THE RELATIVE IMPORTANCE OF WATER HARDNESS AND CHLORIDE LEVELS IN MODIFYING THE ACUTE TOXICITY OF SILVER TO RAINBOW TROUT (ONCORHYNCHUS MYKISS)

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Abstract—Static-renewal 7-d toxicity tests for silver nitrate (AgNO₃) were performed with juvenile rainbow trout (Oncorhynchus mykiss Walbaum). The relative influences of calcium and chloride concentrations on median lethal time (LT50) were assessed. Calcium concentrations were controlled by adding either Ca(NO₃)₂ or CaSO₄, whereas chloride concentrations were adjusted with either NaCl or KCl. For both calcium salts, a 100-fold elevation in concentration increased the LT50 approximately 10-fold. However, a 100-fold elevation in KCl ameliorated silver (Ag) toxicity at least 100-fold, while NaCl protected against Ag toxicity even more substantially, demonstrating the much greater protective effect of chloride relative to calcium. In a separate series of bioassays, fish were exposed to 0.92 μM Ag (100 μg/L as AgNO₃) with varying amounts of NaCl titrated into each tank to alter the free [Ag⁺]. The 7-d LC50 occurred at a [NaCl] of 2.500 μM. Using MINEQL⁺ (a geochemical speciation program), the predicted free [Ag⁺] at this LC50 value is 0.0285 μM. Further bioassays were performed in which [chloride] was maintained at either 50 or 225 μM, while total [Ag] was independently varied from 0.0092 to 0.0694 μM (1.0–7.5 μg/L). The 7-d LC50 value was calculated at 0.0294 μM Ag (3.18 μg/L) at a chloride concentration of 50 μM, very similar to the free [Ag⁺] value of 0.031 μM calculated from an earlier LC50 test at a fixed [chloride] of 730 μM. According to MINEQL⁺, the estimated [Ag⁺] at this LC50 value is 0.0289 μM. Although a 7-d LC50 value could not be determined at 225 μM chloride, it was estimated at slightly above 0.0277 μM Ag⁺. Elevating chloride concentrations from 50 to 225 μM did not alter the accumulation of Ag in the liver. In addition, there were no significant differences in hepatic Ag accumulation between any of the Ag-exposed fish, irrespective of the total Ag concentration used during the exposure. Overall, Ag accumulated to approximately 185 μmol/kg wet weight in all Ag-exposed groups (approximately a 10-fold increase above controls). These results, together with a reanalysis of published data, suggest that Ag toxicity can be correlated with the free Ag ion [Ag⁺], and that any factors altering Ag⁺ availability (i.e., chloride) will be expected to modify acute Ag toxicity.

Keywords—Silver nitrate Rainbow trout LC50 Chloride Hardness

INTRODUCTION

Current water quality criteria established by the U.S. Environmental Protection Agency have assigned a critical role for water hardness (primarily as Ca) in the guidelines designed to protect aquatic life from acute silver (Ag) toxicity [1]. In accordance with this view, the EPA has quantitatively incorporated water hardness into the following equation, which is used by regulatory agencies for enforcing maximum total recoverable silver in water

Max. total recov. Ag (μg/L) = \( e^{0.72[\text{hardness}] - 6.52} \)

where hardness is expressed in units of mg/L of CaCO₃ equivalents. The primary impetus behind the derivation of this equation appears to have come from data tabulated by Lemke [2], all of which were derived from interlaboratory studies of AgNO₃ toxicity in daphnids, fathead minnows, and rainbow trout. In recent years however, there has been an increasing amount of evidence suggesting that in some cases this equation fails to adequately assess the impact of Ag in the aquatic environment. More specifically, it has been proposed that the ambient water quality criteria are overly conservative when applied to waters of low hardness, while underprotective at elevated hardness levels [3].

