

DOE/OR/22012--T15

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

HH
DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

MASTER

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, make any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

**Portions of this document may be illegible
in electronic image products. Images are
produced from the best available original
document.**

- 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]

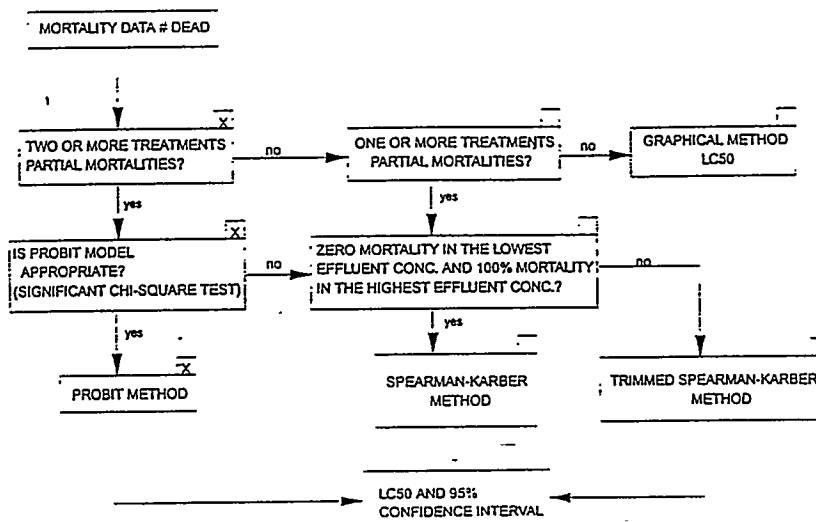
6.0 RESULTS

- 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									
	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	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
			(mg/L)	(mg/L)	(S.U.)	(S.U.)	(µmhos)	(µmhos)	*	*	*	*
CONTROL	24.2	(24.0-24.4)	8.0	7.9	6.9	7.8	348	363	34		89.7	
			(7.5-8.2)	(7.7-8.0)	(6.8-7.0)	(7.8-7.9)	(345-351)	(356-369)	(31-36)		(87.2-90.6)	
0.312 mg/L	24.2	(23.8-24.4)	8.0	7.9	6.7	7.8	350	364	-		-	
			(7.6-8.2)	(7.6-8.0)	(6.6-6.8)	(7.7-7.9)	(347-353)	(356-368)				
0.625 mg/L	24.3	(24.1-24.7)	8.0	7.9	6.6	7.8	350	365	-		-	
			(7.6-8.2)	(7.8-8.0)	(6.6-6.7)	(7.8-7.9)	(347-353)	(360-370)				
1.250 mg/L	24.2	(24.0-24.6)	8.1	7.9	6.7	7.8	349	363	-		-	
			(7.6-8.2)	(7.7-8.0)	(6.6-6.7)	(7.8-7.9)	(346-353)	(355-369)				
2.500 mg/L	24.1	(23.3-24.5)	8.1	7.9	6.5	7.8	353	368	33		92.3	
			(7.7-8.3)	(7.8-8.1)	(6.5-6.6)	(7.7-7.8)	(350-356)	(363-371)	(32-34)		(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		89.6	
			(7.7-8.3)	(7.9-8.0)	(6.5-6.5)	(7.6-7.7)	(354-357)	(363-373)	(26-29)		(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/J</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/JPP</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/JPP</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>DJS</u>	<u>JPP</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 (51% mortality)

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: DJS

*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 Date/Time 5-12-93 10935 Ending 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	<u>Control</u>	11	<u></u>
2	<u>0.312 mg/L Cu</u>	12	<u></u>
3	<u>0.625 mg/L Cu</u>	13	<u></u>
4	<u>1.250 mg/L Cu</u>	14	<u></u>
5	<u>2.500 mg/L Cu</u>	15	<u></u>
6	<u>5.000 mg/L Cu</u>	16	<u></u>
7	<u></u>	17	<u></u>
8	<u></u>	18	<u></u>
9	<u></u>	19	<u></u>
10	<u></u>	20	<u></u>

