

ACUTE AND CHRONIC TOXICITY OF ALUMINUM
TO CERIODAPHNIA DUBIA AT VARIOUS pH'S

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The University of Wisconsin-Superior (UW-S) conducted studies to determine acute-chronic ratio for the effects of aluminum (Al) upon the freshwater cladoceran, Ceriodaphnia dubia. Exposures were attempted at a pH near neutrality and others near pH 8. The test organisms appeared not to tolerate the chronic exposure conditions above pH 8 necessitating a third exposure at a pH slightly less than 8.

METHODS

Biological Conditions

The test organisms (C. dubia) were acquired initially from the U.S. Environmental Protection Agency Environmental Research Laboratory, Duluth, MN stock culture. They were cultured at the University of Wisconsin-Superior (UW-S) culture facility for several generations before use in the Al toxicity testing. Cultures were maintained in either (a) raw Lake Superior water which was acquired at the City of Superior Water Treatment Plant from the Cloquet, MN water intake pipe or (b) water which was Lake Superior water treated at the City of Superior Water Treatment Plant and dechlorinated in the laboratory by charcoal and sodium sulfite addition. Except for the fluoride, the waters from the two water sources were similar in their measured chemical and physical characteristics (Table 1).

Adult cladocerans were cultured individually in 30 mL containers. Cladocerans were transferred to new water every Monday,

TABLE 1. Measured characteristics of the test waters used to expose *Ceriodaphnia dubia* to aluminum. Measurements are of the batch waters prior to addition of aluminum.

Water Characteristic	Raw Lake Superior Water	UW-Superior Laboratory Water
Total Alkalinity (mg.L ⁻¹ as CaCO ₃)	46.0	39.6
Total Hardness (mg.L ⁻¹ as CaCO ₃)	50.0	50.5
Ca Hardness (mg.L ⁻¹ as CaCO ₃)	42.6	37.8
Chloride (mg.L ⁻¹ as Cl)	2.8	3.8
Conductivity (umhos.cm ⁻¹)	87	115
Fluoride (mg.L ⁻¹ as F1)	0.08	1.18
Total Solids (mg.L ⁻¹ at 105 C)	80	70
Total Suspended Solids (mg.L ⁻¹)	2	<1
Total Dissolved Solids (mg.L ⁻¹ , 180 C)	63	60
pH	7.4	7.4

Wednesday and Friday. This technique enabled accurate records of young production which indicated the health of the organisms. A combination of fermented trout chow solution (5 g.L⁻¹ Murray trout chow starter fermented 4 days), yeast solution (5 g.L⁻¹) and green algae (Selenastrum capricornutum) was fed to each container once daily at a rate of 100 uL of 1:1:1 ratio of each component. The 100 uL volume contained approximately 1×10^6 algal cells.

Aluminum exposures were made in 30 mL containers holding 16 mL of test solution. Test solutions of the five Al concentrations and controls were made at least 24 hr before exposures began. These solutions were aerated and had the pH adjusted to the desired exposure pH by the addition of reagent grade HCl or NaOH. Adjustments of pH were made daily prior to test initiation. The pH was adjusted at each renewal for the chronic exposures and at the initiation of the acute exposures.

Acute exposures were conducted with four replicates for each Al concentration and control. All solutions were treated the same except for the Al concentration. Five C. dubia neonates less than 24-hr old were added to each beaker by random selection from a common pool of organisms. Transfer of organisms was accomplished by a fire-polished, wide-mouth pipet. Immobilization was used as the endpoint for the 48-hr EC₅₀ estimates. The organisms were not fed during the exposures.

Chronic exposures were conducted similarly to the acute exposures except that the exposures were of seven-day duration with

ten replicates for each of the five Al exposure concentrations and control. At the initiation of the test, each beaker had a single neonate \leq 16-hr old. Exposures were begun on Monday, Wednesday, or Friday with renewal of test solutions made on these days as well. Each beaker was fed once daily a 100 uL volume of the same food used to culture the organisms. Exposure beakers were checked on Monday, Wednesday, and Friday for deaths or reproduction. Young production was noted when the adults were transferred to a new test solution. Number of young produced per adult surviving 7 days was the test endpoint for determination of the 7-day Maximum Acceptable Toxicant Concentration (MATC).

