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Comparative Aqueous Toxicity of Silver Compounds: Laboratory Studies with Freshwater Species

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The largest user of silver in the world is the photographic industry, which used an estimated 186 million ounces in 1989 (The Silver Institute 1990). As with other uses of silver, the photographic industry, particularly that portion associated with the process of sensitized photographic materials, releases some silver to aquatic environments. Since ionic silver is toxic to some aquatic species (Nebeker et al. 1983), concerns have arisen regarding the environmental fate and effects of silver in aquatic systems (U.S. EPA 1980; Cooley et al. 1988).

Data regarding the comparative toxicity of silver to a variety of aquatic organisms can contribute to our understanding of species at risk, and characteristics of concern when evaluating systems *in situ* (Nehring 1976). The variety of forms of silver utilized in commerce and industry may serve to complicate development of a generic risk characterization scenario. Therefore, it is crucial to examine the bioavailability and toxicity of different compounds of silver. The primary objective of this research was to compare the relative sensitivities of five common laboratory freshwater test species to three silver compounds. Two cladoceran species *(Ceriodaphnia dubia* Richard and *Daphnia magna* Straus), an epibenthic amphipod *(Hyalella azteca* Saussure), the larval stage of a midge *(Chironomus tentans* Fabricius), and a fish (the fathead minnow, *Pimephales promelas* Rafinesque) were used in this study. These freshwater species vary in size, physiology, feeding strategy and habitat preference. The silver compounds chosen for this study, silver nitrate [$AgNO_3$], silver chloride [AgCl], and silver thiosulfate complex [$Ag(S_2O_3)_n$], have different industrial uses, solubilities, potential speciation, and presumably, toxicities.

MATERIALS AND METHODS

Organisms were cultured at the University of Mississippi Ecotoxicology Laboratory (Suede1 et al. 1995). *H. azteca* that passed through a 1.0-mm sieve but were retained by a 0.425-mm sieve (approximately 2-3 weeks old) were collected and used in tests. *C. tentans* used for testing were second instar larvae

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(10-d old). *D. magna* and *C. dubia* were 24-hr old, and fathead minnows, *P. promelas,* were 24-48-hr old at the beginning of tests. Static toxicity tests were conducted for 96 hr and 10 d at 20± 1°C with a LD 16:8 photoperiod. Five concentrations of silver salt and an untreated control were used in toxicity tests. Experiments were started by adding ten *H. azteca,* six *C. tentans,* ten *D. magna* or ten *P. promelas* to each of 4 replicate beakers. Experiments with *C. dubia* were started by adding one neonate to each of 10 replicate beakers per treatment. Aqueous tests were conducted in 250-mL borosilicate glass beakers with 200 mL of UMBFS water, except for *C. dubia* tests, which were conducted in 50-mL beakers with 40 mL of UMBFS water (Table 1). University of Mississippi Biological Field Station (UMBFS) water was used as a control, except for D. *magna* and *C. dubia* tests where adjusted UMBFS pond water was used (Table 1). Hardness and alkalinity of UMBFS water were adjusted up to a total hardness of 70 mg/L as $CaCO_3$ and alkalinity of 80 mg/L as $CaCO_3$ with the addition of 0.1 g/L $NaHC0_3$ and $CaCl_2$ per liter of water (Table 1) because Cladoceran control organisms did not reproduce well in unadjusted water. Glass beads (150-212 µm, Sigma Chemical Co., St. Louis, MO) were used as a substrate in *C. tentans* tests to allow for tube building.

Feeding regimes were as follows: *D. magna* and *C. dubia-0.5* mL and 0.05 mL, respectively, of *Selenastrum capricornutum* algae daily; *H. azteca*- four 7-mm diameter leached maple leaves *(Acer rubrum)* at test initiation; *C. tentans*- 0.1 mL of cerophyll suspension at test initiation and every other day thereafter; *P. promelas*- two drops of newly hatched *(≤* 24-hr old) *Artemia salina (San* Francisco Bay brand) per beaker daily.

