TOXICITY OF ALUMINUM TO FRESHWATER ORGANISMS IN WATER OF pH 6.5 - 8.5

by

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INTRODUCTION

Most studies on the toxicity of aluminum (Al) to aquatic life have been conducted in acidic waters, due to concerns centered around surface water acidification. Fewer studies have been conducted in waters of neutral or alkaline pH. The present study was undertaken to generate Al toxicity information on several freshwater plant and animal taxa at neutral pH or in slightly alkaline waters. The purpose of this work was to provide a broader taxonomic base for the development of Al criteria designed to protect aquatic life in situations where Al may be discharged into such waters.

METHODS

Test Water

Lake Superior water was used for test organism acclimation and Al exposures. It was collected from a tap on the City of Cloquet, MN raw water intake in the City of Superior, WI. The source of the water was from approximately 3200 M offshore on the lake bottom. The water was transported in 20 L polycarbonate containers to the laboratory and mixed into 150 L batches for distribution to the various exposure chambers.

Six batches of water were used for the toxicity tests. Total alkalinity, total hardness, calcium hardness, chloride, sulfate, fluoride, total solids, total suspended solids, and turbidity were measured for each of these batches (Table 1). Generally, measured values varied little between batches and were consistent with historical values for Lake Superior water.

TABLE 1. Mean chemical characteristics of six batches of Lake Superior water used for aluminum exposures.

	Mean	Standard Deviation	Range
Total alkalinity ($mg \cdot L^{-1}$ as $CaCO_3$)	41.6	± 1.27	39.1-42.5
Total hardness ($mg \cdot L^{-1}$ as $CaCO_3$)	47.4	± 4.51	44.1-56.3
Calcium hardness ($mg \cdot L^{-1}$ as $CaCO_3$)	36.5	± 5.67	33.5-48.0
Chloride ($mg \cdot L^{-1}$)	1.5	± 0.42	1.2-2.2
Sulfate ($mg \cdot L^{-1}$)	2.7	± 0.82	<1-3
Fluoride ($mg \cdot L^{-1}$)	0.04	± 0.00	0.04-0.04
Total solids ($mg \cdot L^{-1}$	61	± 5	54-68
Total suspended solids ($mg \cdot L^{-1}$)	<1.0	± 0.00	<1.0-<1.0
Turbidity (turbidity units)	1.2	± 0.73	0.4-2.4

General Approach

Exposure and control tanks were prepared a minimum of 48 hr in advance of organism introduction to the tanks. The water volume was measured, then added to each exposure tank, and aluminum introduced from a 50,000 $mg \cdot L^{-1}$ stock solution. The Al stock solution was made using anhydrous, reagent grade $AlCl_3$ from MCB Manufacturing Chemists, Inc., lot no. B 7N14 with a purity of 99.7%. Exposure solutions were pH adjusted if the pH differed from the desired test pH by more than 0.2 units. Adjustment of pH was done when necessary using either NaOH or HCl of ACS grade purity. After pH adjustment, the solutions were aerated for a minimum of 48 h. One test was run using rainbow trout, snails, and Ceriodaphnia sp. as test organisms with exposure water prepared as described, then aerated for 18 days. All tests with fish were also aerated for the duration of the exposures. Similarly, tests with scuds (Gammarus),

stoneflies (Acronuria), and snails (Physa) were aerated. Tests with the cladoceran Ceriodaphnia, the duckweed Lemna, and the green alga Selenastrum were not aerated during the exposures.

Acute Toxicity Tests with Fish

Rainbow trout (Salmo gairdneri) - pH 6.5, 7.5 and 8.5

Fingerling trout were exposed to Al at three nominal pH levels of 6.5, 7.5, and 8.5. Nominal exposure concentrations were 0, 3.12, 6.25, 12.5 and 25 $mg \cdot L^{-1}$, in duplicate. Trout were received from Fattig Fish Hatchery, Brady, NE. Lengths and weights of control fish at the end of each test were 56 ± 6 mm and 1.51 ± 0.47 g at pH 6.5, 57 ± 3 mm and 1.55 ± 0.23 g at pH 7.5, and 56 ± 4 mm and 1.55 ± 0.41 g at pH 8.5. Five trout were randomly introduced into duplicated aquaria (L x W x H equal to 30.5 x 21 x 25.5 cm) containing 12.0 L of water. Temperature was maintained near 17^{o} C by placing the aquaria into a temperature-controlled water bath. Daily measurements of temperature in all aquaria (n=50) produced mean temperature values (ranges in parentheses) of 16.9 ± 0.69^{o} C (15.9-17.8^{o} C) at pH 6.5, 17.3 ± 0.73^{o} C (15.9-18.3^{o} C) at pH 7.5, and 17.5 ± 0.70^{o} C (16.0-18.5^{o} C) at pH 8.5.

pH was monitored daily in all tanks. Mean measured pH values include readings made before and after adjustments. Mean pH values were 6.59 ± 0.15 (n=67), 7.31 ± 0.89 (n=62), and 8.17 ± 0.42 (n=78) for desired levels of 6.5, 7.5 and 8.5, respectively. The aquaria were aerated throughout the tests, and mean dissolved oxygen levels were 8.7 ± 0.64, 8.4 ± 0.40, and 8.6 ± 0.22 $mg \cdot L^{-1}$ for tests conducted at pH 6.59, 7.31, and 8.17, respectively.

Green sunfish (Lepomis cyanellus) - pH 7.7

Young-of-the-year green sunfish (~3 mo. old) were exposed to Al at a nominal pH of 7.7 and nominal Al concentrations of 0, 6.25, 12.5, 25.0, and 50.0 $mg \cdot L^{-1}$, in duplicate. Fish were received from Fattig Fish Hatchery,

Brady, NE. Tests were conducted in 30.5 x 21 x 25.5 cm aquaria containing 12 L of water. Each aquarium contained 8-11 fish. Temperature was maintained near 22° C by placement of the aquaria in a temperature-controlled water bath. The mean water temperature (range in parentheses) was 22.2 ± 4° C (20.9-22.6° C, n=50). The mean pH was 7.55 ± 0.13 (n=50). The mean dissolved oxygen level in the aerated aquaria was 8.1 ± 0.5 $mg \cdot L^{-1}$ (n=24).

Channel catfish (Ictalurus punctatus) - pH 7.5

Young-of-year catfish were exposed to nominal Al concentrations of 0, 12.5 and 50 $mg \cdot L^{-1}$, in duplicate, at the nominal pH of 7.5. Fish were received from the Environmental Research Laboratory-Duluth. Tests were conducted in 30 x 30 x 30 cm chambers containing 22 L of water each. Three fish were added to each chamber. Mean length and weight of control fish at the end of the exposure were 96 ± 13 mm and 6.98 ± 2.98 g, respectively. Aquaria were placed into a temperature-controlled water bath for temperature maintenance near 17° C. The mean measured temperature was 16.7 ± 0.2° with a range from 16.5-16.9° C, (n=30). pH was monitored daily and adjusted when it deviated by more than 0.2 units. The mean pH was 7.54 ± 0.16 (n=42). The mean dissolved oxygen level in the aerated aquaria was 8.9 ± 0.12 $mg \cdot L^{-1}$ (n=30).

