ACUTE TOXICITY OF SILVER TO SELECTED FRESHWATER INVERTEBRATES

by

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INTRODUCTION

Silver is used in jewelry, silverware, coins, electroplating, and photographic processes. It is a waste product in the manufacture of these products and processes. Also, metal mining and milling processes result in silver entering natural waters.

Silver is one of the most toxic metals to freshwater organisms. Davies et al. (1978) reported a maximum acceptable toxicant concentration (MATC) for silver and rainbow trout (Salmo gairdneri) to be 0.09-0.17 $\mu g \cdot L^{-1}$. Lima et al. (1982) reported silver 96 h LC_{50}'s for the flagfish (Jordanella floridae), fathead minnow (Pimephales promelas), and amphipod (Gammarus pseudolimnaeus) at 9.2, 10.7, and 4.5 $\mu g \cdot L^{-1}$, respectively. They also reported a 48 h LC_{50} for midge larvae (Tanytarsus dissimilis) at 3,160 $\mu g \cdot L^{-1}$.

This study reports the results of static acute exposures to silver with a hydroid (Hydra sp.), a leech (Nephelopsis obscura) and a mayfly nymph (Leptophlebia sp.).

METHODS

Analytical Technique

Samples were collected at 48 h intervals using a glass-teflon syringe (Manostat Varipet), to transfer 90 mL of exposure water into a borosilicate beaker. The samples were acidified to 0.5% HNO_3 (v/v) (Baker Instra-Analyzed).

Within two hr of collection, a portion of the acidified sample was filtered using 25 mm 0.45 µm filters (Millipore, type HA) precleaned in dilute nitric acid (1-3% HNO_3), a plastic Swinnex 25 mm filter support without gaskets, and a 30 mL glass-teflon syringe. The filter-support assembly was conditioned with 30 mL of acidified sample and successive aliquots of sample were collected in plastic bottle (Nalgene LDPE). Analyses for silver on both the unfiltered, acidified portion (acid exchangeable) and the acidified and filtered (acid-soluble) portion (U.S. EPA 1985), were completed within 4 h of filtration. A comparison of acid exchangeable and total recoverable metal (U.S. EPA 1975) was made on sample aliquots, and results showed 103.4 ± 6.5% agreement (n=3) between the two forms.

An analytical stock solution (1010 $mg \cdot L^{-1}$) of silver was prepared by dissolving silver nitrate ($AgNO_3$, Fisher, ACS, lot 772279) in deionized water. The stock solution was acidified to 1.0% HNO_3 (v/v) (Baker Instra-Analyzed). The exact purity of the lot was unavailable from the manufacturer; however, a lot analysis indicated that less than 0.005% of the common contaminated anions and cations were present. No corrections for purity have been applied to the analytical standards. Analytical standards were prepared daily from the 1010 $mg \cdot L^{-1}$ stock solution. All analytical standard solutions were 0.5% (v/v) HNO_3 (Baker Instra-Analyzed).

Flame Analysis

An Instrumentation Laboratory (IL) Video 12 atomic absorption spectrophotometer was used to analyze samples containing greater than 20 $\mu g \cdot L^{-1}$ silver. The light source was an IL Visimax II silver hollow cathode lamp. The spectral bandwidth was set at 1.0 nm, at a wavelength of 328.1 nm. A lean air/acetylene flame with a path length of 10 cm, auto integration, double beam mode, scale expansion, and deuterium arc background correction were employed. The minimum detectable concentration was 10 $\mu g \cdot L^{-1}$.

The absence of matrix interference was verified by standard additions. The mean agreement between the concentration from the calibration curve and standard additions was 93.0% (n=2).

At least 10% of the samples were analyzed in duplicate and 10% were spiked with a known concentration of silver. The mean agreement of duplicate analyses was 96.7 ± 3.3% (n=10); and the mean spike recovery was 99.1 ± 4.1% (n=12). EPA reference sample, WP 681 ICAP3, diluted to yield a "true value" of 80 $\mu g \cdot L^{-1}$, was determined to contain 82 ± 2.7 $\mu g \cdot L^{-1}$ (n=10).

