	24	5	5	6	6
Rep 2: # Alive	10	10*	10*	10*	9*	9	9	4	4	2
# Stressed		0	0	0	1	1	0	5	0	2
# Dead		0	0	0	0	0	1	1	6	6
Rep 3: # Alive	10	10*	10*	10*	9**	9	9	9	5	4
# Stressed		0	0	0	0	0	0	0	1	1
# Dead		0	0	0	1	1	1	1	4	5
Rep 4: # Alive	10	10*	10*	9*	8*	8	8	5	4	1
# Stressed		0	0	0	1	1	1	3	0	2
# Dead		0	0	1	1	1	1	2	6	7

* all closed, but some move a little. By day 3, no movement.

JPP JPP JPP JPP JPP JPP JPP JPP JPP JPP JPP

** Rep 4 - saw two mussels in Rep 3 moult

TREATMENT: 2.500 mg/L Cu

DAY:	0	1	2	3	4	5	6	7	8	9
DATE:	5/12	5/13	5/14	5/15	5/16	5/17	5/18	5/19	5/20	5/21
Rep 1: # Alive	10	*4	6*	*5	2*	1	0			
# Stressed		1	0	1	3	0	0			
# Dead		5	4	4	5	9	10			
Rep 2: # Alive	10	*7	3*	*2	1	0	0			
# Stressed		1	0	0	1	2	0			
# Dead		2	7	8	8	8	10			
Rep 3: # Alive	10	8	8*	*4	4*	0	1*	1*	0	
# Stressed		1	0	3	0	3	0	0	0	
# Dead		1	2	3	6	7	9	9	10	
Rep 4: # Alive	10	7	5*	5*	4*	1*	1*	1*	1*	1*
# Stressed		-	0	0	1	3	0	0	0	0
# Dead		3	5	5	5	6	9	9	9	9

* all closed

** must seen with some video movement

TREATMENT: 5.000 mg/L Cu2

DAY:	0	1	2	3	4	5	6	7	8	9
DATE:	5/12	5/13	5/14	5/15	5/16	5/17	5/18	5/19	5/20	5/21
Rep 1: # Alive	10	7*	3*	0						
# Stressed		0	0	0						
# Dead		3	7	10						
Rep 2: # Alive	10	*1*	1*	0						
# Stressed		0	0	0						
# Dead		9	9	10						
Rep 3: # Alive	10	3*	2*	0						
# Stressed		1	0	0						
# Dead		6	8	10						
Rep 4: # Alive	10	4*	2*	*1	0					
# Stressed		2	0	0	0					
# Dead		4	9	9	10					

* all closed, inactive

DS
AME

DS JPP DS

EPA PROBIT ANALYSIS PROGRAM
 USED FOR CALCULATING EC VALUES
 Version 1.4

Actual Conc.

reftox2

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
0.3400	40	0	0.0000	0.0000	0.0042
0.6700	40	6	0.1500	0.1500	0.1275
1.3000	40	24	0.6000	0.6000	0.6281
2.6000	40	39	0.9750	0.9750	0.9685
5.7000	40	40	1.0000	1.0000	0.9998

Chi - Square Heterogeneity = 0.547

Mu = 0.049708
 Sigma = 0.196472

Parameter	Estimate	Std. Err.	95% Confidence Limits	
Intercept	4.746997	0.147103	(4.458675,	5.035319)
Slope	5.089777	0.674041	(3.768658,	6.410896)

Theoretical Spontaneous Response Rate = 0.0000

refloxx2

Estimated EC Values and Confidence Limits

Point	Conc.	95% Confidence Limits	
		Lower	Upper
EC 1.00	0.3914	0.2640	0.4993
EC 5.00	0.5328	0.3961	0.6444
EC10.00	0.6279	0.4902	0.7407
EC15.00	0.7016	0.5648	0.8156
EC50.00	1.1213	0.9832	1.2806
EC85.00	1.7920	1.5376	2.2381
EC90.00	2.0022	1.6923	2.5796
EC95.00	2.3599	1.9446	3.1936
EC99.00	3.2119	2.5086	4.7948

