

Prepared for
Grand Canyon National Park
P.O. Box 129
Grand Canyon, Arizona 86023



Grand Canyon National Park

Water-Effect Ratio Studies – Round 1 Acute Toxicity of Copper to *Ceriodaphnia dubia* in Site and Reconstituted Laboratory Water Under Static Test Conditions

May 2008

ENSR Corporation

Project ID: 08503-130-409 (021,022)

July 15, 2008

ENSR | AECOM

Study Title

Acute Toxicity of Copper to *Ceriodaphnia dubia* in Site and Reconstituted Laboratory Water
Under Static Test Conditions (Round 1)

Study Periods

Start: May 29, 2008

End: May 31, 2008

Performing Laboratory

ENSR

Fort Collins Environmental Toxicology Laboratory

4303 West LaPorte Avenue

Fort Collins, CO 80521

Telephone: (970) 416-0916

FAX: (970) 490-2963

Laboratory Project ID

8503-130-409-021, 022

SUMMARY

Study Director	Gina R. McNerney
Test Facility	ENSR Corporation 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; ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$; 100.3%); (CAS# 10125-13-0)
Subject	Static Toxicity Test
Test Dates	May 29 – 31, 2008
Length of Study	48 Hours
Test Species	<i>Ceriodaphnia dubia</i>
Source of Organisms	ENSR/FCETL in-house cultures
Age of Test Organisms	<24 hours
Test Concentrations	Nominal Copper Concentrations: Site: 0, 28, 47, 78, 130, 216, 360, and 600 $\mu\text{g Cu/L}$ Lab: 0, 2, 4, 6, 11, 18, 30, and 50 $\mu\text{g Cu/L}$
Dilution Water	Water collected from Grand Canyon National Park outfall (Site) and Laboratory Reconstituted Water (Lab)
Results	<p>Site Water: 48-Hour LC_{50} Based on Dissolved Copper Concentration: 113.9 $\mu\text{g Cu/L}$; 95% C.I. (103.7 – 125.2)</p> <p>Lab Water¹: 48-Hour LC_{50} Based on Dissolved Copper Concentration: 9.13 $\mu\text{g Cu/L}$; 95% C.I. (8.08 – 10.31)</p> <p>Species Mean Acute Value (SMAV): 48-Hour SMAV Based on Dissolved Copper Concentration: 25.43 $\mu\text{g Cu/L}$</p> <p>Site/Lab Water effect ratio (dissolved): $113.9 / 9.13 = 12.48$ Site/SMAV Water effect ratio (dissolved): $113.9 / 25.43 = 4.479$</p>

¹ The Lab Water LC_{50} s was not hardness adjusted to the Site water hardness because the waters were of similar hardness (112 and 116 mg/L as CaCO_3 , respectively).

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	ENSR Corporation Fort Collins Environmental Toxicology Laboratory 4303 W. LaPorte Avenue Fort Collins, Colorado 80521 Fax: (970) 490-2963
Study Director	Gina McNERney (970) 416-0916 (gmcnerney@ensr.aecom.com)
Report Author	Rami B. Naddy (970) 416-0916 (rnaddy@ensr.aecom.com)

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: May 29, 2008 @ 1645 / May 31, 2008 @ 1610 Lab: May 29, 2008 @ 1645 / May 31, 2008 @ 1600
Test Length	48 hours
Species	<i>Ceriodaphnia dubia</i>
Test Waters	Site and Laboratory waters
Location	Grand Canyon National Park, Arizona
Collection / Receipt Date	May 27, 2008 / May 29, 2008 (see Appendix A)
Concurrent Control Water	Test water without the addition of copper chloride
Test Concentrations	Site: 0 (control), 28, 47, 78, 130, 216, 360, and 600 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	May 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
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 ENSR 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
ENSR No.	21789	---
Reconstituted water (RW) No.	---	8684 ¹

