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DATE: July 25, 1985

TO: Mr. Charles Stephan
U.S. Environmental Protection Agency
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Duluth, MN

FROM: Larry Brooke L.J.B.
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SUBJECT: Results of acute exposures to aluminum at pH >6.5 with planaria and daphnids

Acute, static exposures with aluminum were completed at the campus of the University of Wisconsin-Superior (UW-S) using two species of freshwater organisms (brown planaria, Dugesia tigrina; daphnid, Daphnia magna). The studies were done by Jeffrey Lange to complete his credit requirements for his undergraduate degree in biology. He was supervised/advised by Dr. Daniel Call, Mr. Steven Poirier, and myself to insure compliance with the quality assurance requirements of the testing. Ms. Carol Lindberg, staff chemist, provided the analytical results for the aluminum concentrations in the test solutions.

METHODS

Test Organisms

Brown planaria were acquired from Biological Resource Center, Amery, WI. and acclimated in Lake Superior water for 7 days at 20-23 C. Acclimation occurred in beakers containing 150 mL water and 15 individuals. During acclimation they were fed adult brine shrimp (Artemia sp.) ad libitum. Organisms selected for testing were the larger individuals and were uniform in size.

Daphnids were cultured at the UW-S campus laboratory. The culture was originally obtained from the U.S. Environmental Protection Agency, Environmental Research Laboratory-Duluth, MN (U.S. EPA ERL-D). They were acclimated in Lake Superior water for approximately two months and fed three times weekly a ration

of trout chow and a green alga. The trout chow was from the Murray Feed Company, Murray, UT and added to the culture water to a concentration of 5 $mg \cdot L^{-1}$. Algae (Selenastrum capricornutum) were added to the culture solution to a concentration of 10^8 $cells \cdot L^{-1}$. Third generation adults were placed in 100 mL beakers containing 80 mL of Lake Superior water (20-22 C) and neonates <24 h-old were used in the test exposures.

Exposure Conditions

Water for acclimation and aluminum exposures was collected from Lake Superior at the U.S. EPA ERL-D laboratory. Water for the exposures was pH adjusted to 7.5 ± 0.2 daily using either HCl or NaOH (ACS grade). Brown planaria were exposed to aluminum in 100 mL beakers containing 80 mL of test water which had been aged 28 d. Five organisms were placed in each beaker (four replicate beakers·exposure concentration^{-1}) and not fed during the test. Water temperature was monitored daily and was 22 C at initiation of the exposures. It was 24.5 C at 24 and 48 h.

Daphnids were exposed in 100 mL jars suspended in 250 mL beakers containing 160 mL of test solution. Five organisms were placed in each of four replicates·exposure^{-1} concentration. The 100 mL jar had its glass bottom removed and replaced with a screen (58μ Nytex®). The objective of this jar-beaker system was to prevent daphnids from being entrapped by the aluminum floc which settled to the container bottom at aluminum concentrations ≥ 3.12 $mg \cdot L^{-1}$. Test solutions were aerated, pH adjusted, and aged for 48 h before allowing the floc to settle and adding the 100 mL jars and daphnids. Mean test water temperature was 20.5 C.

Certain chemical characteristics of the test water were monitored during the aluminum exposures (Table 1). Conditions were generally uniform between exposure concentrations and tests. Chloride concentrations varied with aluminum concentrations due to the use of Al^{+3}, as $AlCl_3$ and HCl to adjust pH.

The Al stock solution was made using anhydrous, reagent grade $AlCl_3$ from MCB Manufacturing Chemists, Inc., lot No. B7N14 with a purity of 99.7%. The super stock solution was 10,000 $mg \cdot L^{-1}$ in 0.2% HCl.