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>
<u></u>	<u></u>	<u></u>
<u></u>	<u></u>	<u></u>
<u></u>	<u></u>	<u></u>

FOOD/SUBSTRATE PREPARATION:

Phytoplankton Started

5-2-93

Phytoplankton Concentrated

5-6-93

Silt Collected: Date/Location 4/30/93 1 Back 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>1,036.36 mg</u>	<u>2,202.16 mg</u>	<u>1,165.80 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 liquid = 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	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	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
JCP JPP DJS JPP DJS/RES JPP TS TCS DJS										

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

DJS/RES JPP DJS JPP TS JPP TS TCS DJS

*plus not active Very little growth Disturbance in the 10-15 cm. tanks

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 - fine 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

** DAY 4 - saw two muscle A.
Rep 3: 12/21

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	4	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

** mussel seen with some valve 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	9*	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
AMG

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

refloxx

Estimated EC Values and Confidence Limits

Point	Conc.	Lower Upper	
		95% Confidence Limits	
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

PLOT OF ADJUSTED PROBITS AND PREDICTED REGRESSION LINE

Probit

10+

9+

8+

7+

6+

5+

4+

3+

2+

1+

0+o

EC01

EC10

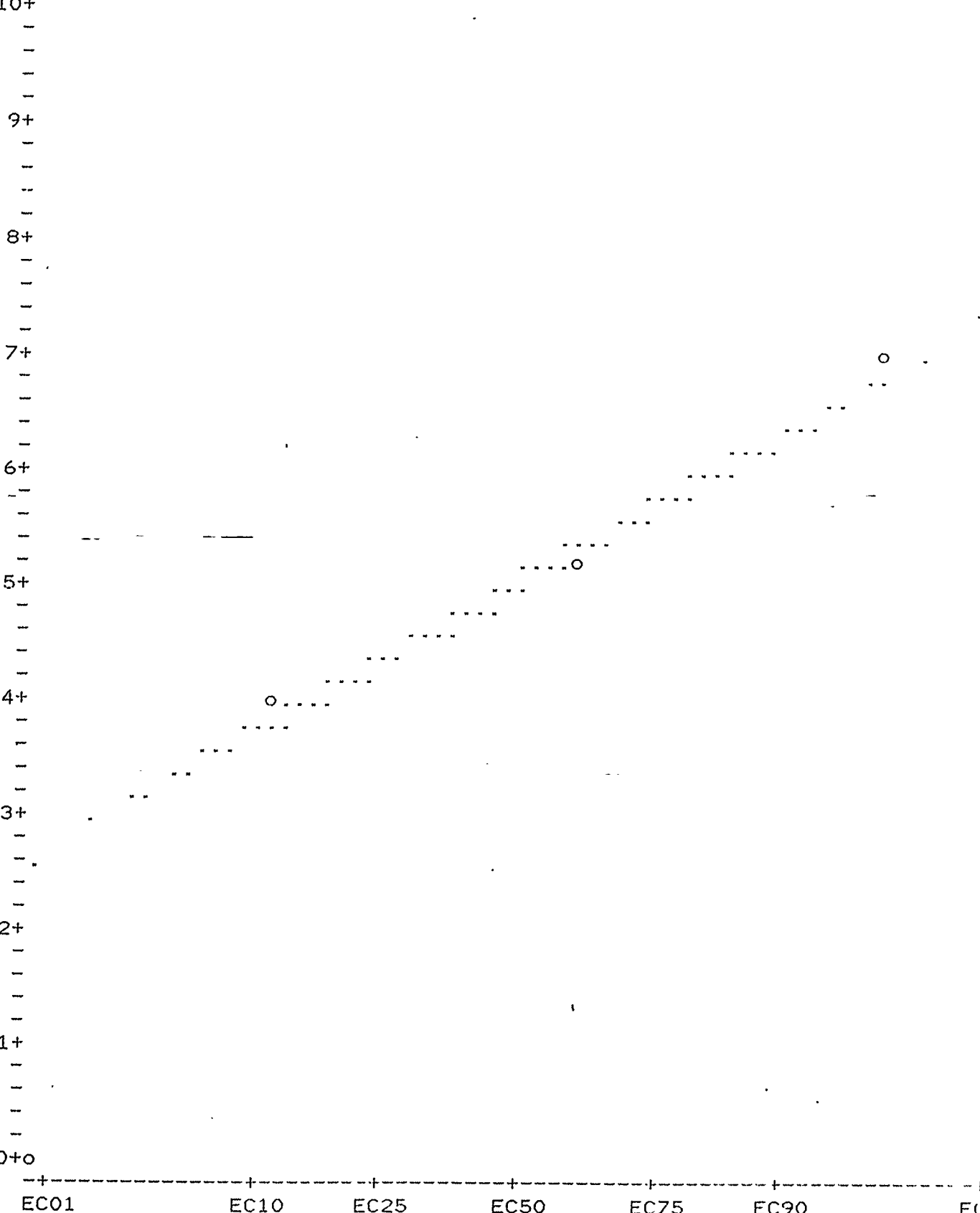
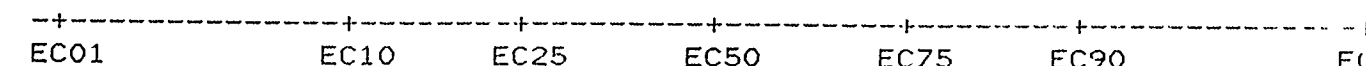
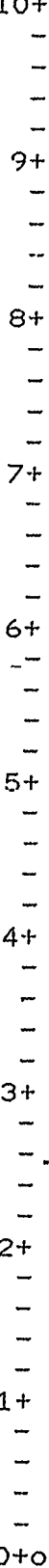
EC25

