



Grand Canyon National Park

Water-Effect Ratio Studies – Round 2 Acute Toxicity of Copper to *Ceriodaphnia dubia* in Site and Reconstituted Laboratory Water Under Static Test Conditions

October 2008

AECOM Environment

Project ID: 08503-133-409 (026,027)

December 30, 2008

AECOM



Study Title

Acute Toxicity of Copper to *Ceriodaphnia dubia* in Site and Reconstituted Laboratory Water
Under Static Test Conditions (Round 2)

Study Periods

Start: October 29, 2008

End: October 31, 2008

Performing Laboratory

AECOM Environment
Fort Collins Environmental Toxicology Laboratory
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Laboratory Project ID

8503-133-409-026, 027

SUMMARY

Study Director	Gina R. McNerney
Test Facility	AECOM Environment 4303 West LaPorte Avenue Fort Collins, Colorado 80521 (970) 416-0916
Location of Data	Data Lock Records Management 328 Link Lane Fort Collins, Colorado 80524
Test Substance	Copper chloride; (CuCl ₂ • 2H ₂ O; 100.3%); (CAS# 10125-13-0)
Subject	Static Toxicity Test
Test Dates	October 29 – 31, 2008
Length of Study	48 Hours
Test Species	<i>Ceriodaphnia dubia</i>
Source of Organisms	AECOM/FCETL in-house cultures
Age of Test Organisms	<24 hours
Test Concentrations	Nominal Copper Concentrations: Site: 0, 31, 51, 86, 143, 238, 396, and 660 µg Cu/L Lab: 0, 2, 4, 6, 11, 18, 30, and 50 µg Cu/L
Dilution Water	Water collected from Grand Canyon National Park outfall (Site) and Laboratory Reconstituted Water (Lab)
Results	<p>Site Water: 48-Hour LC₅₀ Based on Dissolved Copper Concentration: 98.38 µg Cu/L; 95% C.I. (88.48 – 110.0)</p> <p>Lab Water¹: 48-Hour LC₅₀ Based on Dissolved Copper Concentration: 18.28 µg Cu/L; 95% C.I. (16.01 – 20.96)</p> <p>Species Mean Acute Value (SMAV): 48-Hour SMAV Based on Dissolved Copper Concentration: 36.86 µg Cu/L</p> <p>Site/Lab Water effect ratio (dissolved): 98.38 / 18.28 = 5.382 Site/SMAV Water effect ratio (dissolved): 98.38 / 36.86 = 2.669</p>

¹ The Lab Water LC₅₀s did not need any hardness adjustment to the Site water hardness because they were the same hardness (172 mg/L as CaCO₃ for both).

Sponsor and Laboratory Information

Sponsor	Grand Canyon National Park P.O. Box 129 Grand Canyon, Arizona 86023
Project Officer	Ms. Alicia Beale (928) 638-7627 bealeta@msn.com
Testing Facility	AECOM Environment Fort Collins Environmental Toxicology Laboratory 4303 W. LaPorte Avenue Fort Collins, Colorado 80521 Fax: (970) 490-2963
Study Director	Gina McNerney (970) 416-0916 (gina.mcnerney@aecom.com)
Report Author	Gina McNerney

Test Information

Test Purpose	To determine the water-effect ratio for copper to <i>Ceriodaphnia dubia</i> in site and laboratory water
Basis	ASTM (2001) Method E 729-96, USEPA WER guidance (1994a and 2001)
Protocol No.	CD2AZ.WER409.001
Test Initiation / Termination	Site: October 29, 2008 @ 1600 / October 31, 2008 @ 1615 Lab: October 29, 2008 @ 1600 / October 31, 2008 @ 1615
Test Length	48 hours
Species	<i>Ceriodaphnia dubia</i>
Test Waters	Site and Laboratory waters
Location	Grand Canyon National Park, Arizona
Collection / Receipt Date	October 28, 2008 / October 29, 2008 (see Appendix A)
Concurrent Control Water	Test water without the addition of copper chloride
Test Concentrations	Site: 0 (control), 31, 51, 86, 143, 238, 396, and 660 µg Cu/L Lab: 0 (control), 2, 4, 6, 11, 18, 30, and 50 µg Cu/L