Physiological studies observing the effects of AgNO₃ exposure in rainbow trout [4] suggest that the toxic mechanism involves a large inhibition of active Na⁺ and Cl⁻ uptake at the gills. This is further supported by radioisotopic flux studies from Morgan et al. [5], which correlate a decrease in \(^{22}\text{Na}⁺\) uptake with a concurrent inhibition in activity of the branchial transport enzymes Na⁺/K⁺ ATPase and carbonic anhydrase following AgNO₃ exposure. Overall, these transport processes are expected to be influenced only mildly by water hardness. Wood et al. [4] also reported that acute AgNO₃ exposure had no influence on \(^{45}\text{Ca}²⁺\) uptake. These results are consistent with other studies that have shown that most metals exert toxic effects on either Na⁺ [4,6] or Ca²⁺ uptake mechanisms [7,8], but not both. Furthermore, Davies et al. [9] found only a two-fold difference in the 4-d 50% lethal concentration (LC50) for AgNO₃ in trout, when water hardness was varied between 26 and 350 mg/L. Based on the EPA equation, this variation in hardness should have produced a >80-fold difference in the LC50 values. Goettl and Davies [10] observed only negligible effects of water hardness in other fish species exposed to AgNO₃. Even though these data were available when the above equation was derived, they were not used for its formulation due to their lack of correlation with other existing data.

Although it has been suggested that chloride may modify Ag toxicity to some degree [1], extensive correlative studies have not been performed. However, reanalysis of Lemke’s tabulated data suggests that chloride rather than calcium may be...
the true protective agent against Ag toxicity [11]. It is believed that chloride is able to influence toxicity by complexing with Ag⁺, forming species such as Ag(Cl)₂⁻ or cerargyrite [12], both of which have been shown to be far less toxic than Ag⁺, at least on an acute basis [11,13]. Data presented in this manuscript and other citations [14] question the scientific validity or appropriateness of the EPA hardness equation for Ag.

The objectives of this study were to evaluate the relative roles of calcium and chloride as modifiers of AgNO₃ toxicity. In addition, computer-based geochemical speciation modeling with MINEQL⁺ [12] was used in the design of several acute toxicity bioassays to test the assumption that Ag⁺ is the predominately toxic form of the metal. Finally, MINEQL⁺ was employed for the reanalysis of Lemke’s tabulated data to assess further the validity of the hardness equation [1].

MATERIALS AND METHODS

Test organisms

Juvenile rainbow trout (Oncorhynchus mykiss Walbaum; 5.17 ± 0.16 g, n = 600) were obtained from a local hatchery (Rainbow Springs Hatchery, Thamesford, ON, Canada) and placed in a 300-L fiberglass tank (Living Stream, Toledo, OH, USA). Ion-depleted water (not deionized) ([calcium] ~ 15; [chloride] ~ 13; [potassium] ~ 23; [sodium] ~ 13 µM) was produced from Hamilton, Ontario, Canada dechlorinated tap-water by reverse osmosis (Culligan MD-1000). Water was then delivered to a head tank before being fed to the holding tank at a rate of 0.75 L/min. A stock solution containing 16,100 µM NaCl (BDH, Toronto, ON, Canada) and 15,000 µM Ca(NO₃)₂·4H₂O (BDH, analytical-grade salts) was pumped into the water at a rate of 1.75 ml/min. Salts were added at the head tank and well mixed before delivery to the holding tank. Fish were acclimated to this synthetic soft water ([Na⁺] = 50.0, [Cl⁻] = 50.0, [Ca²⁺] = 50.0 µM, 15.5 ± 0.9°C) for at least 2 weeks prior to experimentation. Fish were fed dry trout pellets (Ziegler’s Bros., Gardners, PA, USA) to satiation daily. The particle-reactive nature of Ag required that fish feeding be discontinued 2 d prior to the start of experimentation and during the bioassays. This ensured that the measured concentrations of Ag in the water were maintained near nominal levels.

Experimental design

For each series of bioassays, five treatments plus a simultaneous control were performed in duplicate. Ten fish per replicate (20 per treatment) were tested in each bioassay.

Experimental protocol

All bioassays were performed in 60-L covered tanks. Each was filled with 50 L of synthetic soft water (as described above) and provided with gentle aeration. Bioassays were static renewal with 80% (40 L) of this water renewed daily. Water samples were taken daily, both before and after each water replacement for analysis of total and dissolved silver and total chloride and calcium. Ten fish were randomly placed in each tank approximately 30 min prior to the start of experiments, at which time the appropriate amount of AgNO₃ stock was added to each tank. Henceforth, fish mortality was monitored continuously over the first 4 h, after which observations were made three times daily for a total of 7 d. Cessation of opercular movement and lack of movement during gentle prodding verified mortality. Dead fish were immediately removed and their weights and lengths recorded.