Fathead minnow (Pimephales promelas) - pH 7.5 and 8.2

Fathead minnows (32-33 d old) were exposed to Al at nominal pH values of 7.5 and 8.2. Mean measured pH values were 7.61 and 8.05 for nominal values of 7.5 and 8.2, respectively. At pH 7.5, nominal Al concentrations of duplicate exposures were 0, 12.5, 25.0, and 50.0 $mg \cdot L^{-1}$, while at pH 8.2 nominal Al concentrations were 0, 3.12, 6.25, 12.5, 25, and 50 $mg \cdot L^{-1}$. Fish were reared in our culture unit from embryos. Mean control fish lengths and weights at the end of the 96 hr exposure were 16 ± 3 mm and 0.056 ± 0.032 g at pH 7.5 and

15 ± 2.4 mm and 0.052 ± 0.027 g at pH 8.2. Tests were conducted in covered 1 L glass beakers each containing 900 mL of water. Beakers were placed into a 22° C water bath for temperature control. Mean measured water temperatures were 22.9 ± 0.24 (n=11) and 22.9 ± 0.25 (n=13) at pH 7.5 and 8.2, respectively. Mean dissolved oxygen levels were 8.1 ± 0.17 $mg \cdot L^{-1}$ (n=6) and 8.1 ± 0.07 $mg \cdot L^{-1}$ (n=6) at lower and higher pH, respectively.

Yellow Perch (Perca flavescens) - pH 7.6

Young-of-year yellow perch were exposed to Al at a nominal pH of 7.6. Nominal exposure concentrations were 0, 12.5 and 50 $mg \cdot L^{-1}$. Perch were collected in the Superior, WI harbor, and acclimated in the laboratory for several days prior to use. Mean length and weight of control fish were 84 ± 6 mm and 4.85 ± 1.20 g. Duplicate Al exposures were prepared in a total volume of 20 L of Lake Superior water. Three fish were added per replicate (30 x 30 x 30 cm test chamber). Test aquaria were placed into a temperature-controlled water bath for maintenance of temperature near 17° C. The mean measured water temperature was 16.6 ± 0.17 (n=30), with a range from 16.4 to 16.9° C. The mean dissolved oxygen level was 9.8 ± 0.10 $mg \cdot L^{-1}$ (n=18). Mean measured pH was 7.55 ± 0.12 (n=30).

Toxicity Tests with Invertebrates

Scud (Gammarus pseudolimnaeus) - pH 7.5

Scuds collected from the Eau Claire River in Douglas County, WI and acclimated for several weeks in laboratory water were used as test organisms. They were exposed to Al at nominal concentrations of 0, 3.12, 6.25, 12.5 and 25.0 $mg \cdot L^{-1}$ and at a nominal pH of 7.5. Tests were conducted with 9 animals per exposure, each isolated in a 250 mL glass beaker to prevent cannabalism. Each beaker contained two opposing 2.5 cm diameter holes in the sides covered with Nytex® screen. Nine beakers per exposure concentration were suspended

in a 30.5 x 21 x 25.5 cm aquarium containing 12 L of test water. A single aspen (Populus tremuloides) aged leaf disc 1.5 cm in diameter was added to each beaker to serve as shelter and a possible substrate for food organisms.

The aquaria were placed into a temperature-controlled water bath maintained near 18° C. Mean measured daily temperature was 18.1 ± 0.48° C (n=25) with a range from 17.4 to 18.7° C. The mean measured dissolved oxygen level during the test was 9.4 ± 0.28 $mg \cdot L^{-1}$ (n=20). Mean measured pH was 7.53 ± 0.07 (n=29).

Stonefly (Acronuria sp.) - pH 7.6

Stoneflies collected from the Eau Claire River in Douglas County, WI and acclimated to laboratory water for several days were used as test animals. They were exposed to Al at nominal concentrations of 0, 6.25, 12.5 and 25.0 $mg \cdot L^{-1}$ in test water maintained near pH 7.6. Five stoneflies were added per exposure. Tests were conducted in 5 x 15.5 x 15 cm glass containers (Nytex® screened at both ends) which were placed into aquaria containing 12 L of water. The aquaria were placed into a temperature-controlled water bath maintained near 16° C. The mean measured temperature was 16.4 ± 0.10 (n=20) with a range from 16.3° C to 16.6° C. The mean measured dissolved oxygen concentration was 9.7 ± 0.10 $mg \cdot L^{-1}$ (n=12). The mean measured pH was 7.46 ± 0.18 (n=22).

Snail (Physa sp.) - pH 6.5, 7.5 and 8.5

Snails were received from Fattig Fish Hatchery, Brady, NE and acclimated in the laboratory for several days prior to use. At a nominal pH of 7.5, snails were exposed to duplicate Al concentrations of 0, 6.25, 12.5, and 50.0 $mg \cdot L^{-1}$ in tests run simultaneously with green sunfish. Five snails with mean weight of 0.033 ± 0.013 g were placed into stainless steel mesh wire cylinders (8-10 cm length x 3 cm diameter) closed at either end with neoprene stoppers which had been pre-soaked in laboratory water for 2-3 days. The cylinders were laid horizontally on the floors of glass aquaria containing 12 L of water.

Measurements of pH, temperature and dissolved oxygen were the same as presented for the green sunfish test. Mean values for these parameters were 7.55, 22.2° C, and 8.1 $mg \cdot L^{-1}$, respectively.

Snails were also run simultaneously with rainbow trout at nominal pH values of 6.5 and 8.5. At both pH levels, the nominal duplicated Al concentrations were 0, 3.12, 6.25, 12.5 and 25.0 $mg \cdot L^{-1}$. At the end of the 96 hr exposures, control snails from both tests combined averaged 0.9 ± 0.02 g in weight. Measurements of pH, temperature, and dissolved oxygen that were previously reported for the trout test also pertain to these snail tests. Mean pH values were 6.59 and 8.17, with corresponding mean temperatures of 16.9 and 17.5° C. Mean dissolved oxygen levels were 8.7 and 8.4 $mg \cdot L^{-1}$ at pH 6.59 and 8.17, respectively.

Due to the nature of the test containers, snails were not visible during the 96 hr exposure. To avoid any additional handling stress on the test animals, mortality observations were not made at intermediate exposure intervals of 24, 48, and 72 hr, only at test termination (96 hr). At 96 hr, snails were removed from the cylinders, placed into crystallizing dishes, and examined for movement under a dissecting microscope.

Cladoceran (Ceriodaphnia sp.) - pH 7.5

Ceriodaphnia less than 24 hr old were used to start a 48 hr acute test with Al at a nominal pH of 7.5. Nominal Al concentrations were 0, 3.12, 6.25, 12.5 and 25 $mg \cdot L^{-1}$. Five animals were placed into each 30 mL beaker containing 15 mL of water. Four replicates per concentration were used. Test solutions in Lake Superior water had been aerated for at least 48 hr prior to the start of the test, but were not aerated during the actual tests. Temperature was maintained between 23.2 and 25° C. Dissolved oxygen levels ranged from 8.1 to 8.3 $mg \cdot L^{-1}$. pH was measured at each exposure level initially and at 48 hr.

The mean measured pH was 7.68 ± 0.17 (n=10).

A chronic test was also conducted in 30 mL beakers, each containing 15 mL of water. One young daphnid (<16 hr old) was placed into each of 10 beakers per exposure concentration. Solutions were renewed on days 2, 4, and 6, at which time food was added. Food consisted of a mixture of yeast, Cerophyll®, and fermented trout chow, and was provided in equal amounts (2 drops per beaker) to all test beakers. Nominal Al concentrations were 0, 1.56, 3.12, 6.25, 12.5, and 25.0 $mg \cdot L^{-1}$. pH was measured initially and at the end of 7 days. Mean measured pH was 7.58 ± 0.07 (n=48). The combined mean initial and final dissolved oxygen level was 6.92 ± 0.57 $mg \cdot L^{-1}$ (n=48). The test was run at room temperature of approximately 20-22° C.

