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A Physiologically Based Biotic Ligand Model for Predicting the Acute Toxicity of Waterborne Silver to Rainbow Trout in Freshwaters

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An early silver−gill binding model using conditional equilibrium binding constants (*K*) was fitted to actual toxicity data for rainbow trout (*Oncorhynchus mykiss*) and subsequently modified to produce a mechanistically based acute toxicity model for predicting silver toxicity. The model used an "off the shelf" aquatic geochemistry software program (MINEQL$^+$) and physiologically based log *K* values to predict the acute effects of waterborne silver in rainbow trout. The final version of the model does not predict total gill−silver loading, as the early model did, but rather predicts the binding of Ag^+ to key toxic sites on the gill and incorporates the effects of cation competition at these sites. The acute toxicity model for Ag^+ provided the best fit to toxicity data when a log *K* value for the affinity of these sites was 7.6 with cationic competition log *K* values for Na^+ and Ca^{2+} of 2.9 and 2.3, respectively. A log *K* for Ag−DOM of 9.0 was used representing strong Ag^+ binding to dissolved organic matter. The model we present is easy to use and provides a good match with previously published acute $AgNO_3$ toxicity data for rainbow trout from 31 data sets in 10 studies. The modified model is now ready for full verification with a greater range of laboratory and natural waters.

Introduction

Modeling interactions of metals with biological surfaces, particularly the fish gill, has recently been advocated as a method for predicting the acute toxicity of metals in freshwater systems (*1*−*4*). These biotic ligand models are based on the gill surface interaction model for trace metal toxicity proposed by Pagenkopf (*5*). The applicability of these approaches is based on the premise that waterborne metals, particularly in the free cation form, bind to specific sites on the gills, impair physiological processes related to ionic uptake, and result in acute toxicity (*6*). This gill modeling or tissue residue-based approach has the potential to provide a relatively simple, inexpensive, mechanistically based and scientifically defensible method for predicting the acute toxicity of metals on a water chemistry-specific basis.

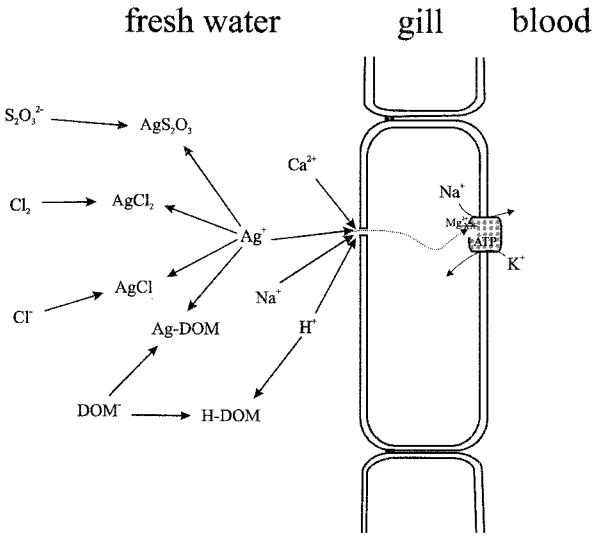

FIGURE 1. Schematic diagram of the Ag−gill modeling approach of Janes and Playle (*16*). The diagram illustrates the role of cationic competition with Ag^+ binding at the site of Ag−gill binding as well as complexation of Ag^+ with anions in the water column such as dissolved organic matter (DOM), Cl^-, and thiosulfate ($S_2O_3^{2-}$).

The data available for the acute toxicity and gill binding of silver for freshwater rainbow trout, while not extensive, are complementary and provide a reasonably complete understanding of silver toxicity (*7*, *8*). The mechanism of acute Ag^+ toxicity is inhibition of Na^+/K^+ ATPase activity in the gills and associated ionoregulatory failure (*9*−*12*). The influence of metal complexation and cationic competition on both acute toxicity and gill Na^+/K^+ ATPase activity has been illustrated in a number of studies (*9*−*11*, *13*−*15*).