¹Prepared to be similar to Site hardness and alkalinity

Test Organism

Species	<i>Ceriodaphnia dubia</i>
Age	<24 hours
Source (Batch No.)	ENSR's FCETL (Batch No. 052808)
Holding conditions	Organisms from FCETL culture conditions ¹
Reference toxicant testing	Initiated May 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	116	112
Alkalinity (mg/L as CaCO ₃) ¹	88	75
pH (Units) ¹	7.6	8.0
Conductivity (µS/cm) ¹	1,097	396
Total Ammonia (mg/L as N) ¹	< 1.0	< 1.0
Total Residual Chlorine (mg/L) ¹	0.02	< 0.02
Calcium (mg/L) ²	29.4	21.9
Magnesium (mg/L) ²	11.2	17.5
Potassium (mg/L) ²	16.0	3.1
Sodium (mg/L) ²	150	34.1
Chloride (mg/L) ²	150	3 B
Sulfate (mg/L) ²	30B	130
TSS (mg/L) ²	52	38
TDS (mg/L) ²	664	242
Dissolved Organic Carbon (mg/L) ²	8	1 U

¹Analyzed by ENSR (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)	19 – 20	19 – 21
Dissolved oxygen (mg/L)	7.2 – 7.8	7.0 – 7.6
Minimum % D.O. saturation	96	93
pH (S.U.)	7.5 – 8.3	8.0 – 8.4
Performance	Acceptable	Acceptable

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

Nominal Conc. (µg Cu/L) ¹	Site	Lab
0 (control)	100	100
28 / 2	100	95
47 / 4	100	100
78 / 6	100	90
130 / 11	55	30
216 / 18	10	0
360 / 30	0	0
600 / 50	0	0

¹Site / 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	17.5 / ---	17.5	---	---	---	---
28	---	---	---	---	---	---
47	---	---	---	---	---	---
78	72.8 / 77.2	75.0	96	---	---	---
130	106.0 / 110.0	108.0	83	142.0	109	75
216	153.0 (163.0) ¹ / 156.0	154.5	72	233.0	108	66
360	237.0 / 217.0	227.0	63	---	---	---
600	---	---	---	---	---	---
Lab						
0	0.5 U / ---	0.5 U	---	---	---	---
2	---	---	---	---	---	---
4	4.3 / 4.2	4.25	106	---	---	---
6	6.9 / 5.8	6.35	106	---	---	---
11	11.3 / 10.4	10.8	99	11.6	105	97
18	18.9 (19.1) ¹ / 13.8	16.4	91	19.7	109	96
30	---	---	---	---	---	---
50	---	---	---	---	---	---

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.

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	299.3	13.28	S-K	---	---
	Dissolved	225.0	11.91	S-K	---	---
48 hours	Total Recoverable ³	159.9	10.11	T-S-K	15.82	5.793
	Dissolved	113.9	9.13	T-S-K	12.48	4.479

¹ Values were not hardness adjusted to the Site water hardness because values were very similar (116 and 112 mg/L, for Site and Lab, respectively).

² Site / Lab LC50s

³ TR LC50s were estimated from diss/TR ratios of initial dissolved values

T-S-K = Trimmed Spearman-Kärber

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 27.6 and 25.43 $\mu\text{g/L}$, respectively (USEPA 2001).

Acute Reference Toxicant Test Results for *Ceriodaphnia dubia*

48-hour LC ₅₀	ENSR FCETL Historical 95% Control Limits	
	Low	High
1,690	1,305	1,852

Note: Values are expressed as mg/L chloride

Protocol Deviations

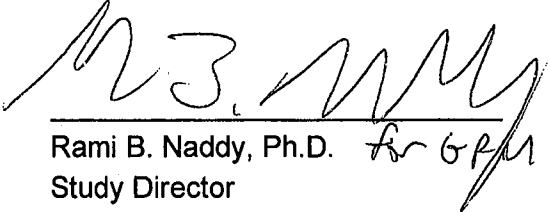
To the best of the Study Director's knowledge, there were no deviations from the test protocol (Appendix B) during this study.

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.
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- 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 PROCEDURAL COMPLIANCE

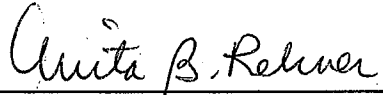
I certify that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge, accurate and complete.


Rami B. Naddy, Ph.D.
Study Director

July 14, 2008
Date

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. This report is an accurate reflection of the raw data generated at the FCETL.


Anita B. Rehner, M.S.
Quality Assurance Unit

July 14, 2008
Date