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	371
-----	------	-----	-----	-----

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	346 347	347 350	348 349	347 348	351 352	358 357		
Hardness	5.2					5.2		
x 17.1	88.9					88.9		
Alkalinity	3.1					2.6		
x 10	31					26		
Chlorine								
Ammonia								

NOTES: ML H1330

INITIAL CHEMISTRYProject: Mussel Ref Tox May 1993Personnel: SimbeckBeginning Date: 5-12-93PoseyEnding Date: 5-26-93SeslerDate: 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 # 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: met # 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: _____

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

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: _____

(PLARC501-130)

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: _____

(PLARC501-130)

FINAL CHEMISTRYProject: Mussel Ref Tox May 1993Personnel: SimbeckBeginning Date: 5-12-93PoseyEnding Date: 5-21-93SeslerDate: 5-17-93Replicate: #1

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	24.2			
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: _____

(PLARC501-130)

PROJECT REAGENT RECORD SHEET

Project/Study: Mussel Reef Tax Map 93
Personnel: Simbert Pascep Seiler

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

WINKLER TITRATION METHOD

Alkaline-Iodide-Azide:

Brand RICCA FISHKILL
Lot # 912254 2075
Exp. NA

Manganous Sulfate:

Brand RICCA
Lot # B275
Exp. NA

Sodium Thiosulfate:

Brand RICCA
Lot # H267
Exp. 4-94

Sulfuric Acid:

Brand FISHKILL
Lot # 854507
Exp. NA

Thyodene:

Brand FISHKILL
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 µmhos:

Brand BIOPHARM
Lot # A2920
Exp. NA 2/94

720 µmhos:

Brand BIOPHARM
Lot # L222C
Exp. 12/94

200 µmhos:

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: Hardness Indicator:

Brand Calgon
Lot # R2037700
Exp. NA

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 Muskel Kopp in May 25

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

W.K.'s
Labels 1Date submitted 5-18-93

Laboratory No. _____

Date wanted 6-18-93

Date reported _____

Charge to account No. 4210 011200-85555, 7, u. 1/3GENERAL DESCRIPTION: Mussel Reference Toxicant Test