reftox2

PLOT OF ADJUSTED PROBITS AND PREDICTED REGRESSION LINE

Probit

10+

9+

8+

7+

6+

5+

4+

3+

2+

1+

0+0

EC01

EC10

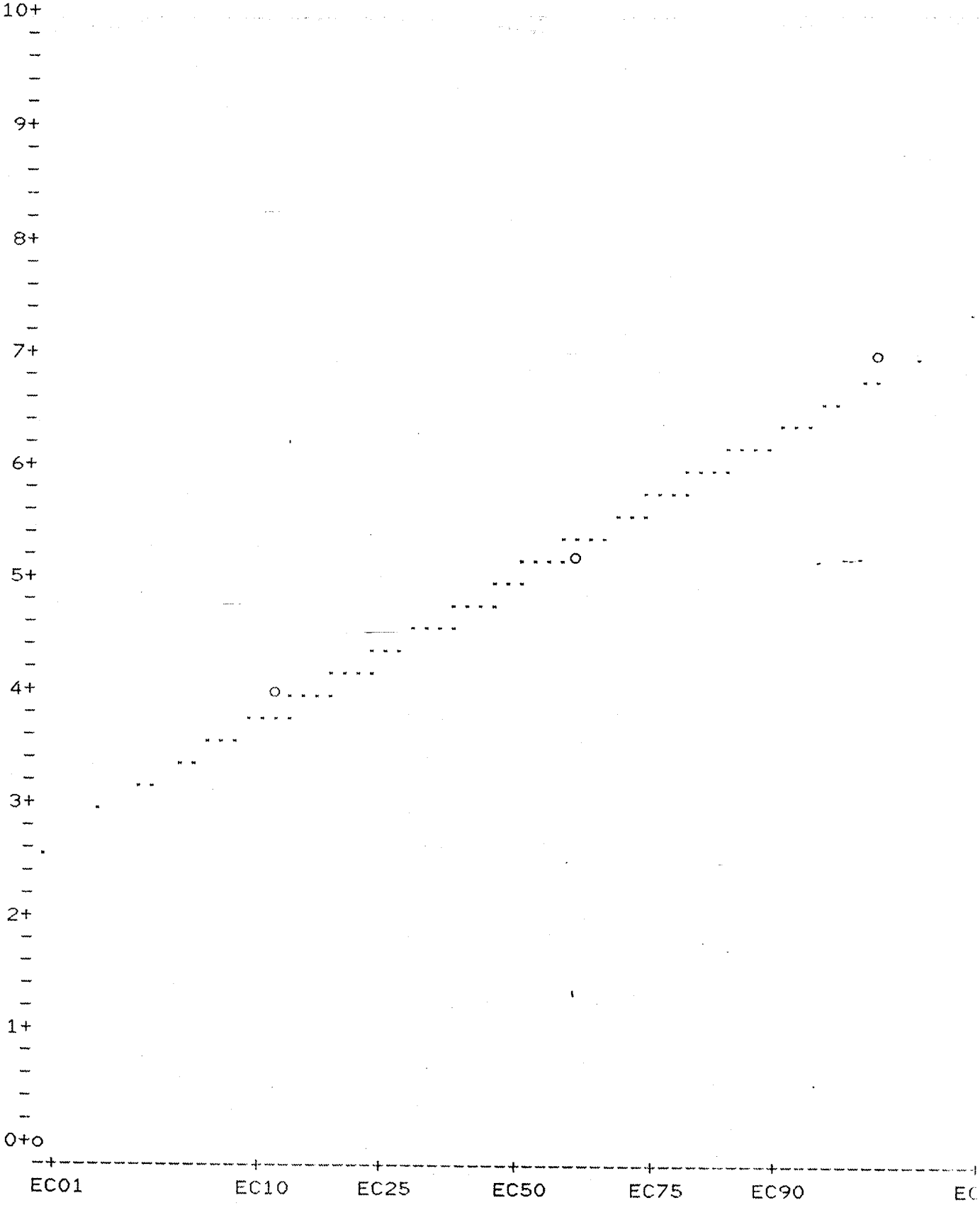
EC25

EC50

EC75

EC90

EC



CR-ERP-1 -- May 12-21, 1993 -- INITIAL AND FINAL CHEMISTRY

INITIAL CHEMISTRY

CONTROL

DAY	TEMP	DO	pH	COND	HARD	ALK	CHLORINE
0	24.1	7.9	7.0	347	88.9	31	--
1	24.2	7.8	6.8	345	90.6	31	--
2	23.9	8.1	6.9	345	90.6	34	--
3	24.3	7.5	6.9	346	87.2	33	--
4	24.0	8.0	6.8	346	88.9	36	--
5	24.3	7.9	6.9	350	90.6	34	--
6	23.9	8.2	6.9	350	90.6	35	--
7	24.3	8.1	6.9	350	90.6	34	--
8	24.1	8.2	6.8	351	88.9	34	--
MEAN	24.1	8.0	6.9	348	89.7	34	--
MIN	23.9	7.5	6.8	345	87.2	31	--
MAX	24.3	8.2	7.0	351	90.6	36	--

1.250 mg/L

DAY	TEMP	DO	pH	COND	HARD	ALK	CHLORINE
0	24.0	8.1	6.7	348	-	-	-
1	24.0	8.1	6.6	346	-	-	-
2	24.0	8.1	6.6	346	-	-	-
3	24.3	7.6	6.7	348	-	-	-
4	24.3	8.2	6.7	347	-	-	-
5	24.4	8.0	6.6	352	-	-	-
6	24.1	8.2	6.7	351	-	-	-
7	24.3	8.1	6.7	351	-	-	-
8	24.4	8.2	6.6	353	-	-	-
MEAN	24.2	8.1	6.7	349	0.0	0	--
MIN	24.0	7.6	6.6	346	0.0	0	--
MAX	24.4	8.2	6.7	353	0.0	0	--

0.312 mg/L

DAY	TEMP	DO	pH	COND	HARD	ALK	CHLORINE
0	24.3	8.0	6.7	350	-	-	-
1	24.0	8.0	6.6	347	-	-	-
2	24.0	8.1	6.7	347	-	-	-
3	24.2	7.6	6.7	349	-	-	-
4	24.3	8.1	6.7	349	-	-	-
5	24.5	7.9	6.7	353	-	-	-
6	24.0	8.2	6.7	352	-	-	-
7	24.3	8.1	6.8	352	-	-	-
8	24.3	8.2	6.7	353	-	-	-
MEAN	24.2	8.0	6.7	350	0.0	0	--
MIN	24.0	7.6	6.6	347	0.0	0	--
MAX	24.5	8.2	6.8	353	0.0	0	--

2.500 mg/L

DAY	TEMP	DO	pH	COND	HARD	ALK	CHLORINE
0	24.1	8.1	6.5	352	-	-	-
1	24.1	8.1	6.5	350	-	-	-
2	23.9	8.2	6.5	350	-	-	-
3	24.2	7.7	6.5	352	-	-	-
4	24.3	8.3	6.5	351	-	-	-
5	24.5	8.1	6.5	356	88.9	32	-
6	24.1	8.2	6.5	355	94.1	33	-
7	24.3	8.1	6.6	355	92.3	34	-
8	24.2	8.3	6.6	355	94.1	34	-
MEAN	24.2	8.1	6.5	353	92.3	33	--
MIN	23.9	7.7	6.5	350	88.9	32	--
MAX	24.5	8.3	6.6	356	94.1	34	--

0.625 mg/L

DAY	TEMP	DO	pH	COND	HARD	ALK	CHLORINE
0	24.0	8.1	6.7	349	-	-	-
1	24.0	8.0	6.6	347	-	-	-
2	24.0	8.1	6.6	347	-	-	-
3	24.2	7.6	6.6	349	-	-	-
4	24.3	8.1	6.6	348	-	-	-
5	24.4	8.0	6.6	353	-	-	-
6	24.2	8.2	6.7	353	-	-	-
7	24.3	8.1	6.7	353	-	-	-
8	24.3	8.2	6.6	353	-	-	-
MEAN	24.2	8.0	6.6	350	0.0	0	--
MIN	24.0	7.6	6.6	347	0.0	0	--
MAX	24.4	8.2	6.7	353	0.0	0	--

5.000 mg/L

DAY	TEMP	DO	pH	COND	HARD	ALK	CHLORINE
0	24.0	8.1	6.5	357	88.9	26	-
1	24.0	8.1	6.5	354	92.3	27	-
2	23.9	8.3	6.5	355	88.9	28	-
3	24.4	7.7	6.5	356	87.2	29	-
4	24.2	8.3	6.5	356	90.6	28	-
5	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-
MEAN	24.1	8.1	6.5	356	89.6	28	-
MIN	23.9	7.7	6.5	354	87.2	26	-
MAX	24.4	8.3	6.5	357	92.3	29	-

FINAL CHEMISTRY

CONTROL

DAY	TEMP	DO	pH	COND
1	24.1	7.8	7.8	356
2	24.1	8.0	7.9	361
3	24.0	8.0	7.8	358
4	24.2	8.0	7.8	365
5	24.2	7.9	7.8	360
6	24.0	7.9	7.8	369
7	24.4	7.9	7.8	369
8	24.3	7.7	7.9	363
9	24.1	7.9	7.9	363
MEAN	24.2	7.9	7.8	363
MIN	24.0	7.7	7.8	356
MAX	24.4	8.0	7.9	369

0.312 mg/L

DAY	TEMP	DO	pH	COND
1	24.3	7.6	7.7	356
2	23.8	8.0	7.8	365
3	23.8	8.0	7.8	362
4	24.3	8.0	7.8	365
5	24.1	8.0	7.8	367
6	24.3	7.9	7.8	366
7	24.3	7.9	7.8	367
8	24.2	7.8	7.9	368
9	24.4	7.9	7.9	363
MEAN	24.2	7.9	7.8	364
MIN	23.8	7.6	7.7	356
MAX	24.4	8.0	7.9	368

0.625 mg/L

DAY	TEMP	DO	pH	COND
1	24.1	7.8	7.8	366
2	24.1	8.0	7.8	360
3	24.2	8.0	7.8	368
4	24.2	8.0	7.8	360
5	24.3	8.0	7.8	360
6	24.4	8.0	7.8	369
7	24.6	7.9	7.8	368
8	24.2	7.8	7.9	370
9	24.7	7.9	7.9	366
MEAN	24.3	7.9	7.8	365
MIN	24.1	7.8	7.8	360
MAX	24.7	8.0	7.9	370

1.250 mg/L

DAY	TEMP	DO	pH	COND
1	24.6	7.7	7.8	355
2	24.0	8.0	7.8	360
3	24.1	7.9	7.8	366
4	24.1	8.0	7.8	358
5	24.2	8.0	7.8	360
6	24.3	8.0	7.8	365
7	24.5	7.8	7.8	367
8	24.2	7.8	7.9	369
9	24.1	7.9	7.9	368