Bioassay for AgNO₃ (effects of NaCl and Ca(NO₃)₂)  

The relative roles of calcium and chloride in ameliorating AgNO₃ toxicity were tested at various water qualities. Eleven tanks were used in this study; nine were spiked with 100 ml of an AgNO₃ stock solution (460.0 µM; 78.7 mg/L) (Fisher Scientific, Phillipsburg, NJ, USA; analytical grade) to give a final total [Ag] of 0.92 µM. Chloride (as NaCl) was added to four of these tanks yielding chloride concentrations of 100.0, 500.0, 1,000.0, and 5,000.0 µM. In four other tanks, Ca (as Ca(NO₃)₂) was independently varied to yield the same concentrations (100.0, 500.0, 1,000.0, 5,000.0 µM). Ag (0.92 µM) was also added to a tank containing only synthetic soft water (50.0 µM of chloride and Ca). Two control tanks containing no AgNO₃ and either 5,000.0 µM of chloride or Ca were tested. The bioassay was performed over 7 d as outlined in the experimental protocol section.

Bioassay for AgNO₃ (effects of KCl and CaSO₄)

To verify that the ameliorating effects on AgNO₃ toxicity could be solely attributed to Ca and chloride, irrespective of their counterions used, the same experiment was performed using CaSO₄ (Sigma Chemical, St. Louis, MO, USA) and KCl (BDH) salts. As before, Ca and chloride concentrations were independently varied from 50.0 to 5,000.0 µM. AgNO₃ was added to each of these nine tanks to a final concentration of 0.92 µM Ag. Two control tanks were employed with calcium and chloride concentrations independently maintained at 5,000.0 µM. Fish mortality was observed over a 7-d period.

AgNO₃ LC₅₀ at varying [chloride]

Geochemical speciation modeling with MINEQL⁺ [12] was used to estimate the [Ag⁺], [AgCl]⁺, and cerargyrite formed at each of the Ag concentrations used in an LC₅₀ test for AgNO₃ on juvenile rainbow trout, recently performed in our laboratory [11]. The test had yielded a 7-d LC₅₀ value of 0.0842 µM Ag (9.1 µg/L; as AgNO₃) in Hamilton city water ([Na⁺] = 600.0, [Cl⁻] = 730.0; [Ca²⁺] = 1,900.0, [HCO₃⁻] = 1,900.0 µM; pH 7.9–8.2). According to MINEQL⁺, an ionic Ag⁺ concentration of 0.031 µM Ag⁺ would be expected at this total [Ag] in Hamilton water. With this information in mind, two separate series of bioassays were designed. In the first, AgNO₃ was added to give a total [Ag] of 0.92 µM, while the [chloride] was manipulated appropriately to give free Ag⁺ concentrations, bracketing the 0.031 µM 7-d LC₅₀ value. The concentrations of free Ag⁺ tested were 0.0190, 0.0255, 0.0319, 0.0451, and 0.0712 µM. These concentrations were obtained by altering the chloride concentrations to 3,750, 2,800, 2,230, 1,560, and 1,000 µM, respectively, using NaCl. Except for variations in Na⁺ and Cl⁻ concentration, the ionic composition of the bioassay water was identical to that of the synthetic soft water used previously.

In the second series, the [chloride] was maintained at either 50 or 225 µM, while concentrations of total Ag (i.e., AgNO₃) were varied to give [Ag⁺] surrounding the 7-d LC₅₀ value. The total Ag concentrations tested were 0.0092, 0.0167, 0.0296, 0.0518, and 0.0694 µM (1.0, 1.8, 3.2, 5.6, and 7.5 µg/L). These yielded nominal free Ag⁺ concentrations of 0.0084, 0.0150, 0.0267, 0.0468, 0.0626 µM (at 50 µM chloride), and 0.0062, 0.0111, 0.0198, 0.0346, and 0.0463 µM (at 225 µM chloride) in the two water qualities, respectively. These concentrations used a logarithmic scale to adhere to general procedures for performing bioassays, as outlined by Sprague [15].
Analysis of Ag in fish liver