Graphite Furnace

Samples of less than 20 $\mu g \cdot L^{-1}$ were analyzed using a graphite furnace technique. Analysis was completed on either the IL Video 12 system or a Perkin-Elmer (PE) 306 system. The conditions for the PE system included: Model 306 atomic absorption spectrophotometer equipped with the HGA 2100 graphite furnace, deuterium arc background correction, pyrolytically coated graphite, 2 second analyte signal integration and manual injection of 20 μL or 50 μL. A Jarrell-Ash multielement lamp supplied the 328.1 nm silver line. The spectral bandwidth used was 0.7 nm. The detection limit was 0.5 $\mu g \cdot L^{-1}$ for 20 μL injections and 0.3 $\mu g \cdot L^{-1}$ for 50 μL injections.

Analytical conditions using the IL Video 12 system were: IL Video 12 atomic absorption spectrophotometer equipped with Constant Temperature Furance (CTF) model 655, Fastac II autosampler, pyrolytically coated graphite, deuterium arc background correction, 3 second analyte signal integration and aerosol depositions of 10 seconds. The light source was an IL Visimax II silver hollow cathode lamp at a wavelength of 328.1 nm and a spectral bandwidth of 0.5 nm. The detection limit was 0.2 $\mu g \cdot L^{-1}$ for a 10 second deposit.

EPA reference sample WP681 ICAP #3 which was diluted to yield a "true value" of 0.010 $mg \cdot L^{-1}$ was determined to contain 0.010 ± 0.0006 $mg \cdot L^{-1}$ (n=7). In addition to the reference sample, approximately 10% of the samples were analyzed in duplicate and 10% were spiked with known concentrations of silver. The mean percent agreement of duplicate samples was 92.1 ± 4.3% (n=10) and the mean percent recovery of the spikes was 101.9 ± 7.0% (n=10). All exposure concentrations were corrected for the mean spike recovery derived from the analytical data for the individual biological tests.

To minimize losses of silver to the glass surfaces, all exposure chambers were preconditioned at or above the nominal concentrations of the test. Even with preconditioning, some loss with time occurred with the acid exchangeable and acid soluble silver concentrations. A trend of loss of concentration as a function of time and an increasing percentage loss with increasing concentration was evident.

Exposure Conditions

The water supply for organism acclimation and testing was the municipal water supply for the City of Superior, WI. The water was dechlorinated by charcoal filtration and sodium sulfite addition. Water temperature was controlled using a constant temperature water both for leech and mayfly tests and a controlled temperature room for hydra tests.

All acute toxicity tests were conducted with duplicate controls and exposure treatments except for the hydra test which had quadruplicate exposure concentrations. All tests consisted of at least five toxicant treatments except the leech test which had three. The exposure concentrations differed from one another by a dilution factor of 0.5.

Test chambers were of varying sizes. The leech test was conducted in 30 x 30 x 30 cm chambers containing 16 L of solution. The mayfly test was conducted in 1 liter beakers containing 1 L of solution plus two leaf discs (*Betula* sp. and *Populus* sp.) 20 mm in diameter. No feeding on the leaf discs was observed. The hydra test was conducted in 100 mL beakers using 80 mL of solution. The number of organisms per test chamber consisted of five for the leech and hydra test and ten for the mayfly test.

Test chambers were conditioned for at least 72 h with the same concentrations to be tested. Test solutions were gently aerated during the leech and mayfly tests. Dissolved oxygen was measured in control, low, middle and high exposures at the initiation of each test and every 48 h thereafter (Table 1). Total hardness (EDTA), total alkalinity and pH were measured at least once during each test on control low, middle and high exposures (Table 1) in accordance with Standard Methods (APHA 1980). Temperatures were recorded daily with mean temperatures calculated (Table 1). Dissolved oxygen (mean percent saturation) ranged from 86.5 to 106.0% for the three tests; mean total (EDTA) hardness ranged from 43.5 to 49.7 $mg \cdot L^{-1}$ as $CaCO_3$; mean total alkalinity ranged from 39.6 to 45.2 $mg \cdot L^{-1}$ as $CaCO_3$; and mean pH from 6.92 to 7.19.