Consignor's Number	Specific Description for Each Sample
3/04550 1	Final / Control 5-18-93
04551 2	Final / 0.312 mg Cu
04552 3	Final / 0.625 mg Cu
04553 4	Final / 1.25 mg Cu
04554 5	Final / 2.5 mg Cu
04555 6	Final / 5.0 mg Cu ✓
04556 7	Blank
Remarks, method, etc.: <u>Total Copper & mix sample before analysis</u>	

ANALYZE FOR

Lab. No.	Consignor's Number	0104-2								

Requested by Damien Simbeck

Notebook page _____

Address ARL-BFN

Analyzed by _____

Request approved by _____

Laboratory

DISTRIBUTION OF COPIES:

- 1- } To Laboratory
 2- } — 2 Returned to Originating Office
 3- Retained by Originating Office

1

SURVEY Mussel Ref Tox

DATE 5-19-93

SHIPPED FROM ARL - BFN

VIA TVH Mail

SHIPPED BY Damien Simbeck

AT 1400

RECEIVED BY _____ AT _____ ON _____
TIME DATE

BOTTLE REQUIREMENT*

SHIPPING

☒

RECEIVING

☒

TAG NO.

① Final / con	X
② Fin / 0.312	X
③ Fin / 0.625	X
④ Fin / 1.25	X
⑤ Fin / 2.5	X
⑥ Fin / 5.0	X
⑦ Blank	X

07-01-2025

10:00

NO. OF ICE CHESTS SHIPPED _____

RECEIVED _____

NO. OF SHIPPING CHESTS SHIPPED 1

RECEIVED _____

NO. OF RACKS SHIPPED _____

RECEIVED _____

NO. OF FIELD SHEETS SHIPPED 2

RECEIVED _____

SIGNATURE OF SHIPPER Dan J. Smith

DATE 5-18-93

*IF THE NUMBER OF SAMPLES OF EACH TYPE RECEIVED IS DIFFERENT FROM THE NUMBER SHIPPED, NOTIFY THE WATER QUALITY AND ECOLOGY BRANCH IMMEDIATELY.

06/10/93

FINAL DATA REPORT

07/15/93

Page 1 of 1

S'INFO : 1
S'CMT : FINAL/CONTROL S-1
S'TYPE : WATER
ACT'NUMB : 4210-011200-8555,F,URS

SAMPLE : 93-04555
LOGDATE : 030520
DUEDATE : 07-11-93
P'LEAD : 905

Lab Sample Number : 93-04555

Project Leader : D. L. H. S. S. S.

Sample ID Information : 1

Sample comments : FINAL/CONTROL S-18-93

Sample type/matrix : WATER

Sample collected by : DANIEL S. HEDER

Sample collection date : 030518

Sample login date : 030520

Sample received by : 030520

Sample account number : 4210-011200-8555,F,URS

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

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

06/10/93

FINAL DATA REPORT

07:15

S'INFO : 2
S'CMT : FINAL/0 312mg Cu
S'TYPE : WATER
ACT'NUMB : 4210-011200-85555 F, WRS

SAMPLE : 010520
LOGDATE : 07/05/93
DUEDATE : 07/11/93
P'LEAD : 0000

Lab Sample Number : 93.04551

Project Leader: William C. Smith

Sample ID Information : 2

Sample comments : FINAL/0 312mg Cu

Sample type/matrix : WATER

Sample collected by : DARIEN SIMBECK

Sample collection date : 070519

Sample login date : 070521

Sample received by Lab : 070520

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

Alt. IDC	Analysis Performed	result	units
01042	Copper, Total in Water	340.	ug/L

TVA Environmental Chemistry

06/10/93

FINAL DATA REPORT

Challenge: 06/10/93

07/15/93

Page 1

S'INFO : 3
S'CMT : FINAL/0 625mg Cu
S'TYPE : WATER
ACT'NUMB : 4210-011200-35555,F,WRS

SAMPLE : 930520
LOGDATE : 930521
DUE DATE : 931121
P'LEAD : @0001

Lab Sample Number : 9304552

Project Leader : William C. [unclear]

Sample ID Information : 7
Sample comments : FINAL/0 625mg Cu
Sample type/matrix : WATER
Sample collected by : DAMIEN SIMBECK
Sample collection date : 930518
Sample login date : 930521
Sample account number : 4210-011200-35555,F,WRS
Sample received by Lab : 930520

Alt. IDC	Analysis Performed	result
01042	Copper, Total in Water	670.