MEAN	24.2	7.9	7.8	363
------	------	-----	-----	-----

MIN	24.0	7.7	7.8	355
MAX	24.6	8.0	7.9	369

2.500 mg/L

DAY	TEMP	DO	pH	COND
1	23.3	7.9	7.7	368
2	24.0	7.9	7.8	363
3	23.7	8.1	7.8	371
4	24.2	8.0	7.8	364
5	24.2	8.0	7.7	365
6	24.2	8.0	7.8	369
7	24.4	7.9	7.8	369
8	24.5	7.8	7.8	371
9	24.0	7.9	7.8	370

MEAN	24.1	7.9	7.8	368
------	------	-----	-----	-----

MIN	23.3	7.8	7.7	363
MAX	24.5	8.1	7.8	371

5.000 mg/L

DAY	TEMP	DO	pH	COND
1	24.1	7.9	7.6	373
2	24.0	7.9	7.7	367
3	24.0	8.0	7.7	369
4	24.3	8.0	7.7	363
5	-	-	-	-
6	-	-	-	-
7	-	-	-	-
8	-	-	-	-
9	-	-	-	-

MEAN	24.1	8.0	7.7	368
------	------	-----	-----	-----

MIN	24.0	7.9	7.6	363
MAX	24.3	8.0	7.7	373

TEMPERATURE: MEAN: 24.2

MIN:	23.3
MAX:	24.7

Reviewed By/Date:

dth 06-14-93

(CR-ERP-1)

INITIAL CHEMISTRYProject: Mussel Ref Tox May 1993Personnel: SimbeckBeginning Date: 5-12-93PoseyEnding Date: 5-21-93SeslerDate: 5-12-93

TREATMENT	Cntrl	0.312	0.625	1.250	2.500	5.000		
Temperature	24.1	24.3	24.0	24.0	24.1	24.0		
D.O. (before air)	8.4	8.5	8.5	8.5	8.5	8.5		
(after air)								
(w/silt/algae)	7.9	8.0	8.1	8.1	8.1	8.1		
pH (before air)	6.9	6.5	6.5	6.6	6.5	6.5		
(after air)								
(w/silt/algae)	7.0	6.7	6.7	6.7	6.5	6.5		
(Aeration time)								
Conductivity	346 ⁵	347 ⁵ 350	345 ⁵ 349	347 ⁵ 348	351 ⁵ 352	358 ⁵ 357		
Hardness	5.2					5.2		
x 17.1	88.9					88.9		
Alkalinity	3.1					2.6		
x 10	31					26		
Chlorine								
Ammonia								

NOTES: Mel #1330

INITIAL CHEMISTRYProject: Mussel Ref Tox May 1993Personnel: SimbeckBeginning Date: 5-12-93PoseyEnding Date: 5-21-93SeslerDate: 5-13-93

TREATMENT	Cntrl	0.312	0.625	1.250	2.500	5.000		
Temperature	24.2	24.0	24.0	24.0	24.1	24.0		
D.O. (before air)								
(after air)								
(w/silt/algae)	7.8	8.0	8.0	8.1	8.1	8.1		
pH (before air)								
(after air)								
(w/silt/algae)	6.8	6.6	6.6	6.6	6.5	6.5		
(Aeration time)								
Conductivity	345	347	347	346	350	354		
Hardness	5.3 5.2					5.4		
x 17.1	90.6					92.3		
Alkalinity	3.1					2.7		
x 10	31					27		
Chlorine								
Ammonia								

NOTES: Med # 1332

INITIAL CHEMISTRYProject: Mussel Ref Tox May 1993Personnel: SimbeckBeginning Date: 5-12-93PoseyEnding Date: 5-21-93SeslerDate: 5-14-93

TREATMENT	Cntrl	0.312	0.625	1.250	2.500	5.000		
Temperature	23.9	24.0	24.0	24.0	23.9	23.9		
D.O. (before air)								
(after air)								
(w/silt/algae)	8.1	8.1	8.1	8.1	8.2	8.3		
pH (before air)								
(after air)								
(w/silt/algae)	6.9	6.7	6.6	6.6	6.5	6.5		
(Aeration time)								
Conductivity	345	347	347	346	350	355		
Hardness	5.3					5.2		
x 17.1	90.6					88.9		
Alkalinity	3.4					2.8		
x 10	34					28		
Chlorine								
Ammonia								

NOTES: Med H 1332

INITIAL CHEMISTRYProject: Mussel Ref Tox May 1993Personnel: SimbeckBeginning Date: 5-12-93PoseyEnding Date: 5-21-93SeslerDate: 5-15-93

TREATMENT	Cntrl	0.312	0.625	1.250	2.500	5.000		
Temperature	24.3	24.2	24.2	24.3	24.2	24.4		
D.O. (before air)								
(after air)								
(w/silt/algae)	7.5	7.6	7.6	7.6	7.7	7.7		
pH (before air)								
(after air)								
(w/silt/algae)	6.9	6.7	6.6	6.7	6.5	6.5		
(Aeration time)								
Conductivity	346	349	349	348	352	356		
Hardness	5.1					5.1		
x 17.1	87.2					87.2		
Alkalinity	3.3					2.9		
x 10	33					29		
Chlorine								
Ammonia								

NOTES: Met # 1335

INITIAL CHEMISTRYProject: Mussel Ref Tox May 1993Personnel: SimbeckBeginning Date: 5-12-93PoseyEnding Date: 5-21-93SeslerDate: 5-16-93

TREATMENT	Cntrl	0.312	0.625	1.250	2.500	5.000		
Temperature	24.0	24.3	24.3	24.3	24.3	24.2		
D.O. (before air)								
(after air)								
(w/silt/algae)	8.0	8.1	8.1	8.2	8.3	8.3		
pH (before air)								
(after air)								
(w/silt/algae)	6.8	6.7	6.6	6.7	6.5	6.5		
(Aeration time)								
Conductivity	346	349	348	347	351	356		
Hardness	5.2					5.3		
x 17.1	88.9					90.6		
Alkalinity	3.6					2.8		
x 10	36					28		
Chlorine								
Ammonia								

NOTES: Med H 1335

INITIAL CHEMISTRYProject: Mussel Ref Tox May 1993Personnel: SimbeckBeginning Date: 5-12-93PoseyEnding Date: 5-21-93SeslerDate: 5-17-93

TREATMENT	Cntrl	0.312	0.625	1.250	2.500	5.000		
Temperature	24.3	24.5	24.4	24.4	24.5	-		
D.O. (before air)								
(after air)								
(w/silt/algae)	7.9	7.9	8.0	8.0	8.1	-		
pH (before air)								
(after air)								
(w/silt/algae)	6.9	6.7	6.6	6.6	6.5	-		
(Aeration time)								
Conductivity	350	353	353	352	356	-		
Hardness	5.3				5.2			
x 17.1	90.6				88.9			
Alkalinity	3.4				3.2			
x 10	34				32			
Chlorine								
Ammonia								

NOTES: Med #1336

INITIAL CHEMISTRYProject: Mussel Ref Tox May 1993Personnel: SimbeckBeginning Date: 5-12-93PoseyEnding Date: 5-21-93SeslerDate: 5-18-93