Culturing and testing was done at 25.0 ± 2.0 C. Water for the exposures with Al was the same as the water used to culture the organisms except that no attempt was made to acclimate the organisms to test pH's. They were placed directly into the exposure pH's from the culturing pH of 7.3-7.6.

Chemical Stock Solutions

Test solutions were prepared by adding aliquots of 5000 mg.L^{-1} Al stock (aluminum chloride, AlCl_3 , MCB Chemicals, lot B7N14, 99.8% purity) to filtered (glass fiber) raw Lake Superior water or dechlorinated tap water. The solution pH's were first adjusted to a pH \leq 2.5 with HCl to match the initial pH of the highest Al concentration exposure solution and samples were then collected to determine the initial amount of aluminum present. The solutions were then adjusted to the test pH using dilute solutions of NaOH

(ACS) or HCl (ACS).

Samples were collected at the initiation, prior to and after solution renewal and at the termination of the tests for acid-exchangeable (i.e. acidified, unfiltered) and acid-soluble Al. Acid soluble is the concentration of metal that can pass a 0.45 μm membrane filter after acidification of the sample to pH of 1.5-2.5 (U.S. EPA, 1980). The stocks of test solutions used for renewals were sampled after pH adjustment and while being vigorously mixed on a magnetic stirrer. Samples were immediately acidified with HNO_3 to 0.1% (v/v, pH range 1.5 to 2.0). After the organisms had been transferred to fresh solutions, the old individual exposure solutions were acidified with HNO_3 to 0.1% (v/v), mixed, and allowed to stand (>1 hr) before equal aliquots were withdrawn and composited. The composites were sampled for both acid exchangeable and acid soluble Al. Prior to analysis, 1 mL of HNO_3 and 2 mL of a 9.5% KCl solution (w/v) were added per 100 mL of sample. Deionized water blanks were carried through all procedures as a check for contamination.

Aluminum Analysis

Calibration standards of 0.50 - 20.0 $\text{mg}\cdot\text{L}^{-1}$ Al in deionized water were prepared from a 1000 $\text{mg}\cdot\text{L}^{-1}$ Al stock (Baker Instra Analyzed AA standard, lot 404175). Each solution was preserved with 1.1 mL HNO_3 and 2.0 mL of a 9.5% KCl solution (w/v) added per 100 mL volume.

Analysis for Al was performed according to method 202.1 in Methods for Chemical Analysis of Water and Wastes (U.S. EPA, 1979). An Instrumentation Laboratory Video 12 atomic absorption spectrophotometer with a fuel-rich nitrous oxide-acetylene flame (path length of 5 cm), deuterium arc background correction, and an Al hollow cathode lamp were employed. The determinations were made at a wavelength of 309.3 nm with a 1 nm spectral bandwidth. A scale expansion factor of 2 was used throughout the analyses. Using the above conditions the detection limit was 0.20 mg.L⁻¹ Al.

Analytical Quality Assurance

Approximately ten percent of the samples were analyzed in duplicate and approximately ten percent of the samples were spiked with a known quantity of Al. The overall mean and standard deviation of duplicate analyses and spike recoveries for acid exchangeable samples were $97.4 \pm 2.5 \%$ (n=17) and $99.2 \pm 8.7 \%$ (n=19), respectively; for acid-soluble analysis $98.0 \pm 2.0\%$ (n=14) and $103.1 \pm 6.8\%$ (n=17), respectively. An EPA Trace Metal Reference solution was analyzed routinely and a mean and standard deviation of 7.69 ± 0.19 mg.L⁻¹ (n=15) was found. The true concentration was 7.29 mg.L⁻¹ Al. Each test was individually corrected for spike recovery. In addition, the lack of an obvious matrix interference was confirmed by standard additions. The agreement of the Al concentrations determined by standard additions, compared to the concentrations determined from the calibration curve was $97.6 \pm 9.8\%$ (n=9).

A ratio of acid soluble Al to acid exchangeable Al concentrations was calculated; an overall agreement of $95.6 \pm 10.0\%$ (n=84) was found. However, an apparent trend of a decrease in the ratio of acid soluble to acid exchangeable Al was noted in the composited samples collected before renewal in the highest exposure in the Lake Superior water tests, where an agreement of $74.2 \pm 6.6\%$ (n=5) was found. All concentrations are reported as acid exchangeable Al.