Test Conditions

Type	Static acute
Test Endpoints	Mortality (no response to stimulus)
Date of test initiation	October 29, 2008
Test duration	48 hours
Test chamber size	60-mL plastic cups
Test solution Volume	40 mL
Replicates per Treatment	5 (replicate E used for chemistry measurements only; no organisms)
Organisms per Replicate	5
Feeding regime	None during testing
Test temperature	20 ± 1°C; see Protocol Deviations
Photoperiod / Lighting	16 hours light : 8 hours dark / fluorescent
Quality Criteria	≥90% control survival <50% mortality in one test treatment other than the control >50% mortality in at least one test treatment
Test Material	Copper chloride dihydrate (CuCl ₂ •2H ₂ O, 100.3%), Stock solution: 50 mg Cu/L (prepared from AECOM Test Substance # 19723, 10,000 mg Cu/L)
Analytical Confirmation	Site/Lab: Dissolved samples at test initiation and test termination bracketing the concentration response. Total recoverable (TR) samples at test initiation to determine TR:dissolved ratio.
Copper Analyses	ICP-MS (EPA Method 200.8), ACZ Laboratories, Inc.
Statistical Analyses	USEPA (1994b) software / USEPA (2002) guidance

Note: See Appendix B for complete test protocol

Identification of Test Waters

Parameter	Site	Lab Match
AECOM No.	22298	---
Reconstituted water (RW) No.	---	8865 ¹

¹Prepared to be similar to Site hardness and alkalinity

Test Organism

Species	<i>Ceriodaphnia dubia</i>
Age	<24 hours
Source (Batch No.)	AECOM's FCETL (Batch No. 102808)
Holding conditions	Organisms from FCETL culture conditions ¹
Reference toxicant testing	Initiated October 1, 2008 using sodium chloride
General organism health	Test organisms appeared to be healthy

¹Target hardness and alkalinity of culture water, 90 and 60 mg/L as CaCO₃, respectively

TEST RESULTS**Characterization of Waters**

Parameter	Site	Lab
Hardness (mg/L as CaCO ₃) ¹	172	172
Alkalinity (mg/L as CaCO ₃) ¹	124	123
pH (Units) ¹	7.8	8.4
Conductivity (µS/cm) ¹	1,224	620
Total Ammonia (mg/L as N) ¹	< 1.0	< 1.0
Total Residual Chlorine (mg/L) ¹	0.03	< 0.02
Calcium (mg/L) ²	41.9	29.0
Magnesium (mg/L) ²	18.9	27.4
Potassium (mg/L) ²	15.1	6.5
Sodium (mg/L) ²	167	59.4
Chloride (mg/L) ²	160	5
Sulfate (mg/L) ²	90	180
TSS (mg/L) ²	5 U	5 U
TDS (mg/L) ²	730	360
Dissolved Organic Carbon (mg/L) ²	8	2 B

¹Analyzed by AECOM (Appendix C / raw data)

²Analyzed by ACZ Laboratories, Inc. (Appendix D)

B-Analyte detected between the method detection limit and the practical quantitation limit

U-Analyte below the method detection limit

Physical and Chemical Data Measured During Toxicity Tests

Parameter	Site	Lab
Solution Temperature (°C)	18 – 21 ¹	19 – 21
Dissolved oxygen (mg/L)	6.7 – 7.2	6.7 – 7.0
Minimum % D.O. saturation	89	89
pH (S.U.)	7.6 – 8.4	8.3 – 8.5
Performance	Acceptable	Acceptable

¹ See Protocol Deviations

Survival (%) of *Ceriodaphnia dubia* at Test Termination (48 hours)

Nominal Conc. (µg Cu/L) ¹	Site	Lab
0 (control)	100	100
31 / 2	100	100
51 / 4	100	100
86 / 6	75	100
143 / 11	10	100
238 / 18	0	50
396 / 30	0	35
660 / 50	0	0

¹ Site / Lab

Note: See Appendix C for a copy of the raw data

Measured Copper Concentrations ($\mu\text{g/L}$)

