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A RELATIONSHIP BETWEEN GILL SILVER ACCUMULATION AND ACUTE SILVER TOXICITY IN THE FRESHWATER RAINBOW TROUT: SUPPORT FOR THE ACUTE SILVER BIOTIC LIGAND MODEL

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Abstract—Rainbow trout were exposed to a range of silver concentrations (as $AgNO_3$) in flowing synthetic soft water (0.05 mM Na^+, 0.05 mM Cl^-, 0.05 mM Ca^{2+}, 0.02 mM Mg^{2+}, 0.02 mM K^+, pH 7.0, approximately 0.7 mg C/L dissolved organic carbon, 10 mg $CaCO_3$/L, 10 ± 2°C) to investigate a possible relationship between short-term gill silver accumulation (3 h or 24 h) and acute silver toxicity (96-h mortality). We also investigated potential relationships between gill silver accumulation and inhibition of Na^+ uptake plus inhibition of gill Na^+K^+–adenosine triphosphatase (ATPase) activity. The 96-h median lethal concentration (LC50) values were 13.3 μg total Ag L^{-1} and 3.3 μg dissolved Ag L^{-1}. A relationship was demonstrated between 3-h and 24-h gill silver accumulation and 96-h mortality. A relationship also was demonstrated between gill silver accumulation and inhibition of Na^+ uptake at 24 h of exposure. No relationship between gill silver accumulation and inhibition of gill Na^+K^+–ATPase activity was found. The 96-h median lethal gill accumulation (LA50) values of 129 (at 3 h) and 191 ng g^{-1} (at 24 h) and a conditional equilibrium binding constant of 8.0 for Ag^+ binding to the gills were calculated. These observations support use of the silver biotic ligand model (BLM) as a regulatory tool to predict acute silver toxicity.

Keywords—Sodium uptake Adenosine triphosphatase activity Water quality criteria Regulations Metal

INTRODUCTION

When present as the free silver ion (Ag^+), silver is one of the most acutely toxic metals to freshwater rainbow trout with 96-h median lethal concentration (LC50) values in the range of 6.5 to 13 μg L^{-1} [1–3]. The Ag^+ noncompetitively inhibits active Na^+ and Cl^- uptake at the gills by inhibiting the gill enzymes Na^+K^+–ATPase and carbonic anhydrase [4], leading to a decline in blood plasma ion levels, circulatory collapse, and death of the fish [5,6]. Complexing ligands such as Cl^-, dissolved organic carbon and sulfide plus competing cations such as Na^+ and H^+, as well as the water hardness cations Ca^{2+} and Mg^{2+}, decrease toxicity by reducing the bioavailability of Ag^+ to toxic sites (i.e., Na^+K^+–ATPase and carbonic anhydrase molecules) at the gills [7].

The U.S. Environmental Protection Agency's current water quality criteria for silver corrects for hardness only [8], although the ameliorating effect of water hardness against silver toxicity is modest, at most, compared to other water chemistry parameters [9–12]. As such, water quality criteria may be over- or under-protective at a particular freshwater site, depending on the water chemistry at that site. Site-specific modifications that take into account other water chemistry parameters can be made but these modifications involve the generation of water-effect ratios, a process that is time-consuming and costly [13].

The biotic ligand model (BLM) [14–16] is a simple and inexpensive alternative to the generation of water-effect ratios for modification of water quality criteria. The BLM uses conditional equilibrium binding constants experimentally determined for the cationic metal (i.e., Ag^+) and for protective cations (e.g., Na^+, Ca^{2+}, H^+, Mg^{2+}) binding to the gills (considered a negatively charged ligand) of reference species such as the rainbow trout, together with the known chemistry of the water at a particular site [17]. These values are inserted into an aquatic chemical equilibrium computer program to estimate metal binding at the gills. The model assumes that the amount of metal binding to the gills in a short time period is proportional to eventual mortality. Complexation by ligands and competition by various cations that reduces Ag^+ binding to the gills [18–20] is considered within the context of the model. Specifically, the BLM predicts the 96-h LC50 of silver (i.e., acute toxicity) in the water quality of interest on the basis of the short-term (3–24 h) gill silver accumulation that is associated with 50% mortality (LA50) at 96 h. The dissolved silver concentration at the LA50 is the predicted LC50 value at that site [reviewed in 14,17].

In practice, there has been difficulty in relating an experimentally determined gill silver load (i.e., LA50) with 96-h toxicity for several reasons. First, the level of silver accumulation on the gills of trout appeared to fluctuate over time during constant exposure conditions [21–23]. Second, several studies [10,24] indicated no relationship between gill silver burden at fixed time points and toxic physiological effects. Thus, there may not be a unique gill silver accumulation associated with a given acute effect. Finally, silver accumulation on the gills has never been experimentally correlated with acute silver toxicity in terms of mortality. As a result, silver versions of the BLM have, to date, used surrogates for gill silver burden. The toxicological BLM of Paquin et al. [15] was calibrated directly to mortality data using assumed gill burdens, while the physiological BLM of McGeer et al. [16] used Na^+K^+–ATPase inhibition (a physiological endpoint associated with mortality) rather than gill silver burden.