Loading requirements were met as stated by ASTM (1980) except for the leech test where loading was 0.91 $g \cdot L^{-1}$ (recommended maximum is 0.8 $g \cdot L^{-1}$). Tests were begun by placing the test organisms in the exposure chambers within 30 min after the toxicant was added except for the hydra tests in which the animals were allowed to acclimate to the test chambers for 24 h before adding toxicant.

The criteria for death were lack of movement, absence of respiratory movement and lack of reaction to gentle prodding. Because death was not readily determined for hydra, an EC_{50} was determined. The hydra EC_{50} was based on whether the foot was attached or not attached to the bottom or walls of the test chambers. This was determined by application of a gentle suction with an eyedropper to each animal. Any animals that were dislodged by the suction or which were already free-floating were considered affected. Exposures were checked every 24 h for death and/or effect.

TABLE 1. Test Water Characteristics for Static Acute Toxicity Tests with Silver. Values are Means ± Standard Deviations, Ranges (in parentheses), and Sample Sizes (m)

Species	Dissolved Oxygen %	Total (EDTA) Hardness ($mg \cdot L^{-1}$ as $CaCO_3$)	Total Alkalinity ($mg \cdot L^{-1}$ as $CaCO_3$)	pH	Conductivity (mhos/cm)	Temperature ^oC
Hydra (*Hydra* sp.)	101.8±5.7 (93.6-108.8) n=8	49.7 - n=1	42.0 - n=1	7.13±0.69 (6.48-7.89) n=8	110 - n=1	24.8±0.6 (23.8-25.4) n=12
Leech (*Nephelopsis obscura*)	105.97±8.5 (90.5-115.5) n=9	48.2±0.4 (47.8-48.6) n=3	39.6±0.4 (39.2-40.0) n=3	69.1±1.1 (6.85-6.98) n=3	96.1±1.1 (95.04-97.2) n=3	16.9±0.8 (15.7-18.3) n=40
Mayfly (*Leptophlebia* sp.)	86.5±4.9 (80.2-94.2) n=12	43.5±5.6 (35.5-48.7) n=8	45.2±6.7 (41.4-58.0) n=8	7.19±0.04 (7.12-7.24) n=8	108±9 (97.2-118.8) n=4	12.4±0.5 (11.8-13.4) n=60

Test Organisms

Test organisms were acquired from a variety of sources. Mayfly nymphs were collected from Chase's Brook, Douglas County, WI; leeches were obtained from a private pond near Superior, WI; and hydra were obtained from the Lemberger Co., Oshkosh, WI. Recommended procedures for care handling and acclimation of test organisms were followed (ASTM 1980). The organisms were acclimated to the test water and exposure temperature for a minimum of 96 h prior to test initiation.

Statistical Treatment of Data

LC_{50}'s and EC_{50}'s and their 95% confidence limits were calculated by the Trimmed Spearman-Karber method (Hamilton et al., 1977). No control organisms died or appeared stressed in any of the tests. No test had less than 100% dead or affected organisms in the highest exposure.

RESULTS

Two methods were used to analyze the silver concentrations - acid exchangeable and acid soluble techniques. The results of both methods are reported and LC_{50} and EC_{50} values are calculated using the results of both methods. Acid exchangeable silver should represent the total silver in the exposures and acid soluble silver should be a better indicator of the biologically available silver.