Livers from the survivors of the second LC50 series (50 and 225 µM chloride series) were removed after completion of the 7-d bioassay. Ag analysis was performed on control, 0.0167, and 0.0518 µM Ag-exposed fish at 50 µM chloride and 0.0167 and 0.0694 µM Ag-exposed fish at 225 µM chloride. Livers were individually homogenized in 1.0 ml of 50 mM Tris-HCl (pH 8.0) (Sigma Chemical), at 0°C in a glass tube fitted with a Teflon®-tipped pestle (Thomas Scientific, Philadelphia, PA, USA). A subsample of each homogenate (400 µl) was withdrawn and transferred to previously acid-washed test tubes. Homogenates were digested for 2 h with five volumes of 70% HNO3 at 120°C. Samples were then allowed to cool to room temperature, after which 0.75 volumes of H2O2 was added. Digests were evaporated to dryness without allowing the digested residue to become ashed. Five milliliters of 0.5% HNO3 was added to each tube and analyzed out allowing the digested residue to become ashed. Five milliliters of 0.5% HNO3 was added to each tube and analyzed for Ag using atomic absorption spectroscopy.

Water and tissue Ag analyses

All water samples were immediately acidified with 0.5% (v/v) of HNO3 (J.T. Baker, Phillipsburg, NJ, USA; trace metal grade). Both water and tissue samples were analyzed for Ag by atomic absorption spectroscopy, using a graphite furnace (Varian GTA-95) for Ag concentrations less than 0.23 µM (25 µg/L) or by flame atomic absorption (AA) (Varian AA 1275) for samples having a concentration greater than 0.23 µM Ag. The graphite furnace operation was set at the following temperature ramping profile: injected at ambient temperature, ambient temperature to 75°C over 5 s, 75 to 90°C over 12 s, 90 to 120°C over 30 s, with atomization occurring at 2,000°C. The graphite tube was flushed with ultrapure N2 gas between samples to eliminate contamination. An automated sample injector was used to dispense 10-µl samples. Both graphite furnace and flame AA analyses were performed at a wavelength of 328.1 nm and band slit width of 0.5 nm [16].

Nonfiltered water samples were used for analysis of total Ag. Water samples were also immediately passed through a 0.45-µm filter (Gelman Sciences, Ann Arbor, MI, USA) for measurement of dissolved [Ag]. Water chloride concentrations were measured in unfiltered samples using the mercuric thiocyanate colorimetric assay [17]. Prior to calcium analysis by flame AA, LaCl3 was added to a final dilution of 0.2% to eliminate Na+ interference.

Statistical methods

Log-probit analysis of mortality plots was employed to determine LT50 values for each exposure [15]. Calculation of 95% confidence limits was performed by the nomographic methods of Litchfield and Wilcoxon [18]. Regression lines were fitted through the probit-versus-dose curves using the method of least squares (Biosoft, Ferguson, MO, USA). LC50 calculations were based on measured total Ag concentrations (e.g., Table 1).

RESULTS

Exposure to 0.92 µM Ag (as AgNO3) was most toxic when water chloride concentrations were low. When both NaCl and Ca(NO3)2 concentrations were maintained at 50 µM, the LT50 was 42 min (95% confidence limits: 34.1–51.7 min) (Fig. 1). The LT50 increased to 6,600 (3,882–11,220) min at an [NaCl] of 1,000 µM ([Ca] unchanged). An LT50 value at 5,000 µM chloride could not be determined because less than 50% mortality was observed by the end of the 7-d. Manipulation of water [Ca] had much less overall effect. Independently varying the [Ca] (using Ca[NO3]2) from 50 to 5,000 µM increased the LT50 approximately 10-fold from 42 (34.1–51.7) min to 470 (395.0–559.3) min (Fig. 1).

Bioassays using KCl and CaSO4 to independently vary [chloride] and [Ca] confirmed that increasing chloride levels decreased Ag toxicity more so than increasing [Ca]. When both [chloride] and [Ca] were maintained at 50 µM the LT50 was 68 (49.3–93.8) min. At a [chloride] of 5,000 µM, the LT50 increased over 100-fold to 7,100 (6,484–7,774) min. Independently varying the [Ca] to 5,000 µM increased only moderately the LT50 value to 540 (461–632) min (Fig. 2), a change of less than 10-fold.