The work of Janes and Playle (*16*) provides a modeling framework using experimentally derived conditional equilibrium binding constants (*K*) to model silver binding to gills of rainbow trout. When the appropriate log *K* values for gill−silver binding are integrated into chemical equilibrium computer programs (e.g., MINEQL$^+$; *17*), the resulting model can be used to predict silver accumulation on trout gills and, by extension, acute silver toxicity. The strengths of this approach are that both the influence of competition with other cations (e.g., Ca^{2+}, H^+, and Na^+) and the complexation with anions such as dissolved organic matter (DOM), thiosulfate, and Cl^- by Ag^+ are incorporated explicitly into the model (Figure 1). This approach has been used for Ag (*18*) as well as for Cu, Cd, Co, and Ni (*4*, *19*−*24*), and the overall approach has been summarized in a recent review (*3*).

The log $K_{Ag-Gill}$ value of 10.0 derived from the work of Janes and Playle (*16*), which modeled short-term silver accumulation on the gills (over 2−3 h), was never experimentally correlated with silver *toxicity*. Indeed, although the connection between metal accumulation on fish gills and toxicity has logical and some empirical support for Cu, Cd, and Ni (*4*, *19*, *24*), the relationships between silver accumulation and toxicity, and especially *predicted* silver accumulation and toxicity, need to be tested thoroughly.

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This issue is particularly important for silver, as some studies indicated that total silver loading at the gill was not correlated with physiological toxicity (*11, 13*).

It was suggested in a recent review (*8*) that predictive models for silver toxicity should be based on the actual mechanism of Ag^+ toxicity, inhibition of gill Na^+/K^+ ATPase activity, rather than on the Ag−gill load. The conditional equilibrium stability constant for Ag^+ causing a 50% inhibition of Na^+/K^+ ATPase activity in vivo ($\log K_{Ag-ATPase}$) was 7.8 (*8*), as calculated from the results of previous studies (*11, 13*). This log *K* value is about 150 times lower than that of the original model ($\log K_{Ag-Gill} = 10.0$; *16*). While the former value better represents the mechanism of silver toxicity, the latter represents short-term accumulation of silver by the gills, and neither has been explicitly validated in a predictive toxicity model. Clearly, these types of metal−gill binding models must be tested against experimentally derived toxicity data from a range of water chemistries and, if necessary, adjusted in a scientifically defensible manner before they can be considered as tools for use in regulating metals in the aquatic environment. One other version of a model for acute silver toxicity has recently been developed (*18*). While this model exploited the complexation and competition framework of the biotic ligand approach, the development of log *K* values was based on fitting the model to silver toxicity data. As such, this was a toxicity based model rather than a mechanistically based model.

The main purpose of our work was to develop a mechanistically based biotic ligand model for the acute toxicity of silver that could be tested against experimentally derived acute toxicity data sets. Using "off the shelf" aquatic geochemistry software with our addition of complexation and competition reactions for gills, Ag^+, other cations, and DOM, we evaluated log *K* values based on gill loading (*16*) or inhibition of gill Na^+/K^+ ATPase activity (*8*) or derived using other published data. The model was developed by comparing predicted LC_{50} values with measured LC_{50} values from published studies under a series of different scenarios, each with a unique combination of log *K* values.

Modeling Scenarios and Inputs

All modeling for acute silver toxicity was done using the geochemical equilibrium modeling software program MINEQL$^+$, version 4.0 (*17*). Modeling simulations were run using components from the existing internal MINEQL$^+$ database that included H_2O, H^+, Ag^+, Ca^{2+}, Cl^-, CO_3^{2-}, Mg^{2+}, Na^+, and $S_2O_3^{2-}$. Two new variables, DOM and ToxAg, were incorporated into the MINEQL$^+$ database, both with an assigned charge of −1. The variable ToxAg represents a population of theoretical sites on the gill where silver binds and causes acute toxicity. The major component of ToxAg is the silver binding sites on branchial Na^+/K^+ ATPase, but other silver binding sites that may also contribute to acute toxicity are also included in ToxAg. Six additional complexation/competition reactions using these new variables were inserted into the program. These additions describe the following reactions: the complexation of Ag^+ with the ToxAg (Ag−ToxAg, which results in acute silver toxicity); the competitive influence of water Na^+, H^+, and Ca^{2+} on Ag−ToxAg interactions (Na−ToxAg, H−ToxAg, and Ca−ToxAg, respectively); and the role of waterborne DOM in complexing Ag^+ and H^+ (Ag−DOM and H−DOM).