Acute Toxicity Tests with Al Solutions Aged for 18 Days - pH 7.6

Rainbow trout, snails (Physa sp.), and Ceriodaphnia sp. were exposed to Al concentrations in which the pH had been adjusted to 7.6-7.7 and the exposure water had been vigorously aerated for 18 days. Nominal Al concentrations in the duplicated trout and snail tests were 0, 5.8, 9.7, 16.2, 27, 45 and 75 $mg \cdot L^{-1}$. Trout were received from Fattig Fish Hatchery, Brady, NE. Tests were conducted in 30.5 x 21 x 25.5 cm aquaria containing 12 L of water. Three trout were added per aquarium. Control fish averaged 5.52 ± 0.29 cm and 1.51 ± 0.33 g at the end of the 96 hr exposure. Snails were tested simultaneously with trout. Five snails were placed into each stainless steel cylinder (8-10 cm long and 3 cm diameter) which was laid horizontally on the bottom of each aquarium. Aquaria were placed in a temperature-controlled water bath where the mean temperature was 15.7 ± 0.43° C. The mean pH for the trout and snail tests as determined from daily measurements was 7.46 ± 0.14 (n=92). The mean dissolved oxygen level

of the aerated test water was 9.91 ± 0.48 $mg \cdot L^{-1}$.

Fish were observed daily for mortalities. Snail mortalities were recorded only at the end of the exposure (96 hr) to minimize handling stress.

Ceriodaphnia were exposed to mean measured total Al concentrations of 0.8, 6.4 and 11.4 $mg \cdot L^{-1}$ in test water at a pH of 7.36. Mortality observations were made at 24 and 48 hr.

Aquatic Plant Toxicity Tests

Duckweed (Lemna minor) - pH 7.6 and 8.2

The procedure used for this test was the Proposed New Standard Practice for Conducting Static Toxicity Tests with Duckweed, a procedure currently being developed for the American Society for Testing and Materials (ASTM). A stock culture of Lemna minor was obtained from Dr. W. Wang, Water Quality Section, Illinois State Water Survey, Peoria, Illinois. The stock culture was maintained in dilution water, as recommended in the proposed procedure, for 7 days before the initiation of the aluminum toxicity test.

Test solutions were prepared by spiking dilution water with an aluminum chloride stock solution to yield nominal test concentrations of 0 (control), 3.12, 6.25, 12.5, 25.0 and 50.0 $mg \cdot L^{-1}$. Each test solution was divided into two containers, the pH of one being adjusted to 7.6 ± 0.05 and that of the other to 8.2 ± 0.05. Each pH adjusted test solution was further divided into three replicates of 200 mL each in 250 mL beakers. Ten colonies consisting of 2 fronds each were added to each beaker. The test beakers were then randomly arranged in the test area. Light intensity was 400 ± 40 ft. candles and temperature was maintained at 24 ± 2° C.

At the end of the 96 hr test exposure, the total number of fronds were counted and the pH of many of the test solutions determined.

Samples were collected at several times before and after the test to determine Al concentrations in the exposure solutions. The first set of samples was collected after the solutions were prepared and before pH adjustment was made. These samples were taken to determine the total Al concentration in the original solutions. A second set of samples was collected immediately before the introduction of the Lemna to the test beakers. These samples were collected after the beakers had been allowed to remain stationary for a minimum of 30 minutes to ensure that the Al floc had settled. This set of samples indicates the amount present in the water column at the initiation of the test. A third set of samples was collected at the end of the 96 hr test. These samples were collected in a manner similar to the previous set and would indicate the amount present in the water column at the end of the test.

Green Algae (Selenastrum capricornutum) - pH 7.6 and 8.2

A protocol used by the EPA Office of Toxic Substances was followed in conducting an algal toxicity screening test. One modification was made in the procedure that involved using a nutrient solution four times as concentrated as the solution designated. The nutrient concentration used was the same as that recommended in the Selenastrum Bottle Test Procedure, Miller et al. (1978). The Selenastrum capricornutum stock culture was obtained from the EPA Environmental Research Laboratory-Duluth and sub-cultured in the above mentioned nutrient solution until utilized in the test.

An initial test was conducted as a range finder. This test consisted of exposing the algae to nominal total Al concentrations of 0, 3.12, 6.25, 12.5, 25.0 and 50.0 $mg \cdot L^{-1}$ at two pH levels, 7.6 and 8.2. The results of this test showed inhibitions of greater than 50% for all concentrations tested.

The results of the range finding test indicated a need to run the second test at lower total Al concentrations. The test solutions were prepared by

spiking the previously described algal nutrient solution with a stock $AlCl_3$ solution ($\sim$10,000 $mg \cdot L^{-1}$) to yield nominal total Al test concentrations of 0 (control), 0.4, 0.8, 1.6, 3.2 and 6.4 $mg \cdot L^{-1}$. The solutions were sampled at this point to determine the total Al concentration. Each test concentration was then divided into two equal portions and the pH of one segment adjusted to 7.6 ± 0.05, while the pH of the second was adjusted to 8.2 ± 0.05. The following morning, the pH values were readjusted to the appropriate levels. After this pH readjustment, the solutions were divided into three replicates of 50 mL each in 125 mL erlenmeyer flasks. Some flasks received more than 50 mL at this point. This allowed for samples to be collected for Al analysis from these flasks before initiation of the test. The flasks were stoppered with foam plugs which allowed for exchange of gases during the test. The flasks and contents were then sterilized by autoclaving for 20 minutes. After cooling, the flasks which had received additional volumes of the test solutions were sampled to indicate the 0 hour Al concentrations in the water columns. The test flasks were then inoculated with Selenastrum capricornutum to yield an initial cell concentration of 10,000 $cells \cdot mL^{-1}$. The 96 hour test was then initiated. The tests were run at 400 ± 40 ft. candles of light intensity, and at a temperature of 25 ± 3° C. The flasks were shaken at a rate of 110 rpm.

At the end of the 96 hour exposures, pH values for many of the flasks were determined. The solutions were then acidified to 1% nitric acid and filtered through prewashed and preweighed 0.45 μ Millipore membrane filters. The filters were then dried at 70° C and reweighed to determine the biomass of algae present in each sample. To determine the actual biomass of algae, a correction for the weight of Al floc on the filters was made. Additional flasks at each test concentration which had not been inoculated with algae were carried through the entire test procedure. These flasks were sampled at the end of the 96 hour test

to determine Al concentrations in the water columns. After the samples had been collected, the remainder of the solution was filtered to determine the weight of Al floc. This weight was then used to correct the weights determined from the inoculated flasks.

Analytical Measurements of Al Concentrations

Samples (15-20 mL) were collected midway between the surface and bottom of the water column in the center of each exposure chamber. A 30 mL adjustable-volume glass-teflon syringe was rinsed three times with the exposure water prior to collecting a sample. All samples were immediately acidified to 1% (v/v) with concentrated nitric acid (Baker Instra analyzed Lot No: 340040). Two mL of potassium chloride solution (95 g $KCl \cdot L^{-1}$) was added per 100 mL sample, as suggested in method 202.1, Methods for Chemical Analysis of Water and Wastewater (U.S. EPA, 1979).

Al was analyzed by atomic absorption spectrophotometry with direct aspiration of the sample into a fuel-rich nitrous oxide-acetylene flame. The instrument was a Perkin Elmer Model 306 Atomic Absorption Spectrophotometer, with a 5.3 cm slot burner head, hollow cathode lamp for Al at 309.3 nm, and a slit width of 1.0 mm. A scale expansion factor of 1.6 was applied to the direct readout of absorbances. Sample absorbances were compared to a curve in the range of 1.00-50.0 $mg \cdot L^{-1}$ of Al.

All standards were prepared from a 1000 mg $Al^{3+} \cdot L^{-1}$ stock solution (Baker InstraAnalyzed, Lot 404175). Ten mL of nitric acid and 20 mL of KCl solution (95 g $KCl \cdot L^{-1}$) were added to each liter of standard prepared.