Table 1. Measured total and dissolved silver concentrations in bioassay water

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Total [Ag] (µM)</th>
<th>Dissolved [Ag] (µM)</th>
<th>% Recovered as Ag</th>
</tr>
</thead>
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<tr>
<td>[Cl] 50 µM</td>
<td>9.2</td>
<td>13.5</td>
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<td>16.7</td>
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<td></td>
<td>51.8</td>
<td>38.5</td>
<td>73.3</td>
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<tr>
<td></td>
<td>69.4</td>
<td>52.4</td>
<td>71.4</td>
</tr>
<tr>
<td>[Cl] 225 µM</td>
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<td>11.4</td>
<td>74.8</td>
</tr>
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<td></td>
<td>16.7</td>
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<td>77.7</td>
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<td>31.6</td>
<td>67.0</td>
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<tr>
<td></td>
<td>69.4</td>
<td>41.6</td>
<td>67.5</td>
</tr>
</tbody>
</table>

*Observed [Ag] represents the arithmetic mean obtained from all water samples taken.

Dissolved [Ag] represents all Ag passing through a 0.45-µm filter.

Fig. 1. Toxicity curves for AgNO3 at varying concentrations of NaCl and Ca(NO3)2 independently varied from 50 to 5,000 µM with juvenile rainbow trout. AgNO3 concentrations maintained constant at 0.92 µM for each tank except for two controls (not plotted). No mortalities were observed in either control. The data are presented as the median lethal times (ET50) ± 95% confidence limit (n = 10) at each concentration. Note the much greater protective effect of elevating water [chloride] versus increasing [Ca] by equivalent amounts.
AgNO₃ LC₅₀ values

When the total silver concentration was maintained at 0.92 μM, while the [chloride] was varied to yield free Ag⁺ concentrations, the resulting 7-d LC₅₀ value occurred at a [chloride] of approximately 2,500 μM (Fig. 3A). The estimated LC₅₀ at the 7-d LC₅₀ value was 0.0285 μM based on speciation modeling with MINEQL⁺ (Fig. 3B).

Seven-day LC₅₀ tests at 50 and 225 μM [chloride] were also performed. At 50 μM [chloride], the LC₅₀ was calculated at 0.0294 μM, while at 225 μM [chloride] an LC₅₀ value could not be directly calculated because there was only 40% mortality at the highest [Ag] (0.0416 μM) (Table 1). The LC₅₀ values for the 50 μM chloride exposure, in terms of Ag⁺ concentration is 0.0289 μM, as determined using measured water quality parameters in MINEQL⁺, while at 225 μM [chloride] it was expected to be slightly above 0.0277 μM Ag⁺ (at [Ag] = 0.0416 μM). Silver analysis of water samples showed that as [chloride] increased the proportion of dissolved Ag decreased (Table 1). The dissolved Ag proportion (as percent recovered) also decreased as total Ag increased regardless of the chloride concentration.

Livers of fish exposed to AgNO₃ during the 7-d bioassays showed a 20-fold increase in [Ag] compared to controls (Table 2). Overall, accumulation was not affected by changes in [chloride] (50 vs 225 μM) with silver levels ranging between 174.29 to 195.61 μmol/kg. Hepatic silver concentrations of control fish were 17.52 ± 4.64 μmol/kg. In addition, there were no significant differences in silver liver burdens when AgNO₃ concentrations were varied between 0.0167 μM and 0.0694 μM.

Speciation modeling

Varying the [chloride] from 50 to 5,000 μM (as performed in initial bioassays) dramatically influenced Ag speciation, decreasing Ag⁺ content from 90% to 1% of the total [Ag] (total [Ag] = 0.92 μM), while cerargyrite and AgCl percentages increased reciprocally. This speciation effect was predominantly observed at a [chloride] ranging from 125 to 800 μM (Fig. 4A–C). In contrast, variations in water [Ca] over the range tested in the bioassay exerted only negligible effects on Ag speciation. Furthermore, the effects of chloride on Ag speciation are also dependent on the total Ag in the water. For instance, at a total [Ag] (as AgNO₃) of 0.092 μM, cerargyrite is not formed between 125 and 800 μM chloride (Fig. 4A). Instead, a greater percentage of Ag will be found as Ag(Cl)₇ species. As [AgNO₃] is increased to 0.46 and 0.92 μM Ag, cerargyrite starts to form at approximately 100 and 200 μM chloride, respectively (Fig. 4B and C).