Previously reported log *K* values available for describing Ag−ToxAg are listed in Table 1, while those calculated in the course of our analysis of previously reported data are given in Table 2. The concentration of the ToxAg variable entered into the model was 1.3 nmol L^{-1}. This value is the molar density of sites available for Ag^+ toxic binding based on the mean site density of 12.5 nmol/g of gill tissue developed for juvenile rainbow trout (*16*), using a gill tissue weight of 0.1 g in 1 L of water. An alternative approach would be to use the number of Ag^+ binding sites on gill Na^+/K^+ ATPase molecules, which is thought to be in the range of 1−4 nmol/g of gill tissue (*8*). In practice, the concentration of ToxAg used in the model was not of primary consideration because acute toxicity predictions were based on the proportional loading (i.e., % saturation) as opposed to actual accumulation of Ag on the ToxAg variable (see below). However, it was important that the concentration of ToxAg remained less than 30 nmol L^{-1} because at higher densities the variable itself had the potential to influence the mass balance of total Ag at equilibrium, an unrealistic scenario for a fish in an open aquatic system. The concentration of Ag binding sites per milligram of carbon on DOM entered into the model was 35 nmol (mg of C)$^{-1}$ (*16*).

TABLE 1. Initial Data Available for Input into the Gill−Ag Toxicity Model As Collected from Literature Sources[a]

model variable	measured response	conditional equilibrium constant	source
Ag−ToxAg	Ag^+-Gill	$\log K_{Ag-Gill} = 10.0^b$	*16*
Ag−ToxAg	Ag^+−$Gill_{ATPase}$	$\log K_{Ag-ATPase} = 7.8^c$	*8*
Na−ToxAg	Na^+−GillAg	$\log K_{Na-GillAg} = 4.7^b$	*16*
H−ToxAg	H^+−GillAg	$\log K_{H-GillAg} = 5.9^b$	*16*
Ca−ToxAg	Ca^{2+}−GillAg	$\log K_{Ca-GillAg} = 3.3^b$	*16*
Ag−DOM	Ag^+−DOM	$\log K_{Ag-DOM} = 9.0^b$	*16*
H−DOM	H^+−DOM	$\log K_{H-DOM} = 4.0^b$	*16*

[a] Binding site density for ToxAg was 1.3 nmol L^{-1}, while for Ag−DOM interactions it was 35 nmol (mg of C)$^{-1}$. [b] Derived from gill−Ag loading experiments. [c] Derived from Ag^+ concentration at IC_{50} in vivo.

TABLE 2. Additional Data Available for Input into the Gill−Ag Toxicity Model As Calculated from Previously Published Data Showing the Toxic Effects of Silver on Rainbow Trout

model variable	measured response	conditional equilibrium constant	source
Ag-ToxAg	Ag^+−$Gill_{ATPase}$	7.6^a	*10, 11*
Ag-ToxAg	Ag^+−LC_{50}	7.8^b	*25*
Ag-ToxAg	Ag^+−LC_{50}	7.3^b	*14*
Ag-ToxAg	Ag^+−LC_{50}	7.6^b	*9*

[a] Derived from Ag^+ concentration at IC_{85}. [b] Derived from Ag^+ concentration at LC_{50}.

With one exception, the additional log *K* values for the Ag−ToxAg variable in the model (Table 2) used the waterborne Ag^+ concentrations at the 50% response level, usually the 96-h LC_{50}. This Ag^+ concentration was subsequently converted to an apparent conditional equilibrium constant by taking the negative log. This is similar to the calculation previously shown for Ag^+−$Gill_{ATPase}$ value (*8*) where inhibition of gill Na^+/K^+ ATPase activity was used as the response directly related to Ag^+ toxicity (Table 1). The exception to this calculation was the Ag^+−$Gill_{ATPase}$ value, where we used the free Ag^+ concentration at 85% inhibition of the gill Na^+/K^+ ATPase activity in vivo (*11*). The 85% inhibition level (IC_{85}) was chosen based on results showing that exposure of rainbow trout to silver (as $AgNO_3$) at a concentration equivalent to the 96-h LC_{50} value in relatively hard Lake Ontario water caused an 85% inhibition, rather than 50% inhibition, of branchial Na^+/K^+ ATPase activity (*10*).