An EPA reference sample was prepared at ten times its normal concentration to bring it into the working range of the tests. The theoretical concentration was 7.3 ± 0.1 $mg \cdot L^{-1}$, and the analytical concentration was 7.7 ± 0.3 $mg \cdot L^{-1}$ (n=15).

In addition to the reference sample, 10% of the total number of samples were duplicate samples and 10% of the samples were spiked with a known quantity of a 1.000 $mg \cdot mL^{-1}$ Al stock solution. Recovery of spiked samples and duplicate agreement were determined at each of three different water pH levels. At nominal pH 6.5, recovery was 105 ± 1.7% (n=4) and duplicate agreement was 97.4 ± 2.4% (n=5); at nominal pH 7.5-7.7, these respective values were 99.3 ± 7.8% (n=37) and 96.2 ± 6.1% (n=42); and at nominal pH 8.2-8.5, the respective values for recovery and agreement were 100 ± 4.3% (n=8) and 98.4 ± 1.4% (n=7). Concentrations of Al reported herein have been corrected for recovery.

Measurements of Water Chemistry Parameters

Water chemistry parameters of total hardness, calcium hardness, total alkalinity, conductivity, dissolved oxygen, chloride, fluorides, sulfates, turbidity, non-filterable residue and total residue were routinely measured for the test water. Total alkalinity and chloride were determined according to procedures in Standard Methods for the Examination of Water and Wastewater, 15th ed. (American Public Health Association et al., 1980). The remaining parameters were measured by procedures in Methods for Chemical Analysis of Water and Wastewater (U.S. EPA, 1979).

RESULTS

Concentrations used in many of the tests ranged downward from a nominal high exposure of 25, 50 or 75 $mg \cdot L^{-1}$ total Al with a 0.5 dilution factor to a low exposure of 1.56 or 3.12 $mg \cdot L^{-1}$. Upon initial pH adjustment prior to the start of organism exposures, a precipitate was readily apparent at concentrations of 3.12 $mg \cdot L^{-1}$ or above. Aeration of the exposure chambers for all tests, with the exception of the daphnid and plant tests which were not aerated, resulted

in a mixing and suspension of the floc in addition to providing an adequate oxygen supply. However, it should be noted that in the large aquaria particularly, vigorous aeration did not suspend all the floc evenly, as floc accumulated in certain areas of the aquaria floors. Thus, organisms that were either placed on or near the exposure chamber floors, such as snails, or organisms that actively sought out these areas, such as scuds, were exposed to a denser floc than those organisms which sought out areas near the water surface.

Daily adjustments of pH were necessary in many of the exposures. Tests run at pH values above neutrality declined in pH in proportion to the total aluminum present. Controls dropped the least or not at all. Tests run at a pH below neutrality (6.5 nominal) rose in pH in all test chambers.

Acute Toxicity Tests with Fish

Rainbow Trout (Salmo gairdneri) - pH 6.5, 7.5 and 8.5

At a nominal pH of 6.5 (mean measured pH of 6.59), rainbow trout deaths occurred at 23.4 $mg \cdot L^{-1}$ within 24 hr, at 11.8 $mg \cdot L^{-1}$ within 48 hr and at 5.4 $mg \cdot L^{-1}$ by 96 hr (Appendix A, Table A1). LC_{50} estimates for pooled replicate data with 95% confidence intervals in parentheses at 48, 72, and 96 hr of exposure were 11.5 (9.1-14.5), 10.0 (8.0-12.3), and 7.4 (5.8-9.4) $mg \cdot L^{-1}$, respectively.

At a nominal pH of 7.5 (mean measured pH of 7.31), the mortality data are presented in Appendix A, Table A2. LC_{50} estimates of 72 and 96 hr were 19.3 (95% confidence interval estimate could not be reliably determined) and 14.6 (9.3-23.1) $mg \cdot L^{-1}$, respectively.

At nominal pH 8.5 (mean measured pH of 8.17), insufficient mortalities occurred for an LC_{50} determination. Only 40% of the trout died after 96 hr of exposure at the highest total Al concentration of 24.7 $mg \cdot L^{-1}$ (Appendix A, Table A3).

Green Sunfish (Lepomis cyanellus) - pH 7.7

No mortalities occurred through 96 hr of exposure to total Al concentrations ranging from 6.4 to 50 $mg \cdot L^{-1}$ at a mean measured pH of 7.55 (Appendix A, Table A5).

Channel Catfish (Ictalurus punctatus) - pH 7.5

No mortalities resulted during 96 hr of exposure to mean total Al concentrations of 12.9 and 47.9 $mg \cdot L^{-1}$ (Appendix A, Table A6).

Fathead Minnows (Pimephales promelas) - pH 7.5 and 8.2

In each of two fathead minnow toxicity tests with mean total Al concentrations in the highest exposure of 48.2 and 49.8 $mg \cdot L^{-1}$ at mean measured pH values of 7.61 and 8.05, respectively, there were no deaths through 96 hr at the highest exposures and only one death in each test at an intermediate concentration (Appendix A, Tables A7 and A8).

Yellow Perch (Perca flavescens) - pH 7.6

No mortalities occurred when yellow perch were exposed to Al concentrations of 12.8 and 49.8 $mg \cdot L^{-1}$ for 96 hr at a mean measured test water pH of 7.55 (Appendix A, Table A9).

Toxicity Tests with Invertebrates

Scud (Gammarus pseudolimnaeus) - pH 7.5

Scuds were exposed to total Al concentrations ranging from 2.6 to 24.1 $mg \cdot L^{-1}$ (Appendix B, Table B1). The 96 hr LC_{50} estimate was 22.0 $mg \cdot L^{-1}$ with a 95% confidence interval of 13.8 to 35.2 $mg \cdot L^{-1}$. A considerable amount of floc occurred at the bottoms of the beakers, particularly at the three highest concentrations. The leaf discs were often situated in the floc with scuds present under the discs. Thus, many of the scuds survived even while apparently spending considerable amounts of time in the Al floc.

Stonefly (Acronuria sp.) - pH 7.6

No deaths occurred when stoneflies were exposed to total Al concentrations of 6.2, 12.3 and 22.6 $mg \cdot L^{-1}$ for 96 hr at a mean measured pH of 7.46 (Appendix B, Table B2).

Snail (Physa sp.) - pH 6.5, 7.5 and 8.5

At nominal pH 6.5 (mean measured pH of 6.59), insufficient mortalities occurred through 96 hr at total Al concentrations up to 23.4 $mg \cdot L^{-1}$ for determination of an LC_{50} value (Appendix B, Table B3). Fifty percent mortality occurred at a mean concentration of 11.8 $mg \cdot L^{-1}$, which seems anomalous due to the fact that only 10% died at 23.4 $mg \cdot L^{-1}$.

At nominal pH 7.5 (mean measured pH of 7.55) snails were exposed to total Al concentrations from 6.4 to 50.3 $mg \cdot L^{-1}$ (Appendix B, Table B4). The 96 hr LC_{50} was 30.6 $mg \cdot L^{-1}$ with a 95% confidence interval of 23.1 to 40.6 $mg \cdot L^{-1}$.

At nominal pH 8.5 (mean measured pH of 8.17), insufficient deaths occurred over a 96 hr exposure to total Al concentrations of up to 24.7 $mg \cdot L^{-1}$ for an LC_{50} determination (Appendix B, Table B5).

Ceriodaphnia sp. - pH 7.5

Ceriodaphnia were exposed to total Al concentrations ranging from 3.1 to 22.8 $mg \cdot L^{-1}$ at a mean pH of 7.68. Complete mortality in all four replicates occurred within 48 hr at exposures of 12.4 and 22.8 $mg \cdot L^{-1}$ (Appendix B, Table B7). Partial mortalities at exposures of 3.12 and 6.25 $mg \cdot L^{-1}$ resulted in an LC_{50} estimate of 3.69 $mg \cdot L^{-1}$ with a 95 percent confidence interval of 3.06 - 4.44 $mg \cdot L^{-1}$.