These observations suggested that gill silver accumulation may not be an appropriate endpoint for predicting acute silver

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toxicity. For this reason, the Science Advisory Board of the U.S. Environmental Protection Agency only conditionally approved the toxicological silver BLM [15] as a regulatory tool, and recommended additional validation and verification [25]. However, based on a recent study [22] we now suspect that the unstable pattern of silver accumulation on the gills seen in earlier studies was an artifact of changing silver bioavailability in closed system exposures, and that under flow-through conditions, gill silver burden increases steadily to a plateau over time. Furthermore, we have demonstrated a relationship between short-term gill silver accumulation and inhibition of Na^+ uptake [22,26], providing evidence, albeit indirect, of a relationship between short-term gill silver accumulation and acute toxicity. Furthermore, it now appears very likely that the neutral complex $AgCl_{aq}$ can enter gills [23] without causing toxicity [7,10,24], so studies that attempt to link silver burden with effect at varying Cl^- levels may not show clear-cut relationships.

Therefore, the objective of the present study was to evaluate under flow-through conditions at constant, low Cl^- concentration, whether there is a relationship between short-term silver accumulation on the gills (3 h or 24 h) and 96-h mortality. If such a relationship exists, it would provide direct, experimental support for the BLM approach for silver. To accomplish this objective, rainbow trout were exposed to a series of water silver concentrations and were monitored for gill silver accumulation at 3 and 24 h and mortality at 96 h. We also investigated possible relationships between gill silver accumulation and inhibition of Na^+ uptake and inhibition of gill Na^+K^+–ATPase activity, as physiological endpoints that may predict silver toxicity.

MATERIALS AND METHODS

Experimental animals and acclimation

Juvenile rainbow trout (*Oncorhynchus mykiss*) were obtained from Humber Springs Trout Hatchery (Orangeville, ON, Canada) and held for two weeks in a 200 L polyethylene tank supplied with flowing, aerated, dechlorinated Hamilton city tap water (ON, Canada) (~ionic composition in mM: 0.5 [Na^+], 0.7 [Cl^-], 1.0 [Ca^{2+}], 0.2 [Mg^{2+}], and 0.05 [K^+]; pH 7.8–8.0; dissolved organic carbon approximately 3 mg C/L; hardness approximately 140 mg/L as $CaCO_3$; temperature 9°C). Fish were then acclimated over a four-week period to synthetic soft water that was generated by reverse osmosis of Hamilton city dechlorinated tap water (Culligan Aqua-Cleer Reverse Osmosis System, Toronto, ON, Canada). Over the first two weeks, the ratio of soft water to tap water that supplied the tank was increased until the desired ionic composition was achieved (~ionic composition in mM: 0.05 [Na^+], 0.05 [Cl^-], 0.05 [Ca^{2+}], 0.02 [Mg^{2+}], and 0.02 [K^+]; pH 7.0; dissolved organic carbon approximately 0.7 mg C/L; sulfide undetectable [<5 nM] [27]; hardness approximately 10 mg/L as $CaCO_3$; temperature 10 ± 2°C). Fish were maintained in this soft water for at least an additional two weeks before experiments were started. During initial holding and soft water acclimation, fish were fed to satiation once daily with commercial trout pellets (Martin Mills, Tavistock, ON, Canada). Feeding was suspended for 1 d before and during the experiments to minimize silver binding to organic matter in uneaten food and waste products during the exposures.

Experimental design

Fish (mean wt 6.3 g) were exposed to nominal silver concentrations of 0, 5, 20, 25, 30, and 40 μg of total silver/L and monitored for gill silver accumulation, whole body Na^+ uptake, gill Na^+K^+–ATPase activity, and mortality. One silver concentration was tested in each of six separate flow-through experiments. Each experiment was performed once.

For each experiment, synthetic soft water (composition as above) was delivered to a primary header tank that overflowed into two separate secondary header tanks, all of which were vigorously aerated. Silver (as $AgNO_3$, Sigma-Aldrich, Oakville, ON, Canada) was added to these two secondary header tanks by a peristaltic pump from a single, light-shielded stock bottle. Each secondary header tank directed soft water at a rate of 130 ml/min to an 80-L polyethylene exposure tank (two exposure tanks in total for each experiment, tank A and tank B) that contained 32 fish in 20 L of aerated, synthetic soft water (i.e., identical loading density in each tank). Each exposure tank had the same concentration of silver. The overflow from each exposure tank drained to waste. The walls of the exposure tanks were pre-equilibrated with $AgNO_3$ at the exposure concentration for at least 24 h to ensure saturation of silver binding sites on the tank walls. The same tanks were used for each experiment.