TREATMENT	Cntrl	0.312	0.625	1.250	2.500	5.000		
Temperature	23.9	24.0	24.2	24.1	24.1	1		
D.O. (before air)								
(after air)								
(w/silt/algae)	8.2	8.2	8.2	8.2	8.2			
pH (before air)								
(after air)								
(w/silt/algae)	6.9	6.7	6.7	6.7	6.5			
(Aeration time)								
Conductivity	350	352	353	351	355			
Hardness	5.3				5.5			
x 17.1	90.6				94.1			
Alkalinity	3.5				3.3			
x 10	35				33			
Chlorine								
Ammonia								

NOTES: med # 1336

INITIAL CHEMISTRYProject: Mussel Ref Tox May 1993Personnel: SimbeckBeginning Date: 5-12-93PoseyEnding Date: 5-21-93SeslerDate: 5-19-93

TREATMENT	Cntrl	0.312	0.625	1.250	2.500	5.000		
Temperature	24.3	—	—	—	—	—		
D.O. (before air)								
(after air)								
(w/silt/algae)	8.1	8.1	8.1	8.1	8.1			
pH (before air)								
(after air)								
(w/silt/algae)	6.9	6.8	6.7	6.7	6.6			
(Aeration time)								
Conductivity	350	352	353	351	355			
Hardness	5.3				5.4			
x 17.1	90.6				92.3			
Alkalinity	3.4				3.4			
x 10								
Chlorine								
Ammonia								

NOTES: Med # 1336

INITIAL CHEMISTRYProject: Mussel Ref Tox May 1993Personnel: SimbeckBeginning Date: 5-12-93PoseyEnding Date: 5-21-93SeslerDate: 5-20-93

TREATMENT	Cntrl	0.312	0.625	1.250	2.500	5.000		
Temperature	24.1	24.3	24.3	24.4	24.2			
D.O. (before air)								
(after air)								
(w/silt/algae)	8.2	8.2	8.2	8.2	8.3			
pH (before air)								
(after air)								
(w/silt/algae)	6.8	6.7	6.6	6.6	6.6			
(Aeration time)								
Conductivity	351	353	353	353	355			
Hardness	5.2				5.5			
x 17.1	88.9				94.1			
Alkalinity	3.4				3.4			
x 10	34				34			
Chlorine								
Ammonia								

NOTES: med # 1336 1337

FINAL CHEMISTRYProject: Mussel Ref Tox May 1993Personnel: SimbeckBeginning Date: 5-12-93PoseyEnding Date: 5-21-93SeslerDate: 5-13-93Replicate: #1

TREATMENT	Cntrl	0.312	0.625	1.250	2.500	5.000		
Temperature	24.1	24.3	24.1	24.6	23.3	24.1		
D.O.	7.8	7.6	7.8	7.7	7.9	7.9		
pH	7.8	7.7	7.8	7.8	7.7	7.6	---	
Conductivity	356	356	366	355	368	373		
Hardness	5.2					5.8		
x 17.1								
Alkalinity	3.4					2.7		
x 10	34					27		
Chlorine								
Ammonia								

NOTES: _____

(PLARC501-130)

FINAL CHEMISTRYProject: Mussel Ref Tox May 1993Personnel: SimbeckBeginning Date: 5-12-93PoseyEnding Date: 5-21-93SeglerDate: 5-14-93Replicate: #2

TREATMENT	Cntrl	0.312	0.625	1.250	2.500	5.000		
Temperature	24.1	23.8	24.1	24.0	24.0	24.0		
D.O.	8.0	8.0	8.0	8.0	7.9	7.9		
pH	7.9	7.8	7.8	7.8	7.8	7.7		
Conductivity	361	365	360	360	363	367		
Hardness	5.4	—				5.6		
x 17.1								
Alkalinity	3.3					2.6		
x 10	33					26		
Chlorine								
Ammonia								

NOTES: _____

(PLARC501-130)

FINAL CHEMISTRYProject: Mussel Ref Tox May 1993Personnel: SimbeckBeginning Date: 5-12-93PoseyEnding Date: 5-21-93SeslerDate: 5-15-93Replicate: #3

TREATMENT	Cntrl	0.312	0.625	1.250	2.500	5.000		
Temperature	24.0	23.8	24.2	24.1	23.7	24.0		
D.O.	8.0	8.0	8.0	7.9	8.1	8.0		
pH	7.8	7.8	7.8	7.8	7.8	7.7		
Conductivity	358	362 371.0	368	366 368.0	371 362.0	369		
Hardness								
x 17.1								
Alkalinity								
x 10								
Chlorine								
Ammonia								

NOTES: _____

FINAL CHEMISTRYProject: Mussel Ref Tox May 1993Personnel: SimbeckBeginning Date: 5-12-93PoseyEnding Date: 5-21-93SeslerDate: 5-16-93Replicate: # 4

TREATMENT	Cntrl	0.312	0.625	1.250	2.500	5.000		
Temperature	24.2	24.3	24.2	24.1	24.2	24.3		
D.O.	8.0	8.0	8.0	8.0	8.0	8.0		
pH	7.8	7.8	7.8	7.8	7.8	7.7	-	-
Conductivity	365	365	360	358	364	363		
Hardness								
x 17.1								
Alkalinity								
x 10								
Chlorine								
Ammonia								

NOTES: _____

FINAL CHEMISTRYProject: Mussel Ref Tox May 1993Personnel: SimbeckBeginning Date: 5-12-93PoseyEnding Date: 5-21-93SeslerDate: 5-17-93Replicate: 41

TREATMENT	Cntrl	0.312	0.625	1.250	2.500	5.000		
Temperature	24.2	24.1	24.3	24.2	24.2			
D.O.	7.9	8.0	8.0	8.0	8.0			
pH	7.8	7.8	7.8	7.8	7.7			
Conductivity	360	367	360	360	365			
Hardness								
x 17.1								
Alkalinity								
x 10								
Chlorine								
Ammonia								

NOTES: _____

(PLARC501-130)

FINAL CHEMISTRYProject: Mussel Ref Tox May 1993Personnel: SimbeckBeginning Date: 5-12-93PoseyEnding Date: 5-21-93SeslerDate: 5-18-93Replicate: #2

TREATMENT	Cntrl	0.312	0.625	1.250	2.500	5.000		
Temperature	24.0	24.3	24.4	24.3	24.2	—		
D.O.	7.9	7.9	8.0	8.0	8.0	—		
pH	7.8	7.8	7.8	7.8	7.8		---	
Conductivity	369	366	369	365	369			
Hardness								
x 17.1								
Alkalinity								
x 10								
Chlorine								
Ammonia								

NOTES: _____

FINAL CHEMISTRYProject: Mussel Ref Tox May 1993Personnel: SimbeckBeginning Date: 5-12-93PoseyEnding Date: 5-21-93SeslerDate: 5-19-93Replicate: #3

TREATMENT	Cntrl	0.312	0.625	1.250	2.500	5.000		
Temperature	24.4	24.3	24.6	24.5	24.4			
D.O.	7.9 8.1 Ts	7.9	7.9	7.8	7.9			
pH	7.8	7.8	7.8	7.8	7.8			
Conductivity	369	367	368	367	369			
Hardness								
x 17.1								
Alkalinity								
x 10								
Chlorine								
Ammonia								

NOTES: _____

FINAL CHEMISTRYProject: Mussel Ref Tox May 1993Personnel: SimbeckBeginning Date: 5-12-93PoseyEnding Date: 5-21-93SeslerDate: 5-20-93Replicate: #4

TREATMENT	Cntrl	0.312	0.625	1.250	2.500	5.000		
Temperature	24.3	24.2	24.2	24.2	24.5			
D.O.	7.7	7.8	7.8	7.8	7.8			
pH	7.9	7.9	7.9	7.9	7.8			
Conductivity	363	368	370	369	371			
Hardness								
x 17.1								
Alkalinity								
x 10								
Chlorine								
Ammonia								