TVA Environmental Chemistry

Charlottesville, Tennessee

06/10/93

FINAL DATA REPORT

07 15

Page 1

S'INFO : 4

SAMPLE 930455

S'CMT : FINAL/1 25mg Cu

LOGDATE 930512

S'TYPE : WATER

DUE DATE 06-11-93

ACT'NUMB : 4210-011200-85555,F,WRS

P'LEAD BURS

Lab Sample Number : 93/0455

Project Leader William F. Smith

Sample ID Information : 4

Sample comments : FINAL/1 25mg Cu

Sample type/matrix : WATER

Sample collected by : DAMIEN SIMBECK

Sample collection date : 930512

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
-------	------------------------	------	------

S'INFO	: 5	SAMPLE	: 9304554
S'CMT	: FINAL/2 Bag Cu	LOGDATE	: 930521
S'TYPE	: WATER	DUE DATE	: 930521
ACT'NUMB	: 4210-011200-25555, F, WRS	P'LEAD	: @UR

Lab Sample Number : 9304554 Project Leader : Hollis

Sample ID Information : 5

Sample comments : FINAL/2 Bag 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	µg/L

S'INFO	: 6	SAMPLE	: 930520
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	: 0025

Lab Sample Number :9304555 Project Leader :William R. Smith

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

ATTACHMENT III

PERSONNEL TRAINING DOCUMENTATION

Position:

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

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 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	9/15	9/15	9/15	JMA	[Signature] 12-3-92
SOP-2 Preparation of Reagent Water					
SOP-3 Glassware Preparation	9/15	9/15	9/15	JMA	[Signature] 12-3-92
SOP-4 Fathead Minnow Culture Methods	9/15	9/15	9/15	JMA	[Signature] 12-3-92
SOP-5 Hatching and Preparation of Live Brine Shrimp (Artemia)	9/15	9/15	9/15	CS	[Signature] 12-3-92
SOP-6 Preparation of Laboratory Reconstituted Water	9/15				
SOP-7 Ceriodaphnia dubia Culture Methods	9/15	9/15	9/15	JMA/DH	[Signature] 12-18-92

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

PLARCS01-975(3)

SOP Section	READ	TRAINED	PROFICIENT	TRAINER (Name)	TRAINEE SIGNATURE AND DATE
SOP-18 Physical/Chemical Analyses		YES	YES	JM	David Smith 10-24-93
SOP-19 Statistics					
SOP-20 Report Preparation		YES	YES	JM	David Smith 10-24-93
SOP-21 Freshwater Mussel Culture Methods	YES	YES	YES	REID	David Smith 10-24-93
SOP-22 Freshwater Mussel Test Methods	YES	YES	YES	REID	David Smith 10-24-93
SOP-23 ROTOX					
SOP-24 Microtox (Microbics Corporation)					
SOP-25 Duckweed Toxicity Test (ASTM)					

PLARC501-975(4)

Training Record for: Tanet Foley Position: Scientific Aide

October 30, 1992
Page 1 of 4

I, Tanet 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	SRP	Tanet Foley 12-2-92
CHAPTER 2 Facility	SRP	Tanet Foley 12-2-92
CHAPTER 3 Personnel	SRP	Tanet Foley 12-2-92
CHAPTER 4 Health and Safety	SRP	Tanet Foley 12-2-92
CHAPTER 5 Quality Assurance	SRP	Tanet Foley 12-2-92