Adult hydra were exposed to five concentrations (<0.5 ± 0.0, 3.5 ± 2.1, 7.5 ± 2.5, 17.0 ± 4.2, 37.0 ± 5.6, and 73.5 ± 2.1 $\mu g \cdot L^{-1}$ for acid exchangeable; <0.5 ± 0.0, 3.2 ± 1.7, 5.7 ± 3.6, 15.0 ± 4.4, 35.0 ± 5.6, and 67.0 ± 6.3 $\mu g \cdot L^{-1}$ for acid soluble) of silver, plus a control, in quadruplicate. Affected hydra lost their ability to stay attached to the bottom or the walls of exposure chambers when gentle suction was applied. The 48 h EC_{50} estimates were 29 and 26 $\mu g \cdot L^{-1}$ for the acid exchangeable and acid soluble forms, respectively (Table 2). Ten percent of the control organisms were affected.

Adult leeches (mean length, 59 ± 11 mm; mean weight 2.90 ± 0.80 g) were exposed to three concentrations (<0.5 ± 0.0, 18 ± 4, 41 ± 5 and 85 ± 4 $\mu g \cdot L^{-1}$ for acid exchangeable; <0.5 ± 0.0, 15 ± 5, 35 ± 8 and 79 ± 5 $\mu g \cdot L^{-1}$ for acid soluble of silver, plus a control, in duplicate. Affected leeches were slow to react to gentle prodding. In addition to becoming lethargic, they would lie on their backs and position themselves in the middle of the exposure chambers. Deaths began occurring within 24 h after initial exposure with all leeches dead in the highest exposure at 96 h. The 96 h LC_{50} estimates were 59 and 53 $\mu g \cdot L^{-1}$ for acid exchangeable and acid soluble silver, respectively. Confidence limits could not be determined because of a lack of partial kills (Table 2). No control organisms were affected.

Mayfly nymphs were exposed to five concentrations (<0.3 ± 0.2, 1.1 ± 0.1, 2.0 ± 0.3, 3.6 ± 1.1, 6.9 ± 2.3 and 11 ± 2.1 $\mu g \cdot L^{-1}$ for the acid exchangeable; <0.3 ± 0.0, 0.44 ± 0.10, 1.0 ± 0.4, 2.1 ± 0.8, 3.9 ± 0.6, and 6.4 ± 1.1 $\mu g \cdot L^{-1}$ for acid soluble) of silver, plus control, in duplicate, except that for the 6.9 $\mu g \cdot L^{-1}$ acid exchangeable exposure, only one chamber was used because of contamination of the other. Affected mayflies layed on their backs and only moved slightly. Deaths began occurring within 48 h after initial exposure with all mayflies dead in the highest exposure at 72 h. The 96 h LC_{50} was 3.9 and 2.2 $\mu g \cdot L^{-1}$ for acid exchangeable and acid soluble silver, respectively (Table 2). No control organisms died.

TABLE 2. LC_{50} and EC_{50} Estimates (95% Confidence Limits in Parentheses) for Acute Static Toxicity Tests with Silver

Test Species	Average Weight ± s.d. (g)	Method of Silver Concentration Determination	LC_{50} ($\mu g \cdot L^{-1}$)			EC_{50} ($\mu g \cdot L^{-1}$)		
			24 h	48 h	96 h	24 h	48 h	96 h
Hydra (*Hydra* sp.)	<0.001	Acid Exchangeable	-	-	-	34 (28-41)	29 (25-34)	-
		Acid Soluble	-	-	-	31 (26-38)	26 (22-31)	-
Leech (*Nephelopsis obscura*)	2.90 ± 0.80	Acid Exchangeable	-	-	59 1/	-	-	59 1/
		Acid Soluble	-	-	53 1/	-	-	53 1/
Mayfly (*Leptophlebia* sp.)	0.011 ± 0.003	Acid Exchangeable	-	8.7 (7.1-11)	3.9 (3.2-4.9)	11 1/	8.7 (7.1-11)	3.9 (3.2-4.9)
		Acid Soluble	-	5.0 (4.0-6.2)	2.2 (1.8-2.7)	6.4 1/	5.0 (4.0-6.2)	2.2 (1.8-2.7)

1/ Ninety-five percent confidence limits were not determined due to a lack of partial mortalities and/or effect at any of the intermediate exposures.

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