Water parameters measured included dissolved oxygen, temperature, pH, conductivity, hardness, and alkalinity (APHA 1992). Dissolved oxygen concentrations did not drop below 40% of saturation in any test; therefore, aeration was not undertaken. Endpoints included survival (all species), reproduction *(C. dubia, D. magna)* measured as mean number of offspring per female, and growth *(C. tentans, P. promelas)* measured as a change in mean dry weight of organisms.

Lethal concentrations (LC_{50} values) and 95% confidence intervals (C.I.) were calculated using the trimmed Spearman-Karber method. Tests for normality and homogeneity of variance were performed using Shapiro-Wilk's test and Bartlett's tests, respectively (Gulley et al. 1989). Analysis of variance (ANOVA) and Dunnett's multiple range test were used to detect differences between control and treatment survival means, and a Bonferroni's t-test was used to detect differences between control and treatment means in terms of growth and reproduction (Gulley et al. 1989). A 5% alpha level was used in all statistical tests.

Three silver salts were evaluated: silver nitrate ($AgNO^3$), silver chloride (AgCl), and silver thiosulfate ($Ag(S_2O_3)_n$). For toxicity testing with $AgNO_3$ a stock solution was prepared using Milli-Q water and $AgNO_3$ (Kodak brand $AgNO_3$

Table 1. Water characteristics for pond water used in toxicity tests with *P. promelas, H. azteca* and *C. tentans,* and adjusted pond water used in experiments with *D. magna* and *C. dubia* exposed to $AgNO_3$, AgCl and $Ag(S_2O_3)_n$.

Parameter	Units	Pond Water	Adjusted Pond Water
Dissolved oxygen	mg/L	$\geq$ 7.5	$\geq$ 7.5
PH	NA	6.9-7.5	7.5-8.2
Hardness	mg/L as CaCO,	10-15	68-70
Alkalinity	mg/L as CaCO,	10-22	73-80
Conductivity	µmhos/cm	40-55	300-330
Suspended solids	mg/L	< 5	< 5

crystals, Cat 173-1082, K P64377-A, Rochester, NY). Appropriate volumes of the stock solution were added to filtered pond water to achieve the desired concentrations. In tests with AgCl, Milli-Q water was used to prepare a stock solution, which was diluted to obtain appropriate test concentrations (AgCl-Sigma Brand, S-2131, Lot # 92H3619). Since AgCl has a solubility of about 1.93 mg/L, aqueous testing in excess of that concentration was not practical or ecologically relevant. Because no commercial source was available, $Ag(S_2O_3)_n$ was prepared for each experiment by adding a concentrated $AgNO_3$ (Kodak CAT 173-1082, KP 64377-A, Rochester, NY) solution to a concentrated solution of $Na_2S_2O_3$ (Aldrich Chemical Co., 10102-17-7, Milwaukee, WI) following the procedures of LeBlanc et al. (1984).

Total silver concentrations were analyzed in test solutions using as appropriate flame or graphite furnace atomic absorption spectroscopy (Buck Model 200-A) (APHA 1992). Water samples were filtered (0.45 µm, Gelman) and acidified with 15 N redistilled $HN0_3$ (Aldrich Chemical Co., Milwaukee, WI) to a pH of l-2 before analysis for total dissolved silver. All results are reported as measured concentrations of silver.

RESULTS AND DISCUSSION

The cladoceran, *C. dubia,* was among the most sensitive of the test organisms with a 96-hr LC_{50} value of 0.92 µg Ag/L as $AgNO_3$ (Table 2). However, *C. dubia* was relatively insensitive to silver in aqueous exposures of AgCl and $Ag(S_20_3)_n$, with 96-hr LC_{50} values greater than 1930 and 12,000 µg Ag/L, respectively. In experiments of longer duration, *C. dubia* was equally sensitive with a 10-d LC_{50} value of 0.92 µg Ag/L as $AgN0_3$ (Table 2) and a 10-d No-Observed-Effect-Concentration (NOEC) value of 0.53 µg Ag/L. Reproduction, a sublethal endpoint for C. *dubia,* was similarly sensitive with a Lowest-Observed-Effect-Concentration (LOEC) of 1.14 µg Ag/L, and an NOEC of 0.53 µg Ag/L for 10-d exposures.