In an 8-day chronic test with Ceriodaphnia, an LC_{50} of 8.6 $mg \cdot L^{-1}$ was obtained, with a 95% confidence interval of 5.2 to 14.2 $mg \cdot L^{-1}$. Brood production was completely eliminated at an exposure of 23.7 $mg \cdot L^{-1}$, and also significantly

reduced ($p \leq 0.01$) at 12.1 $mg \cdot L^{-1}$ (Table 2). The total number of offspring produced per surviving adult was significantly reduced ($p \leq 0.01$) at an exposure of 12.1 $mg \cdot L^{-1}$. Of the five survivors at 12.1 $mg \cdot L^{-1}$, only one individual had a brood. The brood was hatched on day 8 and it consisted of only one offspring. No statistically significant effects upon survival or reproduction were noted at concentrations $\leq$ 4.9 $mg \cdot L^{-1}$. The "no effect-effect" concentration range was from 4.9 to 12.1 $mg \cdot L^{-1}$.

The 8-day LC_{50} of 8.6 $mg \cdot L^{-1}$ was greater than the 48 hr LC_{50} of 3.7 $mg \cdot L^{-1}$. A possible explanation for this difference could be that the introduction of food in the chronic exposure has an effect upon the results. No food was provided to the test animals in the acute test.

Acute Tests with Aged Al Solutions

Rainbow Trout (Salmo gairdneri) - pH 7.5

Measured total Al concentrations and mortalities at daily intervals for rainbow trout exposed to Al which had been "aged" in Lake Superior for 18 days prior to use are presented in Appendix A, Table A4. The mean pH of the test water was 7.46. LC_{50} values with 95% confidence intervals in parentheses at 24, 48, 72, and 96 hr were 13.4 (9.6-18.8), 10.5 (7.9-14.0), 9.7 (7.6-12.4), and 8.6 (6.2-11.9) $mg \cdot L^{-1}$, respectively.

Snails (Physa sp.) - pH 7.5

Snails were exposed to the same set of Al concentrationsand water characteristics as the trout above. Fifty percent of the snails were dead within 96 hr at both of the two highest exposures, 42.7 and 72.1 $mg \cdot L^{-1}$ (Appendix B, Table B6). The Spearman-Karber program provided a 96 hr LC_{50} estimate of 55.5 $mg \cdot L^{-1}$, but the 95% confidence limits were not reliably determined.

TABLE 2. Effects of Al^{3+} Upon Survival and Reproduction in an Eight-Day Chronic Test with Ceriodaphnia sp.

	Al^{3+} Concentration ($mg \cdot L^{-1}$)					
Nominal	0.0	1.56	3.12	6.25	12.5	25.0
Total Measured[a/]	<0.3	1.0	3.0	4.9	12.1	23.7
Number of Survivors[b/]	7	9	6	9	5	1
Days to First Brood (± s.d.)	5.3 (±0.8)	5.8 (±1.5)	5.0 (±0.9)	5.6 (±0.5)	>8.0** (±0.0)	>8.0[c/] (±0.0)
Mean Number of Broods per Surviving Adult (± s.d.)	2.9 (±2.2)	1.9 (±1.0)	2.3 (±0.5)	2.3 (±0.9)	0.2** (±0.4)	0.0[c/] (±0.0)
Mean Total Number of Offspring per Surviving Adult (± s.d.)	6.6 (±5.4)	3.5 (±2.2)	4.8 (±2.3)	4.3 (±1.7)	0.2** (±0.4)	0.0[c/] (±0.0)

a/ Total measured Al is the amount present in the water column upon acidification to a pH where no precipitate was observed.

b/ The test was started with 10 animals per concentration.

c/ This single value was not statistically treatable by one-way ANOVA.

** Significantly different from controls at 99.0% confidence level.

Ceriodaphnia sp. - pH 7.5

Exposure of Ceriodaphnia to total Al concentration of 6.4 and 11.4 $mg \cdot L^{-1}$ resulted in 100% mortality at 48 hr while exposure to 0.8 $mg \cdot L^{-1}$ resulted in no deaths (Appendix B, Table B8). The 48 hr LC_{50} for total Al in "aged" water at a mean pH of 7.36 was 2.3 $mg \cdot L^{-1}$. Ninety-five percent confidence intervals were not reliably determined due to a lack of partial mortalities.

Aquatic Plant Toxicity Tests

Duckweed (Lemna minor) - pH 7.6 and 8.2

Lemna frond production was not significantly affected by Al exposures as high as 45.7 $mg \cdot L^{-1}$ (Table 3). The reduction in fronds produced (percent effect) ranged from 1.6 to 12.1% at pH 7.6 and from 8.8 to 28.2% at pH 8.2. At pH 7.6, the percent effect showed no correlation with total Al concentration. At pH 8.2, Al exposures reduced frond production more than at the lower pH, but again not significantly.

The pH of the test water dropped during the duration of the test, the drop ranging from approximately 0.2 to 1.0 pH unit (Appendix C, Table C1). Concentrations of Al in the water column are also listed in Table C1. With the exception of the controls, in which Al concentrations were below the detection limit (0.3 $mg \cdot L^{-1}$), initial water column concentrations were all approximately 2.0 $mg \cdot L^{-1}$ at pH 7.6, and from 2.5 to 4.7 $mg \cdot L^{-1}$ at pH 8.2. The 96 hr water column concentrations of Al were all at or below the detection limit with the exception of two questionable values.

Total Al concentrations in the samples before pH adjustment and the start of the tests are presented in Table 3. All initial total Al values are within 10% of nominal concentrations.

TABLE 3. Frond Production by Duckweed (Lemna minor) Exposed for 96 Hr to Al^{3+} in Lake Superior Water at Initial pH Values of 7.6 and 8.2.

		Al^{3+} Concentration ($mg \cdot L^{-1}$)					
	Nominal	0.0	3.12	6.25	12.5	25.0	50.0
	Total Measured[a/]	<0.3	2.8	5.9	11.4	23.2	45.7
pH 7.6	Mean Number of Fronds	39.0 ± 3.5	37.0 ± 2.0	38.0 ± 1.7	38.7 ± 1.2	36.7 ± 2.5	38.3 ± 1.2
	Percent Effect[a/]	-	10.5	5.3	1.6	12.1	3.7
pH 8.2	Mean Number of Fronds	42.7 ± 0.6	36.3 ± 3.8	37.0 ± 2.6	38.7 ± 2.9	39.3 ± 3.2	40.7 ± 1.2
	Percent Effect[b/]	-	28.2	25.1	17.6	15.0	8.8

a/ Total measured Al is the initial amount present in the water column prior to pH adjustment and the start of the test. No precipitate was observed.

b/ Percent effect is the increase of fronds in the control (C) minus the increase of fronds in the test concentration (T) divided by the increase of fronds in the control multiplied by 100 ($\% \text{ Effect} = \frac{C-T}{C} \times 100$).

Mean recovery of spiked samples for this test was 106.9% (n=4). The percentage agreement for analysis of duplicates was 99.5% (n=5).