Fish from exposure tank A were used for all physiological measurements. At 3 h and 24 h, eight fish were sampled for gill silver accumulation and whole body Na^+ uptake via ^{24}Na. An additional eight fish from the same tank were sampled at each time point for gill Na^+K^+–ATPase activity. Sampled fish were washed in a silver-free solution containing NaCl (2.9 g/L) to remove any loosely bound silver and/or radioisotope and were euthanized by an overdose of tricaine methane sulphonate (1 g/L). In the case of trout used for silver accumulation and Na^+ uptake measurements, the gills were then immediately dissected from the fish and the gills and carcass counted separately for ^{24}Na radioactivity (and the counts per min [cpm] summed) to determine whole body Na^+ uptake. The gills were then analyzed for gill silver accumulation. In the case of trout used for measurement of Na^+K^+–ATPase activity, the gills were immediately dissected, frozen in liquid nitrogen, and stored at −80°C for later analysis.

Fish from exposure tank B were monitored for mortality over 96 h. The criterion used for mortality was cessation of opercular movement. Dead fish were removed continuously over the exposure. At 1, 3, 24, 48, 72, and 96 h of exposure, a 5-ml nonfiltered water sample (for determination of total silver) and a 5-ml filtered water sample (for determination of dissolved silver, Acrodisc polyethersulfone 0.45 micron syringe filters, Pall Gelman Laboratory, Ann Arbor, MI, USA) were taken from each exposure tank and acidified with 1% HNO_3 (trace-metal grade, Fisher Scientific, Toronto, ON, Canada) for analysis of total and dissolved silver concentrations by graphite furnace atomic absorption spectrophotometry (see below, Varian AA-220, GTA 110, Varian, Mississauga, ON, Canada).

Our experimental design allowed us to correlate possible predictive and physiological measurements (e.g., gill silver burden, Na^+ uptake, and gill Na^+K^+–ATPase activity) at 3 h and 24 h, sampled from tank A with eventual mortality over 96 h in an identical, simultaneous exposure in tank B, served with a common source of exposure water at an identical flow rate. Had we sampled for predictive and physiological measure-

ments and also determined mortality in a common tank, then the mortality data at 96 h would have been biased by the removal of potentially more or less tolerant fish at 3 h and 24 h.

Gill silver accumulation

Once gills had been counted for ^{24}Na activity (see below), gills were weighed and acidified with five times the sample weight of 1 N HNO_3 (trace-metal grade, Fisher Scientific). Acidified samples were digested in an oven over a 24-h period at approximately 60°C, vortexed, and allowed to settle. A portion of the supernatant was diluted with distilled, deionized water and analyzed for total silver concentration by graphite furnace atomic absorption spectrophotometry (Varian AA-220, GTA 110) using a certified standard (Environmental Standard, Inorganic Ventures, Toronto, ON, Canada). Water samples were similarly analyzed.

Na^+ uptake measurements

The Na^+ uptake measurements lasted a total of 30 min, so 30 min before each sample time, eight fish were removed from the exposure tank and placed in a 600-ml Pyrex glass beaker containing continuously aerated water to which the fish had been exposed. The beaker was pre-equilibrated in the silver exposure water to ensure saturation of silver binding sites on the beaker walls and a stable silver concentration over the uptake measurement. For Na^+ uptake measurements conducted under control conditions, fish were placed in a beaker that contained silver-free acclimation water but was not pre-equilibrated in the silver exposure water.

Sodium uptake was determined by the addition of ^{24}Na to the beaker (6.7 μCi, mean specific activity 0.003 μCi/μg Na^+, McMaster University Nuclear Reactor, Hamilton, ON, Canada). A water sample was taken 5 min after the addition of the isotope and again at the end of the measurement for determination of radioactivity by gamma counting (MINAXI Auto-Gamma 5000 gamma counter, Canberra-Packard, Toronto, ON, Canada, corrected for decay to a common reference time) and total Na^+ concentration by flame atomic absorption spectrophotometry (Varian AA-220). At the end of the measurement the fish were sampled as outlined above and then the gills and body were counted separately for radioactivity (as above), and the counts were combined. The whole body Na^+ uptake was calculated from the formula

$$\text{whole body } Na^+ \text{ uptake} = CT/(SA\,w\,t)$$

where CT is the total cpm in the tissue, SA is the measured mean specific activity of the water, w is the wet weight (g) and t is the time of exposure (h). The mean specific activity of the water was calculated as follows:

$$\text{specific activity} = [(cpm_i/[Na^+]_i) + (cpm_f/[Na^+]_f)]/2$$

where cpm_i represents the cpm per ml initially in the water, cpm_f represents the final cpm per ml in the water, and $[Na^+]_i$ and $[Na^+]_f$ represent the initial and final Na^+ concentrations of the water, respectively. The specific activity of sodium in the water decreased, on average by 30% over the course of each measurement.