Table 2. Organism responses to silver aqueous exposures. LC_{50}s (and 95% confidence intervals) expressed in μg Ag/L.

Organism	$AgNO_3$		AgCl		$Ag(S_2O_3)_n$	
	96-hr LC_{50}	10-d LC_{50}	96-hr LC_{50}	10-d LC_{50}	96-hr LC_{50}	10-d LC_{50}
C. dubia	0.92 (0.69-1.23)	0.92 (0.69-1.23)	> 1930	> 1930	> 12000	> 12000
D. magna	1.06 (0.99-1.14)	1.06 (0.99-1.14)	> 1930	⩾ 930	> 12000	> 12000
H. azteca	6.8 (6.22-7.53)	5.8 (5.21-6.51)	> 1930	> 1930	> 12000	> 12000
C. ntans	676 (444-2033)	259 (192-371)	> 1930	> 1930	> 12000	> 12000
P. promelas	11.6 (8.1-30.8)	10.6 (7.5-25.9)	⩾ 1930	⩾ 930	> 12000	> 12000

The microcrustacean, *D. magna,* responded similarly to *C. dubia* with a 96-hr LC_{50} value of 1.06 pg Ag/L as $AgNO_3$ (Table 2). In 10-d exposures, the LC_{50} value was also 1.06 µg Ag/L (Table 2). In exposures using AgCl and $Ag(S_20_3)_n$, the 96-hr and 10-d LC_{50} values were in excess of 1930 and 12,000 µg Ag/L, respectively. It is important to note that the estimated LC_{50} values of these latter two compounds were well in excess of the solubilities of these silver compounds in water. Reproduction of *D. magna* was equally sensitive in 10-d experiments; the LOEC value was 1.22 µg Ag/L and the NOEC was 0.8 µg Ag/L as $AgNO_3$ for *D. magna* reproduction during 10-d exposures.

H. azteca is typically considered an epibenthic amphipod; however, in these experiments *H. azteca* was used in aqueous-only exposures, unconfounded by sediments. These typically benthic organisms are amenable to water-only exposures without apparent stress due to lack of substrate. The 96-hr LC_{50} value for *H. azteca* in $AgNO_3$ exposures was 6.8 µg Ag/L (Table 2). The 10-d NOEC value for survival was 4.0 µg Ag/L as $AgNO_3$. *H. azteca* was relatively insensitive to AgCl and $Ag(S_20_3)_n$ (LC_{50} values of 1930 µg/L and 12,000 µg/L, respectively) at concentrations up to aqueous solubility (Table 2).

The second instar larval stage of the midge, C. *tentans,* was used in aqueous exposures to silver with a glass-bead substrate. Of the five species, *C. tentans* was the least sensitive in silver exposures. The 96-hr LC_{50} value for *C. tentans* exposed to $AgNO_3$ in water only was 676 µg Ag/L, and for 10-d exposure was 259 µg Ag/L (Table 2). The 10-d NOEC value for *C. tentans* exposed to $AgNO_3$ was 125 µg Ag/L. In exposures of 96-hr and 10-d duration using AgCl and $Ag(S_2O_3)_n$, the LC_{50} values were in excess of 1930 and 12,000 µg Ag/L, respectively.