Table 1. Total alkalinity, total hardness, and chloride concentrations in certain aluminum exposure concentrations

Test Organism	Nominal Al^{+3} Concentration ($mg \cdot L^{-1}$)	Total Alkalinity ($mg \cdot L^{-1}$ as $CaCO_3$)	Total EDTA Hardness ($mg \cdot L^{-1}$ as $CaCO_3$)	Chloride ($mg \cdot L^{-1}$)
Brown Planaria (*Dugesia tigrina*)	control	43.1	47.4	-
Daphnid (*Daphnia magna*)	control	40.5	45.4	0.0
	1.56	34.1	47.0	6.2
	12.5	42.7	47.6	11.1

Analytical Procedures

Samples (10-20 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 and 10 mL volumetric pipet was used to sample exposure water. All samples were immediately acidified to 1% (v/v) with concentrated nitric acid (Baker Instra Analyzed Lot No. 340040). Potassium chloride solution (95 g $KCl \cdot L^{-1}$) was added (2.0 $mL \cdot 100\ mL^{-1}$ sample) as suggested in method 202.1, Methods for Chemical Analysis of Water and Wastewater (U.S. EPA, 1979).

Aluminum was analyzed by atomic absorption spectrophotometry with direct aspiration of the sample into a fuel-rich nitrous oxide-acetylene flame. The instrument was an Instrumentation Laboratories Video 12 Atomic Absorption Spectrophotometer, with a 5.0 cm slot burner head, hollow cathode lamp for Al at 309.3 nm, and a slit width of 0.32 nm. A scale expansion factor of 2 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. The minimum detectable limit was 0.3 $mg \cdot L^{-1}$.

All standards were prepared from a 1000 mg $Al \cdot L^{-1}$ stock solution (Baker Instra-Analyzed, 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 $7.3 \pm 0.1\ mg \cdot L^{-1}$, and the analytical concentration was 7.2 $mg \cdot L^{-1}$ (N=1).

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. Mean recovery of spiked samples was 117.8 ± 7.0% (N=5) and the mean percent agreement was 94.4 ± 6.6% (N=9). Concentrations of Al reported herein have been corrected for recovery.

RESULTS

Aluminum exposure concentrations were nominally established from a 0.5 dilution beginning with a high exposure concentration of 25 $mg \cdot L^{-1}$ Al. Concentrations were less than nominal values in the planaria test and in the daphnid exposure jars. However, the concentrations in the daphnid beakers within which the modified exposure jars were suspended remained at or slightly above nominal values. Neither test was aerated. Aluminum concentrations of 3.12 $mg \cdot L^{-1}$ or greater allowed the formation of aluminum floc which precipitated forming a mat on the beaker or container bottoms.

Brown Planaria Exposures

Exposure concentrations averaged 0.3, 1.5, 4.1, 8.7, and 16.6 $mg \cdot L^{-1}$ (quadruplicate exposures). Aluminum concentrations in the control tanks were less than detectable limits (0.3 $mg \cdot L^{-1}$). Hydrogen ion concentration (pH) was measured in all exposure tanks at the initiation of the study (0 h). The arithmetic mean pH of the exposure tanks was 7.48 ± 0.3 (N=6).

No organisms died or showed signs of stress during the 48 h exposure to aluminum. The highest exposure concentration (16.6 $mg \cdot L^{-1}$) did not adversely affect brown planaria.

Daphnia magna Exposures

Daphnids (<24 h old) were exposed to five concentrations of aluminum in quadruplicate (1.53, 3.24, 7.22, 14.53, and 25.3 $mg \cdot L^{-1}$) plus controls (<0.3 $mg \cdot L^{-1}$). The arithmetic mean of test solution pH was 7.61 ± 0.11 (N=12). Aluminum concentrations were lower in the screen bottomed exposure jars (average of 11.1% of the exposure tank concentration). This was due to the absence of aluminum floc in the exposure chambers. Some daphnids were affected at all concentrations except the lowest exposure concentration (1.53 $mg \cdot L^{-1}$). One

daphnid (5%) was affected in the control tank. The 3.24 $mg \cdot L^{-1}$ Al exposure had 5% of the organisms affected, 7.22 $mg \cdot L^{-1}$ Al had 15%, 14.53 $mg \cdot L^{-1}$ Al had 50%, and the 25.3 $mg \cdot L^{-1}$ Al had 25% of the organisms affected. An EC_{50} was not derived due to the lack of monotonically increasing effects.