Gill Na^+K^+–ATPase activity

The gills frozen for determination of Na^+K^+–ATPase activity were kept on ice throughout sample preparation and the assay. Gills were homogenized in 500 μl of SEID buffer (0.5 g of sodium deoxycholate in 100 ml of SEI; SEI = 150 mM sucrose, 10 mM ethylenediaminetetraactic acid, 50 mM imidazole, pH 7.3) using a Teflon®–glass homogenizer, and then the homogenates were assayed for Na^+K^+–ATPase activity using the microplate method of McCormick [28]. Activity was standardized to protein content, measured using the Bradford assay (kit B6916, Sigma, Oakville, ON, Canada) with bovine serum albumin as a protein standard (Sigma).

Statistical analyses

Unless otherwise noted, data have been expressed as means ± standard error of the mean (SEM), n. Linear regression analysis was used to investigate the relationship between gill silver accumulation and mortality, whole body Na^+ uptake, and gill Na^+K^+–ATPase activity, as well as the water total and dissolved silver concentration. Linear regression analyses were done using SPSS® 10 (Chicago, IL, USA) for Windows (Microsoft, Redmond, WA) and the significance of Pearson's correlation coefficient (r) assessed. Prior to regression analyses, percentage data (% mortality, % inhibition of whole body Na^+ uptake, and % inhibition of Na^+K^+–ATPase activity) were transformed using the arcsin square root transformation. The LC50 values and corresponding confidence limits were calculated using the trimmed Spearman–Karber method. The LA50 values were calculated using the equation of the regression line for the 96-h mortality logit versus the 3-h and 24-h log gill silver accumulation, where the mortality logit = log10(M/(1 − M)) and M = the mortality proportion, as outlined by MacRae et al. [29].

RESULTS AND DISCUSSION

96-h LC50 values

The calculated 96-h LC50 value expressed as total silver was 13.3 (95% confidence limits: 10.8–16.3) μg Ag L^{-1} and expressed as dissolved silver was 3.3 (95% confidence limits: 2.6–4.1) μg Ag L^{-1}. The 96-h LC50 value based on ionic silver, as calculated by MINEQL+ [30] was 1.4 (95% confidence limits: 0.86–2.28) μg L^{-1}. The 96-h LC50 value based on total silver calculated in this experiment is approximately two-fold higher than that reported by Davies et al. (6.5 μg/L, [1]) and Nebeker et al. (8.6 μg/L, [3]) in water of similar hardness (26–29 mg $CaCO_3$ L^{-1}). However, the concentration of inorganic ligands (i.e., dissolved organic carbon, sulfide, Cl^-) may have been higher in our experimental water, leading to an increase in the 96-h LC50 value. The 96-h LC50 value based on ionic silver calculated in this study is lower than the 96-h LC50 values determined by Bury et al. (5 μg/L, [11]) and Galvez and Wood (3 μg/L, [9]). The difference in values likely reflects the difference in test conditions between the studies. Our study was performed under flow-through conditions while the studies of Bury et al. [11] and Galvez and Wood [9] were static tests. Static test conditions have been found to reduce acute silver toxicity [31].

Gill silver accumulation and acute silver toxicity

Strong positive relationships existed between silver accumulation on the gills at 3 h and 96-h mortality (Fig. 1A, $r = 0.90$, $p < 0.05$), as well as between silver accumulation on the gills at 24 h and 96-h mortality (Fig. 1B, $r = 0.82$, $p < 0.05$). These data provide the first direct experimental evidence of a relationship between silver accumulation on the gills and acute silver toxicity. The slightly stronger relationship at 3 h than at 24 h may reflect the difference in exposure time because by 24 h some silver may have become associated with phys-