Green Algae (Selenastrum capricornutum) - pH 7.6 and 8.2

Algal biomass and growth inhibition data are presented in Table 4. The amount of inhibition in algal biomass production that was experienced at pH 7.6 and 8.2 at the various total Al concentrations ranged from 32.0 to 76.6% at pH 7.6 and from 39.6 to 93.5% at pH 8.2. The initial total Al concentration and 96 hr percent inhibition data were tested with a Spearman-Karber statistical program to estimate the concentration that would result in a 50% reduction in mean biomass (96 hr EC_{50}). At pH 7.6, an EC_{50} value of 0.57 $mg \cdot L^{-1}$ was obtained with a 95% confidence interval of 0.39 to 0.83 $mg \cdot L^{-1}$. At pH 8.2, the EC_{50} value was 0.46 $mg \cdot L^{-1}$ with a range from 0.41 to 0.52 $mg \cdot L^{-1}$.

The pH in most of the exposure water samples dropped, generally less than one pH unit (Table C2). Total Al concentrations in the samples prior to pH adjustments are listed in Table 4. All values are in good agreement with nominal concentrations. Al concentrations present in the water column at 0 and 96 hr are listed in Appendix C, Table C2. At pH 7.6, the 0 hr water column concentrations increased until the 1.6 $mg \cdot L^{-1}$ nominal concentration was reached, after which decreases occurred at the two higher nominal concentrations. The range of 0 hr water column values was from 0.2 to 0.8 $mg \cdot L^{-1}$. The 96 hr concentrations were at or below the detection limit (0.2 $mg \cdot L^{-1}$) for all but one sample.

Water column Al concentrations in the 0 hr samples at pH 8.2 increased until the 3.2 $mg \cdot L^{-1}$ sample was reached, with a decrease noted for the 6.4 $mg \cdot L^{-1}$ samples. The range of 0 hr Al values was from below the detection limit to 1.3 $mg \cdot L^{-1}$. The 96 hr samples ranged from near or below the detection limit to 1.8 $mg \cdot L^{-1}$.

The mean spike recovery for the Al toxicity tests with algae was 112.5% (n=8). Percent agreement in the analysis of duplicate samples was 98.7% (n=8).

TABLE 4. Biomass and Growth Inhibition of Green Algae (Selenastrum capricornutum) After 96 hr of Exposure to Various Concentrations of Al^{3+}.

		Al^{3+} Concentration ($mg \cdot L^{-1}$)					
	Nominal	0.0	0.4	0.8	1.6	3.2	6.4
	Total Measured[a/]	<0.2	0.4	0.7	1.4	2.8	5.8
pH 7.6	Mean Corrected Algal Biomass (mg)[b/]	5.12 ± 0.67	3.48 ± 0.42	1.20 ± 0.26	1.87 ± 0.21	2.35 ± 1.01[c/]	1.73 ± 0.32
	Percent Growth Inhibition	-	32.0	76.6	63.5	54.1	66.2
pH 8.2	Mean Corrected Algal Biomass (mg)	5.08 ± 0.25	3.07 ± 0.45	1.10 ± 0.20	0.33 ± 0.06	1.38 ± 0.06	1.23 ± 0.15
	Percent Growth Inhibition	-	39.6	78.3	93.5	72.8	75.8

a/ Total measured Al is the initial amount present in the water column prior to pH adjustment and the start of the test. No precipitate was observed.

b/ Mean dry weight of three test replicates corrected for any Al precipitate present.

c/ Median and standard deviation from the median.

APPENDIX A

MEASURED ALUMINUM CONCENTRATIONS AND MORTALITY OBSERVATIONS FOR ACUTE TOXICITY TESTS WITH FRESHWATER FISH

TABLE A1. Cumulative Mortalities[a/] of Rainbow Trout (Salmo gairdneri) Exposed to Al^{3+} in Lake Superior Water at pH 6.59.

Exposure Time (hr)	Al^{3+} Concentrations ($mg \cdot L^{-1}$)									
Nominal	0.0 A	0.0 B	3.12 A	3.12 B	6.25 A	6.25 B	12.5 A	12.5 B	25.0 A	25.0 B
Total Measured[b/]	<0.3	<0.3	2.4	2.6	5.2	5.6	12.0	11.6	23.4	23.5
24	0	0	0	0	0	0	0	0	2	4
48	0	0	0	0	0	0	3	2	5	5
72	0	0	0	0	0	0	4	3	5	5
96	0	1	0	0	1	1	4	5	5	5

a/ Five fish per replicate were tested.

b/ Total measured Al is the amount present in the water column upon acidification to a pH were no precipitate was observed.

TABLE A2. Cumulative Mortalities[a/] of Rainbow Trout (Salmo gairdneri) Exposed to Al^{3+} in Lake Superior Water at pH 7.31.

Exposure Time (hr)	Al^{3+} Concentrations ($mg \cdot L^{-1}$)									
Nominal	0.0 A	0.0 B	3.12 A	3.12 B	6.25 A	6.25 B	12.5 A	12.5 B	25.0 A	25.0 B
Total Measured[b/]	<0.3	<0.3	2.9	1.0	5.2	5.7	17.9	12.4	24.9	24.5
24	0	0	0	0	0	0	0	0	1	0
48	0	0	0	0	0	0	1	3	3	0
72	0	0	0	0	0	0	2	3	4	1
96	1	0	0	0	0	0	2	3	4	4

a/ Five fish per replicate were tested.

b/ Total measured Al is the amount present in the water column upon acidification to a pH where no precipitate was observed.

TABLE A3. Cumulative Mortalities[a/] of Rainbow Trout (Salmo gairdneri) Exposed to Al^{3+} in Lake Superior Water at pH 8.17.

Exposure Time (hr)	Al^{3+} Concentrations ($mg \cdot L^{-1}$)									
	Nominal	0.0 A	0.0 B	3.12 A	3.12 B	6.25 A	6.25 B	12.5 A	12.5 B	25.0 A	25.0 B
	Total Measured[b/]	<0.3	<0.3	3.1	3.5	6.0	6.4	12.1	12.0	12.4[c/]	24.7
24		0	0	0	2	0	0	0	0	1	0
48		0	0	0	2	0	0	0	0	1	0
72		0	0	0	2	0	0	0	1	2	1
96		1	0	0	3	0	0	0	1	2	2

a/ Five fish per replicate were tested.

b/ Total measured Al is the amount present in the water column upon acidification to a pH where no precipitate was observed.

c/ Low measured Al^{3+} concentration due to error in addition of Al^{3+} stock solution.

TABLE A4. Cumulative Mortalities[a/] of Rainbow Trout (Salmo gairdneri) Exposed to Al^{3+} in Aged Lake Superior Water at pH 7.46.

Exposure Time (hr)	Al^{3+} Concentrations ($mg \cdot L^{-1}$)													
	Nominal	0.0 A	0.0 B	5.8 A	5.8 B	9.7 A	9.7 B	16.2 A	16.2 B	27.0 A	27.0 B	45.0 A	45.0 B	75.0 A	75.0 B
	Total Measured[b/]	<0.3	<0.3	6.0	6.0	9.5	9.5	16.2	16.2	26.0	26.0	42.7	42.7	72.1	72.1
24		0	0	0	0	1	2	2	2	2	2	3	3	3	3
48		0	0	0	0	2	2	3	2	2	3	3	3	3	3
72		0	0	0	0	2	2	3	2	3	3	3	3	3	3
96		0	0	1	0	2	2	3	2	3	3	3	3	3	3

a/ Three fish per replicate were tested.

b/ Total measured Al is the amount present in the water column upon acidification to a pH where no precipitate was observed.

TABLE A.5. Cumulative Mortalities[a/] of Green Sunfish (Lepomis cyanellus) Exposed to Al^{3+} in Lake Superior Water at pH 7.55.