The fathead minnow, *P. promelas,* was less sensitive than the crustaceans, but more sensitive than *C. tentans,* with 96-hr and 10-d LC_{50} values of 11.6 and 10.6 µg Ag/L as $AgNO_3$, respectively (Table 2). In 96-hr and 10-d exposures to the other silver compounds, AgCl and $Ag(S_2O_3)_n$ *P. promelas* responded in a similar fashion as the other organisms, with LC_{50} values in excess of 1930 and 12,000 µg Ag/L, respectively.

In aqueous $AgNO_3$ experiments with five species of commonly used toxicity test organisms, the microcrustaceans *C. dubia* and *D. magna* were the most sensitive with LC_{50} values of 0.92 and 1.06 µg Ag/L, respectively (Table 2). *H. azteca* was intermediate in sensitivity, followed by *P. promeh* and finally *C. tentans* (Figure 1). The LC_{50} values for the microcrustaceans were not significantly different for 96 hr and 10 d of exposure. For *H. azteca* and *P. promelas* the LC_{50} values after 10 d were 1 µg Ag/L lower than the 96-hr LC_{50}. The 96-hr and 10-d LC_{50} values for *C. tentans* were significantly different (p=0.05), with a decrease in LC_{50} value from 676 to 259 µg Ag/L, respectively. The 96-hr and 10-d LC_{50} values for *H. azteca* were approximately five times greater than those for the microcrustaceans (Table 2).