Statistical Analysis

Estimates of the 48-hr EC₅₀ Al concentration and the 95% confidence limits were made using the Trimmed Spearman-Kärber method (Hamilton et al. 1977). Replicate exposures were combined to determine the EC₅₀ estimates.

Chronic exposure effects were determined by one-way analysis of variance (ANOVA) tests at $p \leq 0.05$ significance. Specific concentrations causing significant ($p \leq 0.05$) reductions in reproduction were identified using the Dunnett's one-tailed procedure (Steele and Torrie 1980).

RESULTS

Four sets of acute and chronic exposures of C. dubia with Al at different pH's were attempted. Only the sets at pH 7.2-7.4 and 7.6-7.9 were successfully completed. The set at pH 8.1 resulted in a failure of the chronic exposure and the set at pH 8.2 resulted in failure of both the acute and chronic exposures.

pH 7.2-7.4

The 48-hr exposure of C. dubia to five concentrations of Al (1.5, 2.6, 5.0, 9.6, and 16.2 mg.L⁻¹) plus a control all in quadruplicate at a mean pH of 7.42 ± 0.02 resulted in an EC₅₀ estimate of 1.9 mg.L⁻¹ with a 95% confidence interval of 1.78 to 2.04 mg.L⁻¹ (Table 2). No control organisms died. All treatment deaths occurred after 24 hr of exposure to Al.

C. dubia neonates ≤ 16-hrs old were exposed to five concentrations (1.4, 2.6, 5.0, 10.0, and 17.2) plus a control in ten replicates. At the end of the seven-day exposure the mean number of young per adult was higher in the low exposure concentration (1.4 mg.L⁻¹) than the control. However, significant (p ≤ 0.01) reductions in reproduction when compared to the control occurred at concentrations ≥ 2.6 mg.L⁻¹ (Table 2). The geometric mean of the highest no observed effect concentration (1.4 mg.L⁻¹) and the lowest observed effect concentration (2.6 mg.L⁻¹) was 1.9 mg.L⁻¹. This is considered the MATC.

The pH 7.2-7.4 exposures were made in raw Lake Superior water.

TABLE 2. Acute and chronic toxicity of Ceriodaphnia dubia to aluminum on raw Lake Superior water at mean pH of 7.2-7.4. Aluminum concentrations were measured and are expressed as the acid exchangeable form.

48-Hr Acute						
	Exposure (mg.L ⁻¹)					
	Control	1.5	2.6	5.0	9.6	16.2
Mean cumulative percent survival	100	85	5	0	0	0
Estimated 48-Hr EC ₅₀ = 1.9 mg/L (1.78-2.04)						
Mean pH = 7.42 ± 0.02						
Mean DO = 7.0 ± 0.2						
Mean Temp = 24.4 ± 0.3						
Seven-Day Chronic						
	Exposure (mg.L ⁻¹)					
	Control	1.4	2.6	5.0	10.0	17.2
Mean cumulative number of young adult surviving 7 days	3.3 ± 3.6	4.6 ± 2.1	0.3 ± 0.5 **	0 **	0 **	0 **
Mean cumulative number of young per starting adult	2.5 ± 3.3	3.4 ± 2.6	0.2 ± 0.4 **	0 **	0 **	0 **
Percent survival	90	80	60	20	40	80
**Significantly different from the control at 99% C.I.						
Estimated MATC = 1.9 mg/L						
Mean pH = 7.15 ± 0.05						
Mean temp = 24.8 ± 0.2						
Mean DO = 7.1 ± 0.1						

pH 7.6-7.9

The 48-hr exposure of C. dubia to five concentrations of Al (1.2, 2.2, 6.0, 10.6, and 20.6 mg.L⁻¹) plus a control all in quadruplicate at a mean pH of 7.86 ± 0.04 resulted in an EC₅₀ estimate of 1.5 mg.L⁻¹ (Table 3). Confidence limits could not be computed due to the lack of 100 percent survival in any Al exposure concentration. No control organisms died. All treatment deaths occurred between 24 and 48 hrs of exposure to aluminum.