Acute toxicity of silver to rainbow trout was predicted by the model using the assumption that the toxic threshold (i.e., 96-h LC_{50}) occurred at 50% saturation of the theoretical gill sites with Ag^+ (i.e., Ag−ToxAg = 0.65 nmol L^{-1}). The toxic threshold value of 50% saturation is an intrinsically theoretical value and was chosen as being logically analogous to the

TABLE 3. Measured Toxicity Data and Associated Water Chemistry Used To Develop and Validate the Acute Toxicity Model[a]

treatment	reported water chemistry and temperature						96-h LC_{50} for Ag (μg L^{-1})	reference
	Ca^{2+}	Cl^-	Na^+	DOM	pH	temp		
soft water (SW)	48	68	84	0.3	6.2	17	7.5 ± 2.8	Bury et al. (*14*)
SW + 250 Cl^-	52	241	257	0.3	6.3	17	9.2 ± 3.0	Bury et al. (*14*)
SW + 750 Cl^-	65	782	868	0.3	6.6	17	18.5 ± 4.5	Bury et al. (*14*)
SW + 1500 Cl^-	91	1426	1590	0.3	6.7	17	25.6 ± 3.7	Bury et al. (*14*)
SW + 500 Ca^{2+}	499	46	75	0.3	6.6	17	9.9 ± 4.8	Bury et al. (*14*)
SW + 2500 Ca^{2+}	2316	59	86	0.3	6.5	17	10.5 ± 2.3	Bury et al. (*14*)
SW + 1.5 DOM	32	74	111	1.63	6.7	17	18.4 ± 2.7	Bury et al. (*14*)
SW + 5 DOM	35	95	151	5.82	6.7	17	27.7 ± 2.8	Bury et al. (*14*)
Riso soft water	10	10	50	1.3	NR[b]	14	10.2 (9.6−11.3)	Grosell et al. (*33*)
Lake Ontario hard water	1000	700	600	1.3[c]	8.0	15	11.8 (10.9−13.8)	Hogstrand et al. (*9*)
Lake Ontario hard water	1000	700	600	1.3[c]	8.0	15	15.1 ± 5.6	Galvez & Wood, unpublished
soft water	50	50	50	0.3	6.5	NR	4.7[d] (NR)	Galvez & Wood (*15*)
control water (CW)	106	0	0	0	9.3	13	1.5 (1.3−1.6)	Karen et al. (*25*, *26*)
CW + 2.5 humic acid	164	0	0	2.6	9.3	11	5.6 (4.8−6.4)	Karen et al. (*25*, *26*)
CW + 5 humic acid	140	0	0	4.6	9.1	13	9.5 (8.8−10.2)	Karen et al. (*25*, *26*)
CW + 3 Cl^-	121	107	0	0	9.1	13	3.4 (3.0−3.8)	Karen et al. (*25*, *26*)
CW + 20 Cl^-	110	569	0	0	9.2	10	2.4 (2.0−2.9)	Karen et al. (*25*, *26*)
CW + 40 Cl^-	124	1276	0	0	9.0	11	3.8 (3.3−4.3)	Karen et al. (*25*, *26*)
CW + 3 Cl^- + 2.5 humic	162	93	0	2.9	9.0	12	17.1 (14.9−19.6)	Karen et al. (*25*, *26*)
CW + 40 Cl^- + 5 humic	176	1344	0	6.3	9.2	13	28.4 (25.1−32.2)	Karen et al. (*25*, *26*)
CW + Ca^{2+}	228	0	0	0	8.8	13	3.6 (2.9−4.4)	Karen et al. (*25*, *26*)
soft water	269	97	409	0.3[c]	6.9	11	6.5 (5.3−8.1)	Davies et al. (*28*, *29*)
hard water	1893	666	640	0.3[c]	7.9	14	13.0 (3.9−22.1)	Davies et al. (*28*, *29*)
New River, Virginia	35	25	66	0.3[c]	7.8	12	4.8[d] (3.6−6.3)	Diamond et al. (*30*, *31*)
lab 1 continuous flow	337	34	48	3[c]	7.7	14	16.4 (12.8−19.2)	Lemke (*32*)
lab 4 continuous flow	289	313	274	1[c]	7.6	14	8.4 (5.9−11.9)	Lemke (*32*)
lab 5 continuous flow	367	366	265	0.3[c]	7.5[c]	14	9.7 (8.4−11.3)	Lemke (*32*)
lab 6 continuous flow	773	28	387	0.3[c]	7.8	14	9.7 (9.0−10.3)	Lemke (*32*)
rainbow trout 1	90	202	200[c]	0.3[c]	7	12	8.6 (8.0−9.2)	Nebeker et al. (*27*)
steelhead trout	112	202	200[c]	0.3[c]	7	12	9.2 (NR)	Nebeker et al. (*27*)
rainbow trout 2	131	202	200[c]	0.3[c]	7	12	9.7 (8.4−11.3)	Nebeker et al. (*27*)