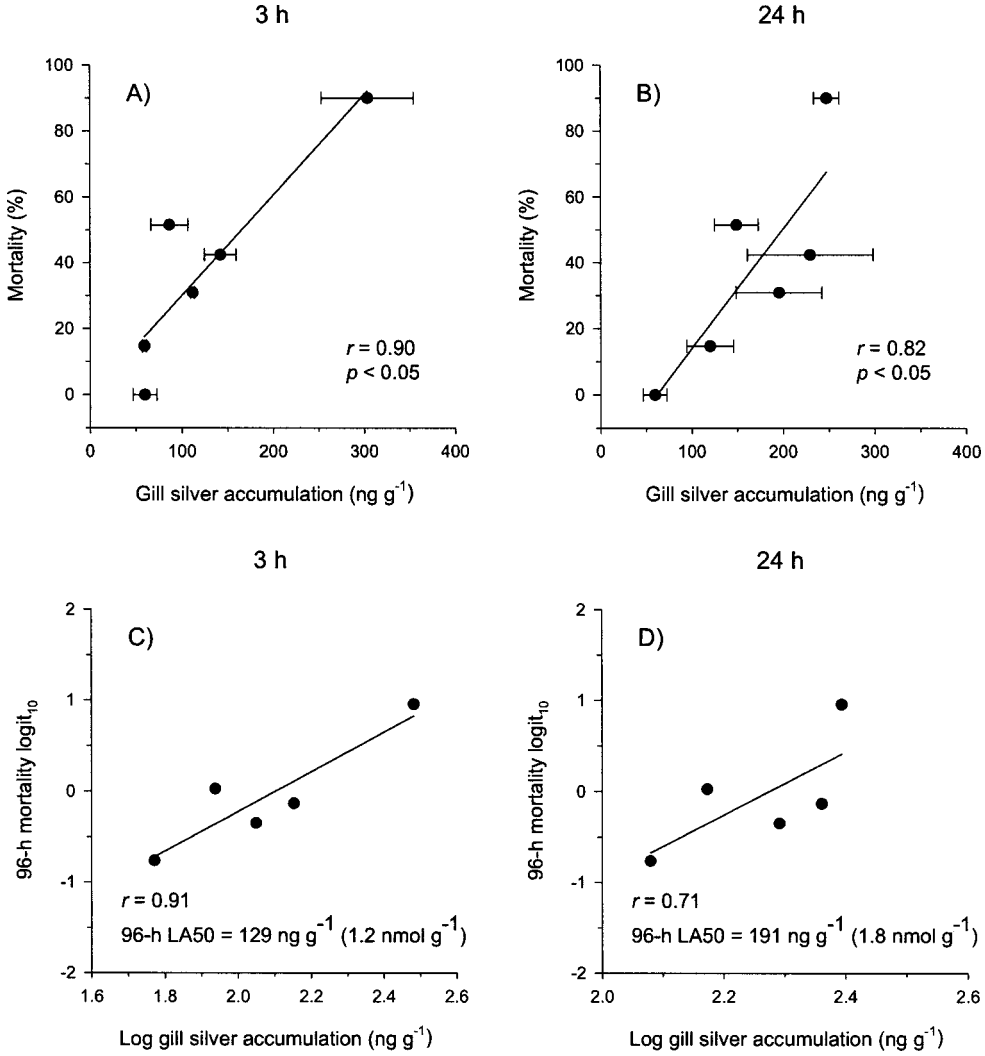

Fig. 1. The relationship between percent mortality of rainbow trout over 96 h and silver accumulation on the gills (**A–B**) and the 96-h mortality logit as a function of log gill silver accumulation (**C–D**) determined at 3 h and 24 h of exposure to $AgNO_3$ in synthetic soft water. Values are means ± 1 standard error of the mean (n = 6–8). For **A** and **B** lines indicate linear regressions with $r = 0.90$, $p < 0.05$ and $r = 0.82$, $p < 0.05$, respectively. For **C** and **D** regression of the plots yields straight lines from which 96-h median lethal accumulation (LA50) values can be calculated. The regression equations are (**C**) log10(M/(1 − M)) = log10 gill silver accumulation·2.17 − 4.57, $r = 0.91$, and (**D**) log10(M/(1 − M)) = log10 gill silver accumulation·3.45 − 7.85, $r = 0.71$, yielding LA50 values of 129 ng g^{-1} (1.2 nmol g^{-1}) and 191 ng g^{-1} (1.8 nmol g^{-1}), respectively.

iologically inert sites at the gills. Notably, Janes and Playle [18] and Schwartz and Playle [19] used 3-h exposures to determine the original gill-binding constants that have been the basis in setting up both silver BLMs [15,16]. The demonstration of a relationship between short-term gill accumulation and 96-h mortality lends support to the use of gill silver accumulation to predict acute toxicity in the silver BLM.

Regression of the 96-h mortality logit versus the 3-h or 24-h log gill silver concentration yields a straight line from which 96-h LA50 (median lethal gill accumulation) values for silver can be calculated (Fig. 1C, D). The 96-h LA50 values, calculated using the 3-h or 24-h gill silver concentrations were 129 ng g^{-1} (1.2 nmol g^{-1}) and 191 ng g^{-1} (1.8 nmol g^{-1}), respectively. These values are approximately 10-fold lower than the LA50 value utilized in the current toxicological version of the silver BLM of 1834 ng g^{-1} (17 nmol g^{-1}) [15] and approximately five-fold lower than the LA50 value used in the physiological BLM of 702 ng g^{-1} (6.5 nmol g^{-1}) [16].