NOTES: _____

FINAL CHEMISTRYProject: Mussel Ref Tox May 1993Personnel: SimbeckBeginning Date: 5-12-93PoseyEnding Date: 5-21-93SeslerDate: 5-21-93Replicate: #1

TREATMENT	Cntrl	0.312	0.625	1.250	2.500	5.000		
Temperature	24.1	24.4	24.7	24.1	24.0			
D.O.	7.9	7.9	7.9	7.9	7.9			
pH	7.9	7.9	7.9	7.9	7.8			
Conductivity	363	363	366	368	370			
Hardness								
x 17.1								
Alkalinity								
x 10								
Chlorine								
Ammonia								

NOTES: _____

PROJECT REAGENT RECORD SHEET

Project/Study: Mussel Reef Tax May 93
 Personnel: Simbert Passey Scales

Beginning Date: 5-12-93
 Ending Date: 5-21-93

WINKLER TITRATION METHOD

Alkaline-Iodide-Azide:

Brand RICCA
 Lot # 24
 Exp. NA

Manganous Sulfate:

Brand RICCA
 Lot # B295
 Exp. NA

Sodium Thiosulfate:

Brand RICCA
 Lot # H267
 Exp. 4-94

Sulfuric Acid:

Brand Fisher
 Lot # 854507
 Exp. NA

Thyodene:

Brand Fisher
 Lot # 912254
 Exp. NA

pH BUFFER SOLUTIONS

pH 4:

Brand RICCA
 Lot # F267
 Exp. 2/94

pH 7:

Brand Spectrum
 Lot # 6K207
 Exp. 7/93

pH 10:

Brand RICCA
 Lot # A037
 Exp. 10/93

pH _____:

Brand _____
 Lot # _____
 Exp. _____

CONDUCTIVITY STANDARD SOLUTIONS

200 μ hos:

Brand BioPharm
 Lot # A2920
 Exp. NA 2/94

720 μ hos:

Brand BioPharm
 Lot # L222C
 Exp. 12/94

200 μ hos:

Brand BioPharm
 Lot # J302C
 Exp. 11/94

ALKALINITY TITRATION

Sulfuric Acid Solution N/50:

Brand Mallinckrodt
 Lot # H366
 Exp. NA

HARDNESS TITRATION

Hardness Titrating Solution:

Brand Calgon
 Lot # R2037700
 Exp. NA

Hardness Indicator:

Brand Calgon
 Lot # 2012
 Exp. NA

Hardness Buffer Solution:

Brand Calgon
 Lot # 26C6B
 Exp. NA

CHLORINE TITRATION

DPD Powder Pillows:

Brand _____
 Lot # _____
 Exp. _____

Potassium Iodide:

Brand _____
 Lot # _____
 Exp. _____

FAS:

Brand _____
 Lot # _____
 Exp. _____

Project Instrument Record Sheet

Project Study Mussel Rep Ten May 93

Beginning Date 5-12-93

Ending Date 5-21-93

DO Meter

Model YSI Model 57

TVA Tag 543348

Calibration Date 8-13-92

pH Meter(s)

Model Orion Research Model SA250

TVA Tag #7413

Calibration Date 4-13-93

Model Orion Research Model 407A

TVA Tag 472857

Calibration Date 4-13-93

Conductivity Meter

Model YSI Model 32

TVA Tag 577779

Calibration Date ~~2-17-93~~ 2-17-93

Thermometer(s)

Model ERTCO X 1494

TVA Tag M 148

Calibration Date 12-8-92

Model _____

TVA Tag _____

Calibration Date _____

ATTACHMENT II

COPPER ANALYSIS REQUEST AND RESULTS

Laboratory No. _____

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Charge to account No. 4210-011200-85555, 7, 000

GENERAL DESCRIPTION: Mussel Reference Toxicant Test

3/04550
04551
04552
04553
04554
04555
04556

ANALYZE FOR

[illegible]

Notebook page _____

Analyzed by _____

Laboratory

DISTRIBUTION OF COPIES:

1

1- { To Laboratory

— 2 Returned to Originating Office

TVA Environmental Chemistry

Chattanooga Tennessee

06/10/93

FINAL DATA REPORT

07-15

S'INFO : 1
S'CMT : FINAL/CONTROL 5-18-93
S'TYPE : WATER
ACT'NUMB : 4210-011200-85555,F,WRS

SAMPLE 93 00550
LOGDATE 930521
DUE DATE 06/10/93
P'LEAD 0000

Lab Sample Number : 93/04550

Project Leader : William P. Scott

Sample ID Information : 1

Sample comments : FINAL/CONTROL 5-18-93

Sample type/matrix : WATER

Sample collected by : DANIEL SURECK

Sample collection date : 930518

Sample login date : 930521

Sample received by lab : 930520

Sample account number : 4210-011200-85555,F,WRS

Alt. IDC	Analysis Performed	result	units
----------	--------------------	--------	-------

01042

Copper, Total in Water

< 10.

ug/l

06/10/93

FINAL DATA REPORT

07:15 |

Page 1 of 1

S'INFO : 2
S'CMT : FINAL/0.312mg Cu
S'TYPE : WATER
ACT'NUMB : 4210-011200-85555,F,WR5

SAMPLE : 9304551
LOGDATE : 030521
DUEDATE : 06/11/93
P'LEAD : 00000

Lab Sample Number : 9304551

Project Leader : William C. Scott

Sample ID Information : 2

Sample comments : FINAL/0.312mg Cu

Sample type/matrix : WATER

Sample collected by : DANIEL SIMBECK

Sample collection date : 030510

Sample login date : 030521

Sample received by Lab : 030520

Sample account number : 4210-011200-85555,F,WR5

Alt. IDC	Analysis Performed	result	units
----------	--------------------	--------	-------

01042

Copper, Total in Water

340.

ug/L

TVA Environmental Chemistry

Chattanooga Tennessee |

06/10/93

FINAL DATA REPORT

07-15 |

Page: 1

S'INFO : 3
S'CMT : FINAL/0.625mg Cu
S'TYPE : WATER
ACT'NUMB : 4210-011200-85555,F,WRS

SAMPLE : 93.04552
LOGDATE : 930521
DUE DATE : 06.11.93
P'LEAD : @WRS

Lab Sample Number : 93/04552

Project Leader : William C. Scott

Sample ID Information : 3

Sample comments : FINAL/0.625mg Cu

Sample type/matrix : WATER

Sample collected by : DANIEL SIMBECK

Sample collection date : 930518

Sample login date : 930521

Sample received by lab : 930520

Sample account number : 4210-011200-85555,F,WRS

Alt. IDC	Analysis Performed	result	units
----------	--------------------	--------	-------

01042

Copper, Total in Water

670.

ug/L

TVA Environmental Chemistry

Chattanooga, Tennessee |

06/10/93

FINAL DATA REPORT

07:15 |

S'INFO : 4
S'CMT : FINAL/1.25mg Cu
S'TYPE : WATER
ACT'NUMB : 4210-011200-85555,F,WRS

SAMPLE : 93.04553
LOGDATE : 930521
DUE DATE : 06/11/93
P'LEAD : @WRS

Lab Sample Number : 93/04553

Project Leader : William F. Scott

Sample ID Information : 4
Sample comments : FINAL/1.25mg Cu
Sample type/matrix : WATER
Sample collected by : DANIEL SINBECK
Sample collection date : 930519
Sample login date : 930521 Sample received by Lab : 930520
Sample account number : 4210-011200-85555,F,WRS