[a] Water chemistry values for Cl^-, Na^+, and Ca^{2+} are in μmol L^{-1} while DOM is mg of C L^{-1}. Measured toxicity in terms of total silver added as $AgNO_3$ is shown ± SEM or (95% CI). [b] NR indicates that information was not reported. [c] Water chemistry values estimated using available information. [d] LC_{50} values are 144 h for Diamond et al. (*30*) and 168 h for Galvez and Wood (*15*).

50% response used in other toxicity tests (e.g., EC_{50}, LC_{50}, LD_{50}, or LT_{50}) under that assumption that all binding of Ag^+ results in toxicity. As such, the model predicts toxicity based on the proportion of sites available for producing toxicity that are complexed by Ag^+ rather than on the predicted total accumulation of silver in the gill tissue. The primary reasons for this simplification was that the time course of accumulation of total silver by the gill can be highly variable (e.g., Figure 4 of ref *8*) so that over time total silver accumulation does not appear to be related to the actual mechanism of Ag^+ toxicity (*11*, *13*). Therefore, the model is an acute silver toxicity prediction model and not a gill−silver loading model and is based on the loading dynamics of a biotic ligand, the theoretical Ag^+ binding sites on the gill that produce acute toxicity.

The process of modeling the various scenarios started with the full model of Janes and Playle (*16*), and then, in progression, a variety of Ag−ToxAg log *K* values were tested. The model was developed by comparing predicted acute toxicity values with the measured 96-h LC_{50} data of Bury et al. (*14*), a toxicity data set in which water chemistry was systematically varied and was well characterized (see Table 3). In combination with the testing of Ag−ToxAg log *K* values, the Na−ToxAg and Ca−ToxAg log *K* values were altered in different modeling scenarios (see Results and Discussion and Table 4) within the constraints of published data (*14*), and in each case calculated and measured acute toxicity were compared by regression analysis. The log *K* values for Ag−DOM and H−DOM (Table 1) taken from Janes and Playle (*16*) were not varied during modeling scenarios; weak Ca^{2+} and Na^+ interactions with DOM were not considered. Our modeling was bound by the requirement that all log *K* values that could be tested had to be based on measured responses to silver that had been reported in the published primary literature.

To develop and test the different log *K* values in the model information on the acute toxicity of silver to juvenile rainbow trout, data were collected from a variety of published studies (see Table 3). However, the development of the model was initially done using only one acute toxicity data set, that of Bury et al. (*14*) as described above. Once the model was developed by fitting to the Bury et al. (*14*) data, it was validated against the full toxicity data set (Table 3).

The criteria for selection of these toxicity studies were as follows: (i) measurements of 96-h (preferably) LC_{50} for silver concentrations for rainbow trout, (ii) relatively comprehensive measurements of test water chemistry, and (iii) acute testing protocols that conformed to standard methods. However, despite a reasonable number of acute silver toxicity studies on trout, complete water chemistry information was sometimes lacking. In particular, DOM was rarely reported. As a result, an effort was made to include more studies in our validation data set by estimating water chemistry based on available information. For example, the study of Hogstrand et al. (*9*) did not report DOM concentration in Hamilton hard water, but values reported in other studies from the same laboratory (*11*, *13*, *14*) would be a reasonable approximation. The study of Karen et al. (*25*) only provided nominal measures of water chemistry, but a full listing of measured water chemistry for the same experiment was found elsewhere (*26*). The hardness values reported in Karen et al. (*25*) were used to estimate the amount of Ca^{2+}, assuming Ca^{2+} and Mg^{2+} were added in equal amounts (*25*). Similarly, the hardness values reported by Nebeker et al. (*27*) were converted to Ca^{2+} concentrations, and the Fort Collins dechlorinated water supply, which is described as being soft