PLARC501-975

Training Checklist
ARL Toxicology Laboratory

I, James P. Poley, 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	JPP	JPP	JPP	JM	James Poley 12-2-92
SOP-2 Preparation of Reagent Water	JPP	JPP	JPP	JM	James Poley
SOP-3 Glassware Preparation	JPP	JPP	JPP	JM	James Poley 12-2-92
SOP-4 Fathead Minnow Culture Methods	JPP	JPP	JPP	TCS	James Poley 12-5-92
SOP-5 Hatching and Preparation of Live Brine Shrimp (Artemia)	JPP	JPP	JPP	RLS	James Poley 12-4-92
SOP-6 Preparation of Laboratory Reconstituted Water			JPP	JM	
SOP-7 Ceriodaphnia dubia Culture Methods					

PLARC501-975(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	Donet Casey 5-25-93
SOP-12 Fathead Minnow, <u>Pimephales promelas</u> , Chronic Toxicity Test	YES	SPP	✓	SM	Donet Casey 5-25-93
SOP-13 Fathead Minnow, <u>Pimephales promelas</u> , Embryo Larval Survival and Teratogenicity Test	YES	SPP	✓	SM	Donet Casey 5-25-93
SOP-14 Daphnid, <u>Daphnia pulex</u> or <u>Ceriodaphnia dubia</u> , Acute Toxicity Test	YES	SPP	✓	SM	Donet Casey 5-25-93
SOP-15 Cladoceran, <u>Ceriodaphnia dubia</u> , Survival and Reproduction Test	YES	SPP	✓	SM	Donet Casey 5-25-93
SOP-16 Algal, <u>Selenastrum capricornutum</u> , Growth Test					
SOP-17 Reference Toxicant Testing	YES	SPP	✓	SM	Donet Casey 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	Quet Pacey 5-25-93
SOP-19 Statistics					
SOP-20 Report Preparation					
SOP-21 Freshwater Mussel Culture Methods	YES	SOP	✓	SM	Quet Pacey 5-25-93
SOP-22 Freshwater Mussel Test Methods	YES	SOP	✓	SM	Quet Pacey 5-25-93
SOP-23 ROTOX	YES	SOP	✓	SM ^{ON} wader	Quet Pacey 5-25-93
SOP-24 Microtox (Microbics Corporation)					
SOP-25 Duckweed Toxicity Test (ASTM)					

PLARC501-975(4)

Training Record for: Teresa A. Sisker Position: Biologist

I, Teresa A. Sisker 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>TS</u>	<u>Teresa A. Sisker 12/2/92</u>
CHAPTER 2 Facility	<u>TS</u>	<u>Teresa A. Sisker 12/2/92</u>
CHAPTER 3 Personnel	<u>TS</u>	<u>Teresa A. Sisker 12/2/92</u>
CHAPTER 4 Health and Safety	<u>TS</u>	<u>Teresa A. Sisker 12/2/92</u>
CHAPTER 5 Quality Assurance	<u>TS</u>	<u>Teresa A. Sisker 12/2/92</u>

PLARC501-975

I, James C. Seiler, 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>JS</i>	<i>JS</i>	<i>JS</i>	<i>James C. Seiler</i>	<i>James C. Seiler 10/22/92</i>
SOP-2 Preparation of Reagent Water					
SOP-3 Glassware Preparation	<i>JS</i>	<i>JS</i>	<i>JS</i>	<i>James C. Seiler</i>	<i>James C. Seiler 10/22/92</i>
SOP-4 Fathead Minnow Culture Methods	<i>JS</i>	<i>JS</i>	<i>JS</i>	<i>James C. Seiler</i>	<i>James C. Seiler 10/28/92</i>
SOP-5 Hatching and Preparation of Live Brine Shrimp (Artemia)	<i>JS</i>	<i>JS</i>	<i>JS</i>	<i>James C. Seiler</i>	<i>James C. Seiler 10/28/92</i>
SOP-6 Preparation of Laboratory Reconstituted Water					
SOP-7 <i>Ceriodaphnia dubia</i> 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	Jenna Decker 5/25/93
SOP-12 Fathead Minnow, <u>Pimephales promelas</u> , Chronic Toxicity Test	yes	yes	✓	JM	Jenna Decker 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	Jenna Decker 5/25/93
SOP-15 Cladoceran, <u>Ceriodaphnia dubia</u> , Survival and Reproduction Test	yes	yes	✓	JM	Jenna Decker 5/25/93
SOP-16 Algal, <u>Selenastrum capricornutum</u> , Growth Test					
SOP-17 Reference Toxicant Testing	yes	yes	✓	JM	Jenna Decker 5/25/93