Gill silver accumulation and physiological toxicity

No significant relationship was found between gill silver accumulation and inhibition of whole body Na^+ uptake after 3 h of silver exposure ($r = 0.55$, $p > 0.05$, Fig. 2A). This is likely because by just 3 h of exposure the silver that was accumulating at the gills had reached the cytosolic carbonic anhydrase but had not yet had enough time for penetration to the basolateral Na^+K^+–ATPase. In fact, evidence suggests that it can take anywhere from 5 to 24 h for the inhibitory effect of silver on Na^+K^+–ATPase to develop fully [22,26]. The time needed for silver penetration to the basolateral membrane could also explain the lack of a significant relationship between gill silver accumulation and gill Na^+K^+–ATPase inhibition after 3 h of exposure ($r = 0.43$, $p > 0.05$, Table 1).

In contrast, however, there was a significant relationship between 24-h gill silver accumulation and inhibition of whole body Na^+ uptake ($r = 0.86$, $p < 0.05$, Fig. 2B). By 24 h, silver accumulation at the gills reached a plateau under flow-through

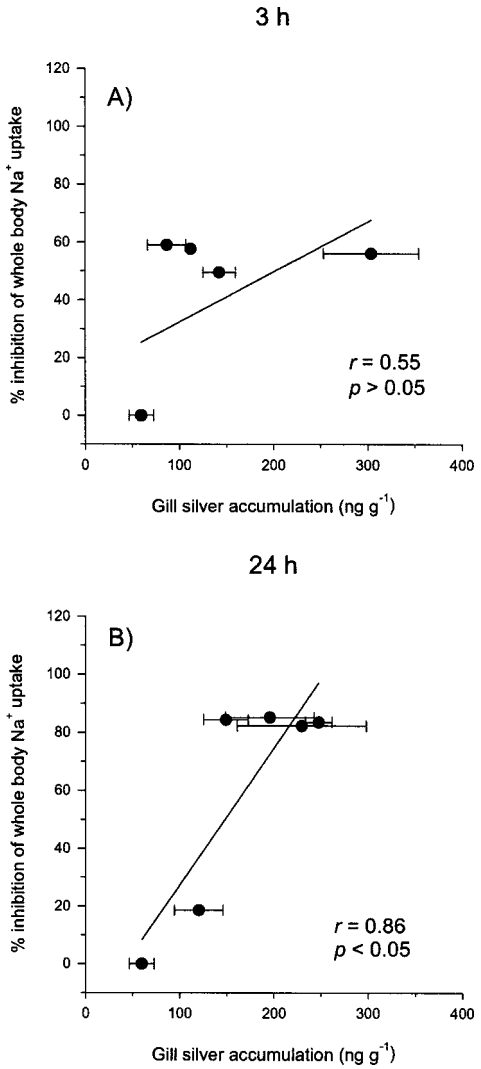

Fig. 2. The relationship between percent inhibition of whole body Na^+ uptake and silver accumulation on the gills of rainbow trout after (**A**) 3 h and (**B**) 24 h of exposure to $AgNO_3$ in synthetic soft water. Values are means ± 1 standard error of the mean (n = 6–8). Lines indicate linear regressions with (**A**) $r = 0.55$, $p > 0.05$ and (**B**) $r = 0.86$, $p < 0.05$.

Table 1. Gill silver accumulation and inhibition of gill Na^+K^+–adenosine triphosphatase (ATPase) activity after 3 h and 24 h of exposure of rainbow trout to $AgNO_3$ in synthetic soft water. Values are means ± 1 standard error of the mean (n = 6–8). There was no significant relationship between gill silver accumulation and inhibition of gill Na^+K^+–ATPase activity after 3 h of exposure ($r = 0.43$; $p > 0.05$) nor after 24 h of exposure ($r = 0.50$; $p > 0.05$). ND = no data

3 h		24 h	
Gill silver accumulation (ng g^{-1})	Inhibition of gill Na^+K^+–ATPase activity (%)	Gill silver accumulation (ng g^{-1})	Inhibition of gill Na^+K^+–ATPase activity (%)
59.09 ± 2.12	0	59.56 ± 13.02	0
59.56 ± 13.02	0	120.19 ± 25.67	10.81
86.68 ± 20.27	28.24	149.00 ± 23.81	36.10
112.02 ± 2.15	27.63	195.70 ± 46.99	32.77
142.26 ± 17.41	0	229.70 ± 68.57	12.07
303.88 ± 50.46	25.34	247.73 ± 13.90	ND

conditions and silver had fully inhibited both carbonic anhydrase and Na^+K^+–ATPase [26], likely because silver equilibrated fully to the basolateral membrane. This result is not surprising in light of other recent studies demonstrating a relationship of gill silver accumulation and inhibition of Na^+ uptake over the same time frame [10,22,26].