Alt. IDC	Analysis Performed	result	units
----------	--------------------	--------	-------

01042	Copper, Total in Water	1300	ug/L
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=====

TVA Environmental Chemistry	Chattanooga, Tennessee
06/10/93	FINAL DATA REPORT
	07-15

===== Page 1 =====

S'INFO : 5	SAMPLE : 93/04554
S'CMT : FINAL/2 Smg Cu	LOGDATE : 930521
S'TYPE : WATER	DUE DATE : 96-11-22
ACT'NUMB : 4210-011200-25555,F,WRS	P'LEAD : @URS

Lab Sample Number : 93/04554 Project Leader : William R. Scott

Sample ID Information : 5

Sample comments : FINAL/2 Smg Cu

Sample type/matrix : WATER

Sample collected by : DANIEL SINBECK

Sample collection date : 930518

Sample login date : 930521 Sample received by lab : 930520

Sample account number : 4210-011200-25555,F,WRS

Alt. IDC	Analysis Performed	result	units
01042	Copper, Total in Water	2600.	ug/L

=====

TVA Environmental Chemistry	Chattanooga, Tennessee
06/10/93	FINAL DATA REPORT 07 15

=====

Page 1

S'INFO : 6	SAMPLE : 93/04555
S'CMT : FINAL/5.0mg Cu	LOGDATE : 930521
S'TYPE : WATER	DUE DATE : 06.11.93
ACT'NUMB : 4210-011200-85555,F,WRS	P'LEAD : QWRB

Lab Sample Number : 93/04555 Project Leader : William R. Scott

Sample ID Information : 6

Sample comments : FINAL/5.0mg Cu

Sample type/matrix : WATER

Sample collected by : DAMIEN SINBECK

Sample collection date : 930518

Sample login date : 930521 Sample received by lab : 930520

Sample account number : 4210-011200-85555,F,WRS

Alt. IDC	Analysis Performed	result	units
----------	--------------------	--------	-------

01042	Copper, Total in Water	5700.	ug/L
-------	------------------------	-------	------

=====

TVA Environmental Chemistry	Chattanooga, Tennessee
06/10/93	07:15
FINAL DATA REPORT	

=====

Page 1

S'INFO : F	SAMPLE : 93/04556
S'CMT : BLANK	LOGDATE : 930521
S'TYPE : WATER	DUE DATE : 06/11/93
ACT'NUMB : 4210-011200-85555,F,WRS	P'LEAD : @URS

Lab Sample Number : 93/04556 Project Leader : William R. Scott

Sample ID Information : F

Sample comments : BLANK

Sample type/matrix : WATER

Sample collected by : DAMIEN SIMBECK

Sample collection date : 930518

Sample login date : 930521 Sample received by lab : 930520

Sample account number : 4210-011200-85555,F,WRS

Alt. IDC	Analysis Performed	result	units
----------	--------------------	--------	-------

01042	Copper, Total in Water	< 10.	ug/L
-------	------------------------	-------	------

ATTACHMENT III

PERSONNEL TRAINING DOCUMENTATION

Training Record for: _____

Position: _____

I, _____, have read the "Referenced Chapters" of the ARL Toxicology Laboratory Quality Assurance Program and Standard Operating Procedures Manual.

The trainee will initial the READ column and will sign and date the TRAINEE SIGNATURE AND DATE column after each referenced chapter.

CHAPTER	READ	TRAINEE SIGNATURE AND DATE
CHAPTER 1 Introduction	MS	Chen / 12-2-92
CHAPTER 2 Facility	MS	Chen / 12-2-92
CHAPTER 3 Personnel	MS	Chen / 12-2-92
CHAPTER 4 Health and Safety	MS	Chen / 12-2-92
CHAPTER 5 Quality Assurance	MS	Chen / 12-2-92

PLARC501-975

I, _____, have read the ARL Toxicology Laboratory Quality Assurance Program and Standard Operating Procedures Manual.

The trainee will initial the READ and TRAINED columns for the referenced SOP after (1) each applicable Standard Operating Procedure has been read, and (2) after training has been received by the Unit Supervisor or approved trainer.

The Unit Supervisor or approved trainer will initial the PROFICIENT column after the calibrations, procedures, and calculations (if applicable) have been satisfactorily performed by the trainee.

After the READ, TRAINED, and PROFICIENT columns have been initialed, the trainee will sign and date the TRAINEE SIGNATURE AND DATE column.

SOP Section	READ	TRAINED	PROFICIENT	TRAINER (Name)	TRAINEE SIGNATURE AND DATE
SOP-1 Sample Collection and Handling	<i>[initials]</i>	<i>[initials]</i>	<i>[initials]</i>	<i>[signature]</i>	<i>[signature]</i> 12-3-92
SOP-2 Preparation of Reagent Water					
SOP-3 Glassware Preparation	<i>[initials]</i>	<i>[initials]</i>	<i>[initials]</i>	<i>[signature]</i>	<i>[signature]</i> 12-3-92
SOP-4 Fathead Minnow Culture Methods	<i>[initials]</i>	<i>[initials]</i>	<i>[initials]</i>	<i>[signature]</i>	<i>[signature]</i> 12-3-92
SOP-5 Hatching and Preparation of Live Brine Shrimp (Artemia)	<i>[initials]</i>	<i>[initials]</i>	<i>[initials]</i>	<i>[signature]</i>	<i>[signature]</i> 12-8-92
SOP-6 Preparation of Laboratory Reconstituted Water	<i>[initials]</i>				
SOP-7 Ceriodaphnia dubia Culture Methods	<i>[initials]</i>	<i>[initials]</i>	<i>[initials]</i>	<i>[signature]</i>	<i>[signature]</i> 12-18-92

PLARCS01-975(2)

SOP Section	READ	TRAINED	PROFICIENT	TRAINER (Name)	TRAINEE SIGNATURE AND DATE
SOP-8 <u>Daphnia pulex</u> Culture Methods					
SOP-9 Algal, <u>Selenastrum capricornutum</u> , Culture, Methods					
SOP-10 Algal Cell Enumeration					
SOP-11 Fathead Minnow, <u>Pimephales</u> <u>promelas</u> , Acute Toxicity Test					
SOP-12 Fathead Minnow, <u>Pimephales</u> <u>promelas</u> , Chronic Toxicity Test	OK	OK	OK	mm	<i>[Signature]</i> 4-21-93
SOP-13 Fathead Minnow, <u>Pimephales</u> <u>promelas</u> , Embryo Larval Survival and Teratogenicity Test					
SOP-14 Daphnid, <u>Daphnia pulex</u> or <u>Ceriodaphnia dubia</u> , Acute Toxicity Test					
SOP-15 Cladoceran, <u>Ceriodaphnia dubia</u> , Survival and Reproduction Test	OK	OK	OK	mm	<i>[Signature]</i> 4-21-93
SOP-16 Algal, <u>Selenastrum capricornutum</u> , Growth Test					
SOP-17 Reference Toxicant Testing					

PLARC501-975(3)

SOP Section	READ	TRAINED	PROFICIENT	TRAINER (Name)	TRAINEE SIGNATURE AND DATE
SOP-18 Physical/Chemical Analyses		MS	MS	MS	David Smith 4-24-93
SOP-19 Statistics					
SOP-20 Report Preparation		MS	MS	MS	David Smith 4-24-93
SOP-21 Freshwater Mussel Culture Methods	MS	MS	MS	MS	David Smith 4-24-93
SOP-22 Freshwater Mussel Test Methods	MS	MS	MS	MS	David Smith 4-24-93
SOP-23 ROTOX					
SOP-24 Microtox (Microbics Corporation)					
SOP-25 Duckweed Toxicity Test (ASTM)					

PLARC501-975(4)

Training Record for:

Janet Foley

Position:

Scientific Aide

ARL Toxicology Laboratory
Training Checklist
October 30, 1992
Page 1 of 4

I, Janet Foley, have read the "Referenced Chapters" of the ARL Toxicology Laboratory Quality Assurance Program and Standard Operating Procedures Manual.