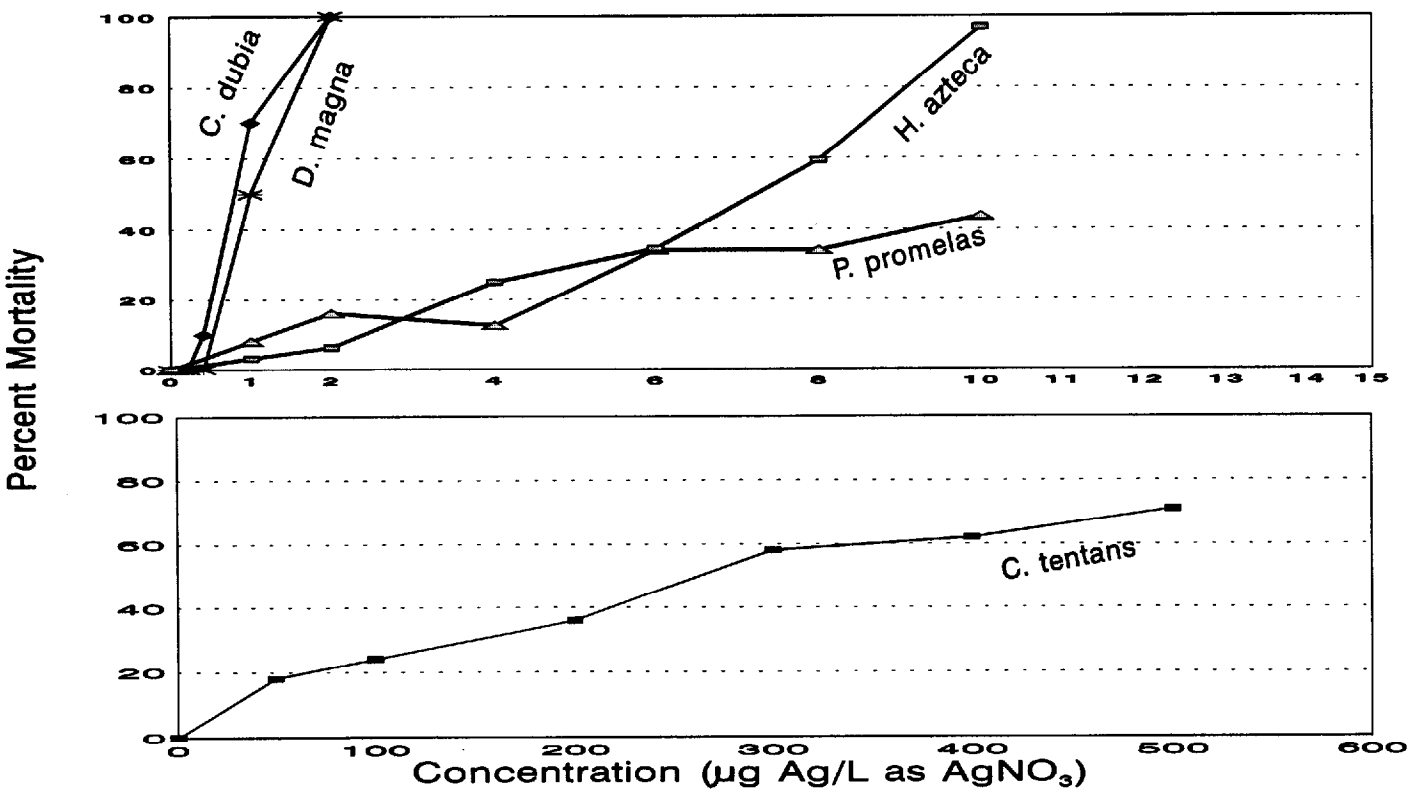

Figure 1. Silver nitrate toxicity curves for 5 species of organisms exposed for 10 days.

P. promelas had 96-hr and 10-d LC_{50} values twice that of *H. azteca* and an order of magnitude greater than those of the microcrustaceans. The LC_{50} values for *C. tentans* were at least 20 times greater than the LC_{50} values for other species tested.

Other authors have reported similar responses for organisms exposed to aqueous $AgNO_3$. Nebeker (1982) reported 48-hr and 21-d LC_{50} values for survival of *D. magna* exposed to aqueous $AgNO_3$ in similar exposure temperatures and for test animals of similar age. Estimates for 48-hr LC_{50} values ranged from 0.6 to 55.0 µg Ag/L as water hardness varied from "relatively soft" to "relatively hard". Unfortunately, hardness and other water characteristics that may influence silver toxicity were not quantitatively specified (Nebeker et al. 1983). Hardness in the present study ranged from 68-70 mg/L as $CaCO_3$ for *D. magna* and *C. dubia* to 10-15 mg/L as $CaCO_3$ for the other test species. The 21-d LC_{50}, values for *D. magna* survival reported by Nebeker et al. (1983) were 2.9-3.7 µg Ag/L, and 21-d NOECs for reproduction were 1.6-20.0 µg/L. The 21-d LC_{50} and NOEC values reported by Nebeker et al. (1983) were similar to *D. magna* data from this study (10-d LC_{50} values of 1.06 µg Ag/L and 10-d NOEC values for reproduction of 0.8 µg Ag/L) with $AgNO_3$.

Lima et al. (1982) exposed fathead minnows to $AgNO_3$ in water with a hardness of 44-48 mg/L as $CaCO_3$ (alkalinity 43 mg/L as $CaCO_3$, pH 7.4) at 25°C and reported 96-hr LC_{50} values of 10.7 µg Ag/L (95% C.I. 10.7-10.8 µg Ag/L). LeBlanc et al. (1984) reported a 96-hr LC_{50} value for fathead minnows exposed to $AgNO_3$ as 16 µg Ag/L (95% C.I. 12-20 µg Ag/L) in water of similar characteristics (36 mg/L as $CaCO_3$ hardness, 38 mg/L as $CaCO_3$ alkalinity, pH 7.2). These values for fathead minnows bracket the 96-hr LC_{50} of 11.6 µg Ag/L obtained in this study.

This study includes toxicity data for three aquatic species *(C. dubia, H. uzteca,* and *C. tentans)* that were not reported previously in the water quality criteria document for silver (U.S. EPA 1987). The water quality criteria document for silver (U.S. EPA 1987) is also based primarily on results of organism exposures to $AgNO_3$. This study includes toxicity data for five species of organisms exposed to silver compounds (e.g., AgCl and $Ag(S_2O_3)_n$) other than $AgNO_3$, and illustrates that there are important differences in exposure to various silver salts.

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