**DISCUSSION**

Elevating the [Ca] (as Ca(NO₃)₂) 100-fold increased survival time approximately 10-fold (Fig. 1). In comparison, only a twofold increase in [chloride] (as NaCl) was required to produce the same protective effect to an acutely toxic exposure of 0.92 μM total Ag (as AgNO₃) and a similar 100-fold elevation of chloride levels increased the LT₅₀ approximately

![Figure 2](image-url)

Fig. 2. Toxicity curves with juvenile rainbow trout for AgNO₃ at varying concentrations of KCl and CaSO₄ independently varied from 50 to 5,000 μM. The total Ag level was held constant at 0.92 μM. The data are presented as the median lethal times (ET₅₀) ± 95% confidence limit (n = 10) at each concentration. Other details as in legend of Fig. 1.

![Figure 3A](image-url)

Fig. 3. (A) Toxicity curve for 7-d bioassay performed on juvenile rainbow trout at a total [Ag] of 0.92 μM at varying concentrations of chloride. Each data point represents the % survivorship (n = 10) for each concentration. Best-fit regression line determined using the method of least squares. The LC₅₀ occurred at 2,500 μM chloride. (B) Toxicity curve for 7-d bioassay performed on juvenile rainbow trout. Each data point represents the % survivorship (n = 10) at the predicted [Ag⁺]. Ionic Ag⁺ concentrations were determined for varying Cl⁻ concentrations (Fig. 3A) at a total [Ag] of 0.92 μM using MINEQL⁺ [12]. Best-fit regression line determined using the method of least squares. The predicted 7-d LC₅₀ was 0.0285 μM Ag⁺.

![Table 2](image-url)

Table 2. Hepatic silver concentrations in juvenile rainbow trout

<table>
<thead>
<tr>
<th>Nominal [Ag] (μM)</th>
<th>Hepatic Ag concentrations (μmol/kg wet weight)</th>
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<tbody>
<tr>
<td>50 μM chloride</td>
<td>17.52 ± 4.64 (n = 9)</td>
</tr>
<tr>
<td>16.7</td>
<td>195.61 ± 87.70 (n = 7)</td>
</tr>
<tr>
<td>51.8</td>
<td>176.23 ± 17.24 (n = 4)</td>
</tr>
<tr>
<td>69.4</td>
<td>193.66 ± 33.10 (n = 2)</td>
</tr>
<tr>
<td>225 μM chloride</td>
<td>na</td>
</tr>
</tbody>
</table>

* Heptic Ag concentration measured as μmol per kg wet weight.  
* Not analyzed.
Calcium and chloride effects on silver toxicity in trout

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Fig. 4. Geochemical speciation modeling using MINEQL+ to determine relative amounts of Ag⁺, Ag\(\text{Cl}\)\(_2\), and cerargyrite (as % of total Ag) at chloride concentrations ranging from 50 to 800 μM. Speciation determined at three different total silver concentrations (as AgNO₃): (A) 0.09 μM, (B) 0.47 μM, and (C) 0.93 μM. Note the decrease in [Ag⁺] as chloride concentrations increase and the lack of cerargyrite formation when total Ag is low.

100-fold. A parallel experiment to this one was performed in which [chloride] and [Ca] were manipulated using KCl and CaSO₄ salts, respectively. Once again, a 100-fold elevation in [calcium] resulted in only a 10-fold increase in LT₅₀, whereas similar increases in [chloride] had greater than a 100-fold protective effect. These results show no differences in acute Ag toxicity between either of the Ca²⁺ salts used, whereas NaCl was shown to have a slightly greater protective effect than KCl.