The trainee will initial the READ column and will sign and date the TRAINEE SIGNATURE AND DATE column after each referenced chapter.

CHAPTER	READ	TRAINEE SIGNATURE AND DATE
CHAPTER 1 Introduction	JP	<u>Janet Foley</u> 12-2-92
CHAPTER 2 Facility	JP	<u>Janet Foley</u> 12-2-92
CHAPTER 3 Personnel	JP	<u>Janet Foley</u> 12-2-92
CHAPTER 4 Health and Safety	JP	<u>Janet Foley</u> 12-2-92
CHAPTER 5 Quality Assurance	JP	<u>Janet Foley</u> 12-2-92

PLRCS01-975

I, Janet Foley, have read the ARL Toxicology Laboratory Quality Assurance Program and Standard Operating Procedures Manual. The trainee will initial the READ and TRAINED columns for the referenced SOP after (1) each applicable Standard Operating Procedure has been read, and (2) after training has been received by the Unit Supervisor or approved trainer.

The Unit Supervisor or approved trainer will initial the PROFICIENT column after the calibrations, procedures, and calculations (if applicable) have been satisfactorily performed by the trainee.

After the READ, TRAINED, and PROFICIENT columns have been initialed, the trainee will sign and date the TRAINEE SIGNATURE AND DATE column.

SOP Section	READ	TRAINED	PROFICIENT	TRAINER (Name)	TRAINEE SIGNATURE AND DATE
SOP-1 Sample Collection and Handling	JPF	JPF	JPF	JM	Janet Foley 12-2-92
SOP-2 Preparation of Reagent Water	JPF	JPF	JPF	JM	Janet Foley
SOP-3 Glassware Preparation	JPF	JPF	JPF	JM	Janet Foley 12-2-92
SOP-4 Fathead Minnow Culture Methods	JPF	JPF	JPF	TCS	Janet Foley 12-5-92
SOP-5 Hatching and Preparation of Live Brine Shrimp (Artemia)	JPF	JPF	JPF	RLS	Janet Foley 12-4-92
SOP-6 Preparation of Laboratory Reconstituted Water			JPF	JM	
SOP-7 Ceriodaphnia dubia Culture Methods					

PLARCS01-975(2)

SOP Section	READ	TRAINED	PROFICIENT	TRAINER (Name)	TRAINEE SIGNATURE AND DATE
SOP-8 <u>Daphnia pulex</u> Culture Methods					
SOP-9 Algal, <u>Selenastrum capricornutum</u> , Culture, Methods					
SOP-10 Algal Cell Enumeration					
SOP-11 Fathead Minnow, <u>Pimephales promelas</u> , Acute Toxicity Test	YES	SPP	✓	SM	Danet Poley 5-25-93
SOP-12 Fathead Minnow, <u>Pimephales promelas</u> , Chronic Toxicity Test	YES	SPP	✓	SM	Danet Poley 5-25-93
SOP-13 Fathead Minnow, <u>Pimephales promelas</u> , Embryo Larval Survival and Teratogenicity Test	YES	SPP	✓	SM	Danet Poley 5-25-93
SOP-14 Daphnid, <u>Daphnia pulex</u> or <u>Ceriodaphnia dubia</u> , Acute Toxicity Test	YES	SPP	✓	SM	Danet Poley 5-25-93
SOP-15 Cladoceran, <u>Ceriodaphnia dubia</u> , Survival and Reproduction Test	YES	SPP	✓	SM	Danet Poley 5-25-93
SOP-16 Algal, <u>Selenastrum capricornutum</u> , Growth Test					
SOP-17 Reference Toxicant Testing	YES	SPP	✓	SM	Danet Poley 5-25-93

PLARC501-975(3)

SOP Section	READ	TRAINED	PROFICIENT	TRAINER (Name)	TRAINEE SIGNATURE AND DATE
SOP-18 Physical/Chemical Analyses	YES	SOP	✓	SM	Janet Pacey 5-25-93
SOP-19 Statistics					
SOP-20 Report Preparation					
SOP-21 Freshwater Mussel Culture Methods	YES	SOP	✓	SM	Janet Pacey 5-25-93
SOP-22 Freshwater Mussel Test Methods	YES	SOP	✓	SM	Janet Pacey 5-25-93
SOP-23 Rotox	YES	SOP	✓	Don Wade	Janet Pacey 5-25-93
SOP-24 Microtox (Microbics Corporation)					
SOP-25 Duckweed Toxicity Test (ASTM)					

PLARCS01-975(4)

Training Record for: Terese N. Sester

Position: Biologist

I, Terese N. Sester have read the "Referenced Chapters" of the ARL Toxicology Laboratory Quality Assurance Program and Standard Operating Procedures Manual.

The trainee will initial the READ column and will sign and date the TRAINEE SIGNATURE AND DATE column after each referenced chapter.

CHAPTER	READ	TRAINEE SIGNATURE AND DATE
CHAPTER 1 Introduction	<u>JS</u>	<u>Terese N. Sester 12/2/92</u>
CHAPTER 2 Facility	<u>JS</u>	<u>Terese N. Sester 12/2/92</u>
CHAPTER 3 Personnel	<u>JS</u>	<u>Terese N. Sester 12/2/92</u>
CHAPTER 4 Health and Safety	<u>JS</u>	<u>Terese N. Sester 12/2/92</u>
CHAPTER 5 Quality Assurance	<u>JS</u>	<u>Terese N. Sester 12/2/92</u>

PLARCS01-975

1. Sharon C. Deibel, have read the ARL Toxicology Laboratory Quality Assurance Program and Standard Operating Procedures Manual.

The trainee will initial the READ and TRAINED columns for the referenced SOP after (1) each applicable Standard Operating Procedure has been read, and (2) after training has been received by the Unit Supervisor or approved trainer.

The Unit Supervisor or approved trainer will initial the PROFICIENT column after the calibrations, procedures, and calculations (if applicable) have been satisfactorily performed by the trainee.

After the READ, TRAINED, and PROFICIENT columns have been initiated, the trainee will sign and date the TRAINEE SIGNATURE AND DATE column.