C. dubia neonates ≤ 16-hrs old were exposed to five concentrations (1.1, 2.4, 5.7, 10.1, and 17.8 mg.L⁻¹) plus controls in ten replicates. At the end of the seven-day exposure the mean number of young per adult was significantly reduced from the control at concentrations of ≥ 2.4 mg.L⁻¹ (Table 3). The geometric mean of the highest no observed effect concentration (1.1 mg.L⁻¹) and the lowest effect concentration (2.4 mg.L⁻¹) was 1.6 mg.L⁻¹. This was considered the MATC.

The pH 7.6-7.9 exposures were made in UW-S dechlorinated tap water.

pH 8.1

The 48-hr exposure of C. dubia to five concentrations of Al (1.3, 2.1, 3.7, 8.5, and 17.7 mg.L⁻¹) plus controls all in quadruplicate at a mean pH of 8.13 ± 0.03 resulted in an EC₅₀ estimate of 2.56 mg.L⁻¹ Al with a 95% confidence interval of 2.14 to 3.06 mg.L⁻¹ (Table 4). No control organisms died. All treatment deaths occurred between 24 and 48 hr of exposure to Al.

TABLE 3. Acute and chronic toxicity of Ceriodaphnia dubia to aluminum in UW-Superior laboratory water at mean pH of 7.6-7.9. Aluminum concentrations were measured and are expressed as the acid exchangeable form.

48-Hr Acute						
	Exposure (mg.L ⁻¹)					
	Control	1.2	2.2	6.0	10.6	¹ 28.6
Mean cumulative percent survival	100	75	0	0	0	0
Estimated 48-Hr EC ₅₀ = 1.5 (95% C.I. are not reliable)						
Mean pH = 7.86 ± 0.04						
Mean Temp = 25.3 ± 0.3						
Mean DO = 25.3 ± 0.3						
Seven-Day Chronic						
	Exposure (mg.L ⁻¹)					
	Control	1.1	2.4	5.7	10.1	17.8
Mean number of young per adult surviving for 7 days	7.2 ± 5.0	3.67 ± 4.2	0.7 ± 1.2 **	0.2 ± 0.4 **	0 **	0 **
Mean number of young per starting adult	6.3 ± 4.5	2.2 ± 3.7 **	0.5 ± 1.1 **	0.2 ± 4.2 **	0 **	0 **
Percent survival	80	60	70	60	0	0
**Significantly different from the control at 99% C.I.						
Estimated MATC = 1.6 mg/L						
Mean pH = 7.61 ± 0.11						
Mean temp = 25.3 ± 0.1						
Mean DO = 7.3 ± 0.1						

TABLE 4. Acute toxicity of aluminum to Ceriodaphnia dubia in raw Lake Superior water at mean pH of 8.1. Aluminum concentrations were measured and are reported as the acid exchangeable form.

	Exposure (mg.L ⁻¹)					
	Control	1.3	2.1	3.7	8.5	17.7
Mean cumulative percent survival	100	100	50	25	0	0
Estimated 48-Hr EC ₅₀ = 2.56 (2.14-3.06)						
Mean pH = 8.13 ± 0.03						
Mean Temp = 25.0 ± 0.1 C						
Mean DO = 7.4 ± 0.1 mg/L						

A seven-day chronic exposure was attempted at pH 8.1. No organisms survived in any exposure concentrations or the controls. The water for the pH 8.1 tests was raw Lake Superior water.

Acute-Chronic Ratios

Exposures were successfully completed to allow the calculation of two acute-chronic ratios. The ratio of 1.0 was computed for the exposure pH's of 7.2-7.4 and 0.9 for the exposure pH's of 7.6-7.9 (Table 5). The ratio of 0.9, if significantly different from 1.0, is then likely the result of food interaction with the Al concentrations. The food used only in the chronic exposures may have sequestered some of the free Al.

An acute-chronic ratio could not be calculated from the exposures of pH 8.1 or 8.2.

TABLE 5. Acute and chronic exposure effects of aluminum to Ceriodaphnia dubia at various water pH's

pH	Toxic Effect (mg.L ⁻¹)			Ratio
	Acute 48-Hr EC ₅₀	Chronic MATC	Acute Chronic	
7.2-7.4	1.9	1.9	1.0	
7.6-7.9	1.5	1.6	0.9	
8.1	2.6	<u>1</u> /	-	

1/ No survival in control or any exposures.

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