Wood et al. [4] have demonstrated that acute Ag exposure (as AgNO₃) resulted in a significant decrease of plasma Na⁺ and Cl⁻ concentrations in freshwater rainbow trout. This ion-regulatory disfunction was attributed to impairment of Na⁺ and Cl⁻ uptake mechanisms at the gill [5]. It seems likely that an increased [Na⁺] in the water might slightly alleviate this ionic disturbance by either competing with Ag⁺ at the gill surface or by creating an improved gradient for Na⁺ entry [13,19,20]. In contrast, acute AgNO₃ exposure did not produce any observable effects on plasma K⁺ levels [4], suggesting that the branchial uptake mechanisms of this electrolyte were unaffected. Consequently, elevating the waterborne [K⁺] would not be expected to decrease Ag toxicity to the same extent as [Na⁺] would. This is supported with results from this study that appear to indicate that chloride is somewhat more protective when introduced as NaCl as compared to KCl. In addition, no effect on either the branchial uptake rate of Ca²⁺ or on plasma Ca levels was produced in response to acute AgNO₃, exposure [4]. Consequently, [Ca] (hardness) should not greatly affect the specific sites of toxicity. Nevertheless, Ca²⁺ may help to decrease ion permeability at epithelial tight junctions due to its role as a gill membrane stabilizer. This tightening of paracellular junctions would result in reduced Na⁺ and Cl⁻ efflux rates [21], possibly helping to counteract the acute toxic effects of AgNO₃ exposure and explaining the small protective effect seen here.

Speciation modeling with MINEQL+ has suggested that Cl⁻ may exert an ameliorating role on Ag toxicity by complexing with Ag⁺, thus reducing the amount of Ag⁺ bioavailable to the organism [11]. Although increasing [chloride] reduces Ag toxicity, it does not appear to affect the rate at which Ag is accumulated in hepatic tissue. In this study, Ag levels in the liver were consistently around 185 μmol/kg wet weight (after 7 d of exposure), regardless of the [chloride] used in the study (50 or 225 μM) (Table 2). Hogstrand et al. [11] observed similar Ag levels in livers of fish exposed to Ag concentrations surrounding the 7-d LC₅₀ value (about 0.0842 μM), at a chloride concentration of 730 μM. In addition, small variations in total Ag concentrations did not appear to alter hepatic Ag accumulation levels. This suggests that Ag uptake kinetics become saturated at relatively low concentrations of Ag.

Several earlier studies have indicated that Ag toxicity is best correlated with free [Ag⁺], rather than total Ag [11,13,22,23]. In the present study, the estimated Ag⁺ concentrations at the calculated LC₅₀ values ranged between 0.0277 and 0.0289 μM. This narrow range was obtained despite LC₅₀ values (as total Ag) varying as much as 31-fold (0.0294–0.92 μM Ag) and chloride concentrations varying at least 50-fold (50–2,500 μM). In comparison, the 7-d LC₅₀ value at 730 μM chloride was 0.0842 μM Ag, translating to an [Ag⁺] of 0.0310 μM [11].

With this background in mind, Lemke’s tabulated data [2] were reanalyzed using MINEQL+ to determine the free Ag⁺ concentrations that should correspond to each of the reported 4-d LC₅₀ values for fathead minnows and rainbow trout, based on the given ionic composition of the test water. Although LC₅₀ values varied substantially (from 0.0361 μM to 2.590 μM total Ag), speciation analyses with MINEQL+ using water chemistry data reported by Lemke [2] gave [Ag⁺] estimates from 0.025 to 0.314 μM, with the majority of values below 0.100 μM (Fig. 5). The relatively close agreement in Ag⁺ concentrations between these data and our own 7-d LC₅₀ re-
sults further support the view that toxicity is best correlated with [Ag⁺].

Free ionic Ag⁺ concentrations ranging from 0.0009 to 1.85 nM have been measured in natural water samples using potentiometric techniques [24]. In all cases, the highest waterborne Ag⁺ concentrations were observed in water bodies receiving industrial discharges. Thus, despite having Ag concentrations as high as 0.35 μM in surface waters of highly polluted sites [25], complexation processes are expected to reduce Ag⁺ concentrations to well below acutely toxic concentrations [14,26]. When LC50 values (as total Ag) are low, Cl⁻ likely ameliorates Ag toxicity by complexing with Ag⁺ to form Ag(Cl)₂ (Fig. 4a). At high LC50 values it is probably cerargyrite formation that is the driving force behind the amelioration of Ag toxicity in response to elevated chloride levels (Fig. 4b and c). Consequently, the present regulatory framework based on the hardness-equation [1] fails to assess correctly the true toxicity of Ag species in the natural environment. Clearly more research is needed on the exact geochemical requirements of future Water Quality Criteria for Ag.

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