SOP Section	READ	TRAINED	PROFICIENT	TRAINER (Name)	TRAINEE SIGNATURE AND DATE
SOP-1 Sample Collection and Handling	<i>SCD</i>	<i>SCD</i>	<i>SCD</i>	<i>Sharon C. Deibel</i>	<i>Sharon C. Deibel 12/2/92</i>
SOP-2 Preparation of Reagent Water					
SOP-3 Glassware Preparation	<i>SCD</i>	<i>SCD</i>	<i>SCD</i>	<i>Sharon C. Deibel</i>	<i>Sharon C. Deibel 12/2/92</i>
SOP-4 Fathead Minnow Culture Methods	<i>SCD</i>	<i>SCD</i>	<i>SCD</i>	<i>Sharon C. Deibel</i>	<i>Sharon C. Deibel 12/8/92</i>
SOP-5 Hatching and Preparation of Live Brine Shrimp (Artemia)	<i>SCD</i>	<i>SCD</i>	<i>SCD</i>	<i>Sharon C. Deibel</i>	<i>Sharon C. Deibel 12/13/92</i>
SOP-6 Preparation of Laboratory Reconstituted Water					
SOP-7 <u>Ceriodaphnia dubia</u> Culture Methods					

PLARCS01-975(2)

SOP Section	READ	TRAINED	PROFICIENT	TRAINER (Name)	TRAINEE SIGNATURE AND DATE
SOP-8 <u>Daphnia pulex</u> Culture Methods					
SOP-9 Algal, <u>Selenastrum capricornutum</u> , Culture, Methods					
SOP-10 Algal Cell Enumeration					
SOP-11 Fathead Minnow, <u>Pimephales promelas</u> , Acute Toxicity Test	yes	yes	✓	JM	Jean Dehn 5/25/93
SOP-12 Fathead Minnow, <u>Pimephales promelas</u> , Chronic Toxicity Test	yes	yes	✓	JM	Jean Dehn 5/25/93
SOP-13 Fathead Minnow, <u>Pimephales promelas</u> , Embryo Larval Survival and Teratogenicity Test					
SOP-14 Daphnid, <u>Daphnia pulex</u> or <u>Ceriodaphnia dubia</u> , Acute Toxicity Test	yes	yes	✓	JM	Jean Dehn 5/25/93
SOP-15 <u>Cladoceran</u> , <u>Ceriodaphnia dubia</u> , Survival and Reproduction Test	yes	yes	✓	JM	Jean Dehn 5/25/93
SOP-16 Algal, <u>Selenastrum capricornutum</u> , Growth Test					
SOP-17 Reference Toxicant Testing	yes	yes	✓	JM	Jean Dehn 5/25/93

PLARC501-975(3)

SOP Section	READ	TRAINED	PROFICIENT	TRAINER (Name)	TRAINEE SIGNATURE AND DATE
SOP-18 Physical/Chemical Analyses	yes	JCB	✓	JM	Deena Decker 5/25/93
SOP-19 Statistics	yes	JCB	✓	JM	Deena Decker 5/25/93
SOP-20 Report Preparation					
SOP-21 Freshwater Mussel Culture Methods					
SOP-22 Freshwater Mussel Test Methods	yes	JCB	✓	JD	Deena Decker 5/25/93
SOP-23 Rotox	yes	JCB	✓	JP	Deena Decker 5/25/93
SOP-24 Microtox (Microbics Corporation)					
SOP-25 Duckweed Toxicity Test (ASTM)					

PLARC501-975(4)

Training Record for: Jennifer Meese

Position: Unit Supervisor

I, Jennifer Meese, have read the "Referenced Chapters" of the ARL Toxicology Laboratory Quality Assurance Program and Standard Operating Procedures Manual.

The trainee will initial the READ column and will sign and date the TRAINEE SIGNATURE AND DATE column after each referenced chapter.

CHAPTER	READ	TRAINEE SIGNATURE AND DATE
CHAPTER 1 Introduction	JM	Jennifer Meese, 12-2-92
CHAPTER 2 Facility	JM	Jennifer Meese, 12-2-92
CHAPTER 3 Personnel	JM	Jennifer Meese, 12-2-92
CHAPTER 4 Health and Safety	JM	Jennifer Meese, 12-2-92
CHAPTER 5 Quality Assurance	JM	Jennifer Meese, 12-2-92

PLARCS01-975

I, _____, have read the ARL Toxicology Laboratory Quality Assurance Program and Standard Operating Procedures Manual. The trainee will initial the READ and TRAINED columns for the referenced SOP after (1) each applicable Standard Operating Procedure has been read, and (2) after training has been received by the Unit Supervisor or approved trainer.

The Unit Supervisor or approved trainer will initial the PROFICIENT column after the calibrations, procedures, and calculations (if applicable) have been satisfactorily performed by the trainee.

After the READ, TRAINED, and PROFICIENT columns have been initialed, the trainee will sign and date the TRAINEE SIGNATURE AND DATE column.

SOP Section	READ	TRAINED	PROFICIENT	TRAINER (Name)	TRAINEE SIGNATURE AND DATE
SOP-1 Sample Collection and Handling	TM	TM	✓		Amelia Mesa, 12-2-92
SOP-2 Preparation of Reagent Water	TM	TM	✓		Amelia Mesa, (12-1-92)
SOP-3 Glassware Preparation	TM	TM	✓		Amelia Mesa, 12-2-92
SOP-4 Fathead Minnow Culture Methods	TM	TM	✓		Amelia Mesa, 12-8-92
SOP-5 Hatching and Preparation of Live Brine Shrimp (Artemia)	TM	TM	✓		Amelia Mesa, 12-8-92
SOP-6 Preparation of Laboratory Reconstituted Water	TM	TM	✓		Amelia Mesa, 12-8-92
SOP-7 Ceriodaphnia dubia Culture Methods	TM	TM	✓		Amelia Mesa, 12-21-92

PLARCS01-975(2)

SOP Section	READ	TRAINED	PROFICIENT	TRAINER (Name)	TRAINEE SIGNATURE AND DATE
SOP-6 <u>Daphnia pulex</u> Culture Methods	TM	TM			Donna M. Moore (03-31-92)
SOP-9 Algal, <u>Selenastrum cadricornutum</u> , Culture, Methods	TM	TM			Donna M. Moore (10-01-92)
SOP-10 Algal Cell Enumeration	TM	TM			Donna M. Moore (03-31-92)
SOP-11 Fathead Minnow, <u>Pimephales</u> <u>promelas</u> , Acute Toxicity Test	TM	TM			Donna M. Moore (10-01-92)
SOP-12 Fathead Minnow, <u>Pimephales</u> <u>promelas</u> , Chronic Toxicity Test	✓	✓	✓		Donna M. Moore 4-21-93
SOP-13 Fathead Minnow, <u>Pimephales</u> <u>promelas</u> , Embryo Larval Survival and Teratogenicity Test	TM	TM	✓		Donna M. Moore (03-31-92)
SOP-14 Daphnid, <u>Daphnia pulex</u> or <u>Ceriodaphnia dubia</u> , Acute Toxicity Test	TM	TM	✓		Donna M. Moore (10-01-92)
SOP-15 <u>Cladocera</u> , <u>Ceriodaphnia dubia</u> , Survival and Reproduction Test	✓	✓	✓		Donna M. Moore 4-21-93
SOP-16 Algal, <u>Selenastrum cadricornutum</u> , Growth Test	TM	TM	✓		Donna M. Moore (10-01-92)
SOP-17 Reference Toxicant Testing	TM	TM	✓		Donna M. Moore (03-31-92)

PLARC501-975(3)

SOP Section	READ	TRAINED	PROFICIENT	TRAINER (Name)	TRAINEE SIGNATURE AND DATE
SOP-18 Physical/Chemical Analyses	✓	✓	✓		<i>[Signature]</i> 10-31-92
SOP-19 Statistics					
SOP-20 Report Preparation	✓	✓	✓		<i>[Signature]</i> 10-31-92
SOP-21 Freshwater Mussel Culture Methods					
SOP-22 Freshwater Mussel Test Methods					
SOP-23 Rotox®					
SOP-24 Microtox (Microbics Corporation)					
SOP-25 Duckweed Toxicity Test (ASTM)					

PLARCS01-975(4)