Exposure Time (hr)	Al^{3+} Concentration ($mg \cdot L^{-1}$)									
	Nominal	0.0 A	0.0 B	6.25 A	6.25 B	12.5 A	12.5 B	25.0 A	25.0 B	50.0 A	50.0 B
	Total Measured[b/]	<0.3	<0.3	6.4	6.3	12.2	12.2	24.6	25.0	49.9	50.0
24		0	0	0	0	0	0	0	0	0	0
48		0	0	0	0	0	0	0	0	0	0
72		0	0	0	0	0	0	0	0	0	0
96		0	0	0	0	0	0	0	0	0	0

a/ Ten fish per replicate were tested.

b/ Total measured Al is the amount present in the water column upon acidification to a pH where no precipitate was observed.

TABLE A6. Cumulative Mortalities[a/] of Channel Catfish (Ictalurus punctatus) Exposed to Al^{3+} in Lake Superior Water at pH 7.54.

Exposure Time (hr)	Al^{3+} Concentrations ($mg \cdot L^{-1}$)					
Nominal	0.0 A	0.0 B	12.5 A	12.5 B	50.0 A	50.0 B
Total Measured[b/]	<0.3	<0.3	13.0	12.8	47.8	48.0
24	0	0	0	0	0	0
48	0	0	0	0	0	0
72	0	0	0	0	0	0
96	0	0	0	0	0	0

a/ Three fish per replicate were tested.

b/ Total measured Al is the amount present in the water column upon acidification to a pH where no precipitate was observed.

TABLE A7. Cumulative Mortalities[a/] of Fathead Minnows (Pimephales promelas) Exposed to Al^{3+} in Lake Superior Water at pH 7.61.

Exposure Time (hr)	Al^{3+} Concentrations ($mg \cdot L^{-1}$)							
Nominal	0.0 A	0.0 B	12.5 A	12.5 B	25.0 A	25.0 B	50.0 A	50.0 B
Total Measured[b/]	<0.3	<0.3	12.2	12.4	24.6	24.4	48.6	47.9
24	0	0	0	1	0	0	0	0
48	0	0	0	1	0	0	0	0
72	0	0	0	1	0	0	0	0
96	0	0	0	1	0	0	0	0

a/ Five fish per replicate were tested.

b/ Total measured Al is the amount present in the water column upon acidification to a pH where no precipitate was observed.

TABLE A8. Cumulative Mortalities[a/] of Fathead Minnows (Pimephales promelas) Exposed to Al^{3+} in Lake Superior Water at pH 8.05.

Exposure Time (hr)	Al^{3+} Concentrations ($mg \cdot L^{-1}$)											
	Nominal	0.0 A	0.0 B	3.12 A	3.12 B	6.25 A	6.25 B	12.5 A	12.5 B	25.0 A	25.0 B	50.0 A	50.0 B
	Total Measured[b/]	<0.3	<0.3	2.9	2.9	5.9	5.9	13.2	12.7	25.3	24.8	49.2	50.5
24		0	0	0	0	0	0	0	0	0	1	0	0
48		0	0	0	0	0	0	0	0	0	1	0	0
72		0	0	0	0	0	0	0	0	0	1	0	0
96		0	0	0	0	0	0	0	0	0	1	0	0

a/ Five fish per replicate were tested.

b/ Total measured Al is the amount present in the water column upon acidification to a pH where no precipitate was observed.

TABLE A9. Cumulative Mortalities[a/] of Yellow Perch (Perca flavescens) Exposed to Al^{3+} in Lake Superior Water at pH 7.55.

Exposure Time (hr)	Al^{3+} Concentrations ($mg \cdot L^{-1}$)					
Nominal	0.0 A	0.0 B	12.5 A	12.5 B	50.0 A	50.0 B
Total Measured[b/]	<0.3	<0.3	12.9	12.8	49.3	50.2
24	0	0	0	0	0	0
48	0	0	0	0	0	0
72	0	0	0	0	0	0
96	0	0	0	0	0	0

a/ Three fish per replicate were tested.

b/ Total measured Al is the amount present in the water column upon acidification to a pH where no precipitate was observed.

APPENDIX B

MEASURED ALUMINUM CONCENTRATIONS AND MORTALITY OBSERVATIONS FOR TOXICITY TESTS WITH FRESHWATER INVERTEBRATES

TABLE B1. Cumulative Mortalities[a/] of Scuds (Gammarus pseudolimnaeus) Exposed to Al^{3+} in Lake Superior Water at pH 7.53.

Exposure Time (hr)	Al^{3+} Concentrations ($mg \cdot L^{-1}$)				
Nominal	0.0	3.12	6.25	12.5	25.0
Total Measured[b/]	<0.3	2.6	5.9	12.4	24.1
24	0	0	0	0	1
48	0	2	0	0	2
72	0	4	0	0	4
96	0	4	0	0	5

a/ Nine organisms were tested per exposure concentration.

b/ Total measured Al is the amount present in the water column upon acidification to a pH where no precipitate was observed.

TABLE B2. Cumulative Mortalities[a/] of Stoneflies (Acronuria sp.) Exposed to Al^{3+} in Lake Superior Water at pH 7.46.

Exposure Time (hr)	Al^{3+} Concentrations ($mg \cdot L^{-1}$)			
Nominal	0.0	6.25	12.5	25.0
Total Measured[b/]	<0.3	6.2	12.3	22.6
24	0	0	0	0
48	0	0	0	0
72	0	0	0	0
96	0	0	0	0

a/ Five organisms were tested per exposure concentration.

b/ Total measured Al is the amount present in the water column upon acidification to a pH where no precipitate was observed.

TABLE B3. Mortalities[a/] of Snails (*Physa* sp.) Exposed to Al^{3+} for 96 hr in Lake Superior Water at pH 6.59.

Exposure Time (hr)	Al^{3+} Concentrations ($mg \cdot L^{-1}$)									
Nominal	0.0 A	0.0 B	3.12 A	3.12 B	6.25 A	6.25 B	12.5 A	12.5 B	25.0 A	25.0 B
Total Measured[b/]	<0.3	<0.3	2.4	2.6	5.2	5.6	12.0	11.6	23.4	23.5
96	0	0	0	0	0	0	4	1	1	0

a/ Five organisms per replicate were tested.

b/ Total measured Al is the amount present in the water column upon acidification to a pH where no precipitate was observed.

TABLE B4. Mortalities[a/] of Snails (Physa sp.) Exposed to Al^{3+} for 96 hr in Lake Superior Water at pH 7.55.

Exposure Time (hr)	Al^{3+} Concentrations ($mg \cdot L^{-1}$)									
Nominal	0.0 A	0.0 B	6.25 A	6.25 B	12.5 A	12.5 B	25.0 A	25.0 B	50.0 A	50.0 B
Total Measured[b/]	<0.3	<0.3	6.4	6.3	12.3	12.3	24.8	25.2	50.2	50.4
96	0	0	0	0	0	0	3	0	4	5

a/ Five organisms per replicate were tested.

b/ Total measured Al is the amount present in the water column upon acidification to a pH where no precipitate was observed.

TABLE B5. Mortalities[a/] of Snails (Physa sp.) Exposed to Al^{3+} for 96 hr in Lake Superior Water at pH 8.17.

Exposure Time (hr)	Al^{3+} Concentrations ($mg \cdot L^{-1}$)									
Nominal	0.0 A	0.0 B	3.12 A	3.12 B	6.25 A	6.25 B	12.5 A	12.5 B	25.0 A	25.0 B
Total Measured[b/]	<0.3	<0.3	3.1	3.5	6.0	6.4	12.1	12.0	12.4[c/]	24.7
96	0	0	0	0	0	0	1	1	0	0

a/ Five organisms per replicate were tested.

b/ Total measured Al is the amount present in the water column upon acidification to a pH where no precipitate was observed.

c/ Low measured Al concentration due to error in preparation.

TABLE B6. Mortalities[a/] of Snails (*Physa* sp.) Exposed to Al^{3+} in Aged Lake Superior Water at pH 7.46.