It is puzzling, however, that there was not a significant relationship between gill silver burden and Na^+K^+–ATPase inhibition at 24 h ($r = 0.50$, $p > 0.05$, Table 1). However, as noted earlier, some silver may have become associated with physiologically inert sites at the gills by 24 h, explaining the lack of correlation with Na^+K^+–ATPase inhibition. Similarly, McGeer and Wood [24] were unable to demonstrate a significant correlation between gill silver concentration and disruption of Na^+ balance at 48 h. Again, time may be a factor such that the longer-term silver exposure of McGeer and Wood [24] may have masked the relationship by allowing enough time for silver binding to physiologically inert sites on the gills. Regardless, the observed relationship between 24-h gill silver accumulation and the actual toxic mechanism of action of silver (i.e., inhibition of Na^+ uptake) illustrates that gill silver accumulation is a good indicator of the physiological impact of silver and lends additional support to the BLM approach of predicting acute silver toxicity from gill silver accumulation.

Water silver concentration and gill silver accumulation

A strong positive relationship was found between the water total silver concentration averaged over the 96-h exposure period and 24-h silver accumulation on the gills ($r = 0.81$, $p < 0.05$, Fig. 3A). Similarly, a strong positive relationship also existed between the water-dissolved silver concentration averaged over 96-h and 24-h silver accumulation on the gills ($r = 0.75$, $p < 0.05$, Fig. 3B). However, there were no significant relationships between average total or dissolved water silver concentrations over 96-h and 3-h silver accumulation on the gills ($r = 0.69$, $p > 0.05$; $r = 0.35$, $p > 0.05$; Table 2). The dissolved water silver concentration is believed to better represent the bioavailable fraction of silver than the total silver concentration, and is now used in the application of U.S. Environmental Protection Agency water quality criteria [32].

In essence we have demonstrated a relationship between the water bioavailable silver concentration and gill silver accumulation over 24 h. Because a fraction of the bioavailable silver concentration represents Ag^+, this relationship is consistent with the BLM, which assumes that accumulation of silver occurs at the gills as the result of Ag^+ binding and that accumulation will increase as the Ag^+ concentration increases for a specific water chemistry [14,17].

The relationship between the water-dissolved silver concentration and silver accumulation on the gills after 24 h of exposure (Fig. 3B) and the 96-h LA50 value determined from the gill silver accumulation after 24 h (Fig. 1D) can be used to calculate a conditional equilibrium binding constant (K) for Ag^+ binding to the gills of rainbow trout. First, the water-dissolved silver concentration at the 96-h LA50 value (191 ng g^{-1}) was determined using the equation of the regression line of Figure 1D (dissolved [Ag] = 4.26 μg L^{-1}). Second, the ionic silver concentration at the LA50 value was calculated using the aquatic chemistry program MINEQL+ (Ver 4.0) [30] based on the known chemistry of the water ([Na^+] 0.05 mM, [Cl^-] 0.05 mM, [Ca^{2+}] 0.05 mM, [Mg^{2+}] 0.02mM, [K^+] 0.02 mM, [dissolved organic carbon] 0.7 mg C/L, sulfide undetectable) and appropriate binding constants from Janes and