Nominal Conc.	Diss. Conc. Initial / Final	Avg. Diss.	% of Nom.	Total Rec. Conc. Initial	% of Nom.	Diss. / TR (%) ²
Site						
0	15.9 / ---	15.9	---	---	---	---
31	---	---	---	---	---	---
51	56.4 / 59.5	57.95	114	---	---	---
86	84.0 / 83.1	83.55	97	---	---	---
143	128.0 / 136.0	132.0	92	140.0	98	91
238	192.0 (193.0) ¹ / 193.0	192.5	81	215.0	90	89
396	---	---	---	---	---	---
660	---	---	---	---	---	---
Lab						
0	0.5 U / ---	0.5 U	---	---	---	---
2	---	---	---	---	---	---
4	---	---	---	---	---	---
6	---	---	---	---	---	---
11 ³	10.0 / 9.9	9.95	90	11.6	105	86
18 ³	16.2 (12.4) ⁴ / 16.2	16.2	90	18.3	102	89
30	22.9 / 22.9	22.9	76	---	---	---
50	41.0 / 41.3	41.2	82	---	---	---

¹ Duplicate analysis

² Calculated as initial dissolved divided by initial total recoverable (D1/T1); average diss/TR ratios were used to estimate TR analytical concentrations to determine TR LC50s.

³ The values obtained from the original analyses for initial and final dissolved copper, as well as total recoverable copper, were suspect and therefore re-analyzed. The values obtained from the re-analysis were used.

⁴ Duplicate analysis; this value agrees with the initial and final dissolved values obtained from the original analysis

Note: See Appendix D for a copy of raw data (ACZ Laboratories, Inc.)

Median Lethal Copper Concentrations ($\mu\text{g/L}$) and Water Effect Ratios (WERs)

Time (h)	Sample	$\mu\text{g Cu / L}$		Method	Site/Lab Calculated WER ²	Site/SMAV Calculated WER
		Site	Lab ¹			
24 hours	Total Recoverable ³	138.9	32.84	S-K	---	---
	Dissolved	127.6	28.73	S-K	---	---
48 hours	Total Recoverable ³	107.8	20.92	Probit	5.153	2.694
	Dissolved	98.38	18.28	Probit	5.382	2.669

¹ Values did not need hardness adjustment to the Site water hardness because values were the same (172 mg/L for both).

² Site / Lab LC50s

³ TR LC50s were estimated using diss/TR ratios and initial dissolved values

S-K = Spearman-Kärber

Note: WERs were also calculated using the hardness adjusted Species Mean Acute Values for total recoverable and dissolved copper. The SMAVs were 40.01 and 36.86 $\mu\text{g/L}$, respectively (USEPA 2001). See Appendix C.

Acute Reference Toxicant Test Results for *Ceriodaphnia dubia*

48-hour LC ₅₀	AECOM/FCETL Historical 95% Control Limits	
	Low	High
1,835	1,265	1,914

Note: Values are expressed as mg/L chloride

Protocol Deviations

On the day of test termination, temperature measured in old test solution from the 31, 51, and 86 $\mu\text{g Cu/L}$ nominal treatments from the SITE water study were 18°C, which is slightly below the recommended range of 19 to 21°C. Since the temperature differential (maximum minus minimum) did not exceed 3°C, this test met the temperature requirements of USEPA (2002). To the best of the Study Director's knowledge, no further deviations from the test protocol (Appendix B) occurred during this study.

References

- ASTM. 2001. Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians. E 729-96. Annual Book of ASTM Standards, Volume 11.05, Section 11, Water and Environmental Technology.
- USEPA. 1994a. Interim Guidance on Determination and Use of Water-Effect Ratios for Metals. EPA-823-B-94-001. U.S. Environmental Protection Agency. Office of Water, Office of Science and Technology. February.
- USEPA. 1994b. USEPA Toxicity Data Analysis Software. Version 1.5. USEPA, Cincinnati, OH.
- USEPA. 2001. Streamlined Water-Effect Ratio Procedure for Discharges of Copper. EPA-822-R-01-005. U.S. Environmental Protection Agency. Office of Water, Office of Science and Technology. Washington, DC. March.
- USEPA. 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fifth Edition, EPA-821-R-02-012. U.S. Environmental Protection Agency. Office of Research and Development. Washington, D.C. October.

Statement of Quality Assurance

The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures, and that the resulting data and report meet the requirements of the NELAC standards. This report is an accurate reflection of the raw data.

Anita Rehner
Quality Assurance Unit

December 30, 2008
Date

Gina McNerney
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December 30, 2008
Date