Exposure Time (hr)	Al^{3+} Concentrations ($mg \cdot L^{-1}$)													
	Nominal	0.0 A	0.0 B	5.8 A	5.8 B	9.7 A	9.7 B	16.2 A	16.2 B	27.0 A	27.0 B	45.0 A	45.0 B	75.0 A	75.0 B
	Total Measured[b/]	<0.3	<0.3	6.0	6.0	9.5	9.5	16.2	16.2	26.0	26.0	42.7	42.7	72.1	72.1
96		0	0	0	0	2	0	0	0	2	0	4	1	3	2

a/ Five organisms per replicate were tested.

b/ Total measured Al is the amount present in the water column upon acidification to a pH where no precipitate was observed.

TABLE B7. Cumulative Mortalities[a/] of Ceriodaphnia sp. Exposed to Al^{3+} in Lake Superior Water at pH 7.68.

Exposure Time (hr)	Al^{3+} Concentrations ($mg \cdot L^{-1}$)																			
	Nominal	0.0				3.12				6.25				12.5				25.0			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
	Total Measured[b/]	<0.3				3.1				6.2				12.4				22.8			
24		0	0	0	0	0	0	0	0	0	1	2	5	1	2	1	2	0	0	0	1
48		0	0	0	0	3	1	0	3	5	4	5	5	5	5	5	5	5	5	5	5

a/ Five organisms per replicate were tested.

b/ Total measured Al is the amount present in the water column upon acidification to a pH where no precipitate was observed at the beginning of the test.

TABLE B8. Cumulative Mortalities[a/] of Ceriodaphnia sp. Exposed to Al^{3+} in Aged Lake Superior Water at pH 7.36.

	Al^{3+} Concentrations ($mg \cdot L^{-1}$)															
Nominal	0.0				3.0				9.2				16			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Total Measured[b/]	<0.3				0.8				6.4				11.4			
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	5	5	5	5	5	5	5	5

a/ Five organisms per replicate were tested.

b/ Total measured Al is the amount present in the water column upon acidification to a pH where no precipitate was observed from a pooled sample of the four replicates at test termination.

APPENDIX C

VALUES OF pH AND WATER COLUMN Al^{3+} CONCENTRATIONS IN TOXICITY TESTS WITH FRESHWATER PLANTS

TABLE C1. Measured pH Values and Concentrations of Al^{3+} in the Water Column at 0 and 96 hr in Toxicity Tests with Duckweed (Lemna minor).

Sample	pH 7.6			
	0 hr		96 hr	
	Measured pH	Water Column Al Conc ($mg \cdot L^{-1}$)	Measured pH	Water Column Al Conc ($mg \cdot L^{-1}$)
Control #2	7.58	<0.3	7.42	<0.3
3.12 $mg \cdot L^{-1}$ #1	7.61	2.1	7.30	<0.3
6.25 $mg \cdot L^{-1}$ #1	7.60	1.7	7.21	<0.3
6.25 $mg \cdot L^{-1}$ #2	7.60	1.7	7.18	<0.3
6.25 $mg \cdot L^{-1}$ #3	7.60	1.7	7.17	5.6
12.5 $mg \cdot L^{-1}$ #2	7.65	2.1	7.11	<0.3
25.0 $mg \cdot L^{-1}$ #1	7.57	1.7	6.89	1.3
25.0 $mg \cdot L^{-1}$ #2	7.57	1.7	6.84	<0.3
25.0 $mg \cdot L^{-1}$ #3	7.57	1.7	6.82	<0.3
50.0 $mg \cdot L^{-1}$ #2	7.60	1.8	6.68	<0.3
	pH 8.2			
Control #1	8.24	<0.3	7.25	<0.3
Control #2	8.24	<0.3	7.24	<0.3
Control #3	8.24	<0.3	7.26	<0.3
3.12 $mg \cdot L^{-1}$ #3	8.19	2.5	7.39	<0.3
6.25 $mg \cdot L^{-1}$ #1	8.20	4.1	7.44	<0.3
12.5 $mg \cdot L^{-1}$ #1	8.23	4.7	7.33	<0.3
12.5 $mg \cdot L^{-1}$ #2	8.23	4.7	7.35	<0.3
12.5 $mg \cdot L^{-1}$ #3	8.23	4.7	7.29	<0.3
25.0 $mg \cdot L^{-1}$ #2	8.20	4.2	7.26	<0.3
50.0 $mg \cdot L^{-1}$ #3	8.22	4.2	7.19	0.3

TABLE C2. Measured pH Values and Concentrations of Al^{3+} in the Water Column at 0 and 96 hr in Toxicity Tests with Green Algae (Selenastrum capricornutum).

	pH 7.6			
	0 hr		96 hr	
Sample	Measured pH	Water Column Al Conc ($mg \cdot L^{-1}$)	Measured pH	Water Column Al Conc ($mg \cdot L^{-1}$)
Control #1	7.55		7.89	
Control #2	7.55	<0.2	7.67	
Control #3	7.55		8.56	<0.2
0.4 ppm #2	7.58		7.63	
0.4 ppm #3	7.58	0.4	7.60	
0.8 ppm #1	7.62	0.7	7.50	
1.6 ppm #1	7.56	0.8	7.45	
1.6 ppm #2	7.56	0.8	7.47	<0.2
1.6 ppm #3	7.56	0.8	7.46	
3.2 ppm #2	7.61	0.7	7.51	
3.2 ppm #3	7.61		7.43	
6.4 ppm #1	7.65	0.3	7.29	<0.2
6.4 ppm #2	7.65	0.3	7.24	
6.4 ppm #3	7.65	0.2	7.25	
		Uninoculated		
Control	7.55		7.07	<0.2
0.8 ppm	7.62		7.23	<0.2
1.6 ppm	7.56	0.7	7.18	<0.2
3.2 ppm	7.61		7.12	0.8
6.4 ppm	7.65	0.3	7.05	0.2

TABLE C2 Cont. Measured pH Values and Concentrations of Al^{3+} in the Water Column at 0 and 96 hr in Toxicity Tests with Green Algae (Selenastrum capricornutum).

	pH 8.2			
	0 hr		96 hr	
Sample	Measured pH	Water Column Al Conc ($mg \cdot L^{-1}$)	Measured pH	Water Column Al Conc ($mg \cdot L^{-1}$)
Control #1	8.16	<0.2	7.78	<0.2
Control #2	8.16		8.02	
Control #3	8.16		8.04	
0.4 ppm #3	8.22	<0.2	7.62	
0.8 ppm #2	8.17	0.3	7.52	
1.6 ppm #2	8.16	0.8	7.39	<0.2
3.2 ppm #1	8.19	1.3	7.36	0.2
3.2 ppm #2	8.19	1.3	7.39	
3.2 ppm #3	8.19	1.3	7.38	
6.4 ppm #1	8.21	0.9	7.30	
6.4 ppm #2	8.21	0.9	7.31	<0.2
6.4 ppm #3	8.21	1.0	7.35	
		Uninoculated		
Control	8.16		7.07	<0.2
0.8 ppm	8.17	0.4	7.35	0.3
1.6 ppm	8.16		7.30	0.2
3.2 ppm	8.19	1.6	7.28	0.7
6.4 ppm	8.21	0.7	7.23	1.8

REFERENCES

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1980. Standard Methods for the Examination of Water and Wastewater (15th ed.). American Public Health Association, Washington, D.C.

Miller, W.E., S.C. Greene and T. Shiroyama. 1978. The Selenastrum capricornutum Printz algal assay bottle test. Office of Research and Development, U.S. Environmental Protection Agency, Corvallis, OR. EPA-600/9-78-018.

U.S. Environmental Protection Agency. 1979. Methods for Chemical Analysis of Water and Wastes. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH. EPA 600/4-79-020.