PLARC501-975(3)

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

PLARCS01-975(4)

Training Record for: Domilia Maza Position: Unit Supervisor

I, Domilia Maza, 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	DM	Domilia Maza, 12-2-92
CHAPTER 2 Facility	DM	Domilia Maza, 12-2-92
CHAPTER 3 Personnel	DM	Domilia Maza, 12-2-92
CHAPTER 4 Health and Safety	DM	Domilia Maza, 12-2-92
CHAPTER 5 Quality Assurance	DM	Domilia Maza, 12-2-92

PLARC501-975

1. _____, 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	JM	JM	✓		Denise Moore 12-2-92
SOP-2 Preparation of Reagent Water	JM	JM	✓		Denise Moore (12-1-92)
SOP-3 Glassware Preparation	JM	JM	✓		Denise Moore 12-2-92
SOP-4 Fathead Minnow Culture Methods	JM	JM	✓		Denise Moore 12-8-92
SOP-5 Hatching and Preparation of Live Brine Shrimp (Artemia)	JM	JM	✓		Denise Moore 12-8-92
SOP-6 Preparation of Laboratory Reconstituted Water	JM	JM	✓		Denise Moore 12-8-92
SOP-7 Ceriodaphnia dubia Culture Methods	JM	JM	✓		Denise Moore 12-21-92

PLARC501-975(2)

SOP Section	READ	TRAINED	PROFICIENT	TRAINER (Name)	TRAINEE SIGNATURE AND DATE
SOP-8 <u>Daphnia pulex</u> Culture Methods	TM	TM			Donna Lee Moore (03-31-92)
SOP-9 Algal, <u>Selenastrum capricornutum</u> , Culture, Methods	TM	TM			Donna Lee Moore (10-01-92)
SOP-10 Algal Cell Enumeration	TM	TM			Donna Lee Moore (03-31-92)
SOP-11 Fathead Minnow, <u>Pimephales promelas</u> , Acute Toxicity Test	TM	TM			Donna Lee Moore (10-01-92)
SOP-12 Fathead Minnow, <u>Pimephales promelas</u> , Chronic Toxicity Test	✓	✓	✓		Donna Lee Moore (10-01-92)
SOP-13 Fathead Minnow, <u>Pimephales promelas</u> , Embryo Larval Survival and Teratogenicity Test	TM	TM	✓		Donna Lee Moore (03-31-92)
SOP-14 Daphnid, <u>Daphnia pulex</u> or <u>Ceriodaphnia dubia</u> , Acute Toxicity Test	TM	TM	✓		Donna Lee Moore (10-01-92)
SOP-15 Cladoceran, <u>Ceriodaphnia dubia</u> , Survival and Reproduction Test	✓	✓	✓		Donna Lee Moore (10-01-92)
SOP-16 Algal, <u>Selenastrum capricornutum</u> , Growth Test	TM	TM	✓		Donna Lee Moore (10-01-92)
SOP-17 Reference Toxicant Testing	TM	TM	✓		Donna Lee Moore (03-31-92)

PLARC501-975(3)

SOP Section	READ	TRAINED	PROFICIENT	TRAINER (Name)	TRAINEE SIGNATURE AND DATE
SOP-18 Physical/Chemical Analyses	✓	✓	✓		4-5-92
SOP-19 Statistics					
SOP-20 Report Preparation	✓	✓	✓		4-5-92
SOP-21 Freshwater Mussel Culture Methods					4-5-92
SOP-22 Freshwater Mussel Test Methods					
SOP-23 Rotox®					
SOP-24 Microtox (Microbics Corporation)					
SOP-25 Duckweed Toxicity Test (ASTM)					

PLARC501-975(4)