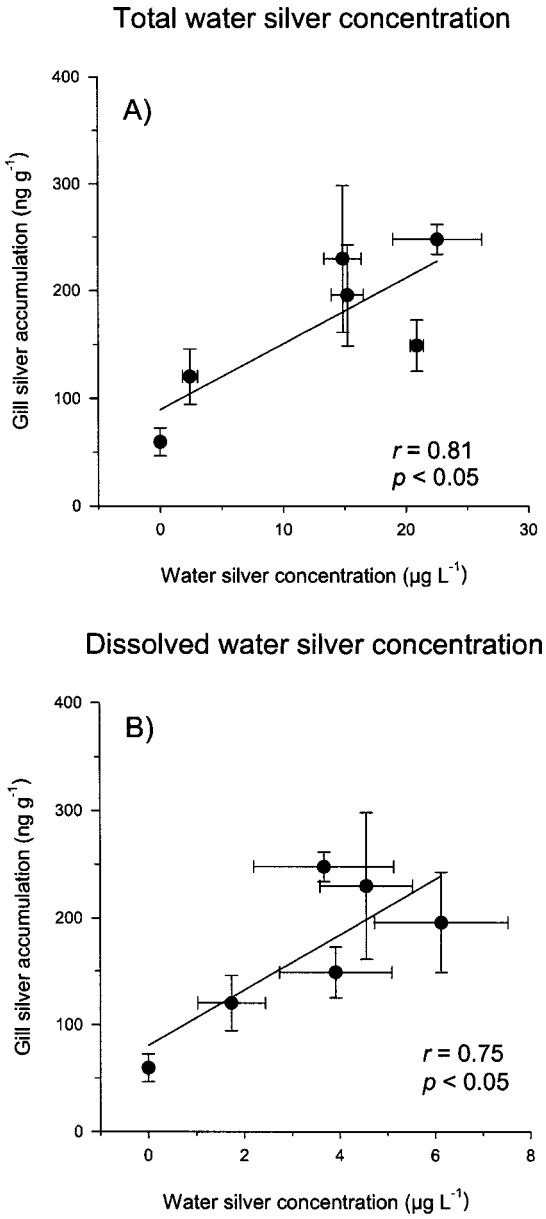

Fig. 3. Rainbow trout 24-h gill silver accumulation as a function of the (**A**) total and (**B**) dissolved water silver concentration averaged over the 96-h exposure period. Values are means ± 1 standard error of the mean (n = 6–8). Lines indicate linear regressions with (**A**) r = 0.81, $p < 0.05$ and (**B**) r = 0.75, $p < 0.05$.

Playle [18]. The water ionic silver concentration ([Ag^+] = 1.02 μg L^{-1}) was converted to a conditional equilibrium binding constant by taking the negative logarithm of the molar value. The log conditional equilibrium binding constant determined by this procedure (log $K_{Ag\text{-}gill}$ = 8.0) is approximately five-fold higher than the log conditional equilibrium binding constant used in the current toxicological version of the BLM (log $K_{Ag\text{-}gill}$ = 7.3) [15], but is closer to the value used in the physiological version of the BLM (log $K_{Ag\text{-}gill}$ = 7.6) [16] for rainbow trout. Inasmuch as silver is three- to five-fold more toxic than copper to trout [1,33], it makes sense that the log $K_{Ag\text{-}gill}$ should be three- to five-fold higher than the log $K_{Cu\text{-}gill}$, which is 7.4 in the current copper BLM [14,34].

For accurate prediction of acute silver toxicity, the binding constant determined in the present study may be more appropriate for use in the silver BLM as this value was determined experimentally and is a directly measured toxicological value associated with short-term (24 h) gill silver load (LA50) at the 96-h LC50 value. In contrast, the binding constant used in the current toxicological silver BLM was determined by fitting the BLM to silver toxicity data [15] and thus represents a toxicity-based binding constant without direct gill measurements. Use of the current toxicological BLM binding constant may underestimate the amount of Ag^+ bound to the gill, leading to an overestimation of the LC50 value. However, it should be noted that the high LA50 value of Paquin et al. [15] may compensate for the low log K in their model, providing a model that can still accurately predict acute silver toxicity.

Table 2. Total and dissolved water silver concentrations (averaged over 96 h) and 3 h-gill silver accumulation during exposure of rainbow trout to $AgNO_3$ in synthetic soft water. Values are means ± 1 standard error of the mean (n = 6 for water silver concentration data and n = 6–8 for gill silver accumulation data). There were no significant relationships between total or dissolved water silver concentrations over 96-h and 3-h gill silver accumulation (r = 0.69; $p > 0.05$ and r = 0.35; $p > 0.05$, respectively)

3 h gill silver accumulation (ng g^{-1})	Water silver concentration (μg L^{-1})	
	Total	Dissolved
59.09 ± 2.12	2.43 ± 0.62	1.73 ± 0.71
59.56 ± 13.02	0	0
86.68 ± 20.27	20.91 ± 0.54	3.91 ± 1.18
112.02 ± 2.15	15.25 ± 1.32	6.13 ± 1.40
142.26 ± 17.41	14.87 ± 1.52	4.56 ± 0.97
303.88 ± 50.46	22.58 ± 3.63	3.67 ± 1.46

CONCLUSION

We have provided the first experimental evidence of a relationship between gill silver accumulation and acute silver toxicity in terms of mortality. We have also demonstrated a relationship between gill silver accumulation and inhibition of Na^+ uptake, the eventual cause of mortality in rainbow trout. In combination with evidence that an equilibrium level of silver accumulation on the gills is reached over time [26], these results justify the use of short-term gill silver accumulation as an endpoint to predict the acute toxicity of silver to rainbow trout, as is done in the current toxicological version of the BLM. The experimentally determined LA50 values and conditional equilibrium binding constant for ionic silver binding to the gills may increase the predictive capabilities of the model. Together, these results may help promote use of the toxicological silver BLM by regulatory agencies such as the U.S. Environmental Protection Agency in the assessment of the toxicity of silver to aquatic organisms, and in the generation of water quality criteria.

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