TABLE 4. Measured Acute Toxicity (96-h LC_{50}) of Ag (As $AgNO_3$) for Rainbow Trout under Eight Different Water Chemistry Treatments from Bury et al. (*14*; see Table 3) and Corresponding Calculated Acute Toxicity Using Different Modeling Scenarios Obtained by Varying log *K* Values[a]

	predicted acute toxicity values for Ag (μg L^{-1}) in modeling scenarios					measd 96-h LC_{50} of Ag (μg L^{-1})
scenario	**1**	**2**	**3**	**4**	**5**	
log $K_{Ag-ToxAg}$	10.0	**7.8**	7.8	**7.6**	**7.3**	
log $K_{Na-ToxAg}$	4.7	4.7	**2.9**	2.9	2.9	
log $K_{Ca-ToxAg}$	3.3	3.3	**2.3**	2.3	2.3	
log $K_{H-ToxAg}$	5.9	5.9	5.9	5.9	5.9	
log K_{Ag-DOM}	9.0	9.0	9.0	9.0	9.0	
treatments						
soft water (SW)	0.6	12.5	4.3	6.2	11.1	7.5 ± 2.8
SW + 250 μmol L^{-1} of Cl^-	1.1	39.2	5.4	7.9	14.8	9.2 ± 3.0
SW + 750 μmol L^{-1} of Cl^-	3.0	NC[b]	10.1	15.4	29.7	18.5 ± 4.5
SW + 1500 μmol L^{-1} of Cl^-	5.0	NC	19.2	29.8	NC	25.6 ± 3.7
SW + 500 μmol L^{-1} of Ca^{2+}	0.6	12.6	3.8	5.4	9.7	9.9 ± 4.8
SW + 2500 μmol L^{-1} of Ca^{2+}	0.8	19.4	4.7	6.9	12.6	10.5 ± 2.3
SW + 1.5 mg L^{-1} of DOM	2.6	19.4	9.1	10.5	14.1	18.4 ± 2.7
SW + 5 mg L^{-1} of DOM	9.7	29.1	23.7	25.7	30.2	27.7 ± 2.8
r	0.91**	0.28	0.96***	0.94***	0.83*	
slope	0.37	0.37	0.92	1.13	0.99	

[a] Altered parameters in each scenario are given in bold type. The regression of calculated against measured LC_{50} values was used to quantify the fit of each model with the *r* (* = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$) and the slope of each scenario shown at the bottom of the column of calculated values. [b] NC indicates that it was not possible to calculate a value due to cerargyrite formation before 50% saturation of ToxAg sites.

(*28*, *29*), was assumed to have a similar Na^+ concentration to the reported Cl^- levels. In the study of Davies et al. (*28*), water chemistry was not reported, but a complete quantification (except DOM) of identical water can be found in an earlier study (*29*). The study of Diamond et al. (*30*) also lacks detail on water chemistry (reported as being soft water), but we were able to find average water ionic concentration for the area just upstream of the test site on the New River (*31*), although DOM again was not reported. Because of the lack of accurate DOM concentrations in the data sets summarized by Lemke (*32*), some generalized assumptions were made. We assigned a DOM content of 0.3 mg of C L^{-1} for all well water sources as well as other waters reported as being soft and values of 3 and 1 mg of C L^{-1} for the Lake Superior (lab 1) and reservoir (lab 4) waters, respectively (*32*).

Results and Discussion

Model Development. Calculated acute toxicity of silver in five modeling scenarios, each run with the eight different water chemistries given by Bury et al. (*14*; see Table 3), are shown in Table 4 and discussed below. These data allow a comparison of measured and calculated acute silver toxicity as water Cl^-, Ca^{2+}, and DOM were systematically varied. As a measure of the performance of the acute toxicity models under each specific combination (scenario) of log *K* values, the slope and correlation coefficient (*r*) of the regression of calculated against measured toxicity were used.

Modeling scenario 1 tested the original conditional equilibrium stability constants from Janes and Playle (*16*), generated using gill total silver burden as an end point. Although this model produced a good correlation between predicted and measured LC_{50} values ($r = 0.91$), the low slope (0.37) suggested that the model was overly sensitive (i.e., calculated LC_{50} values were consistently lower than measured LC_{50} values). This result suggested that a better prediction of acute silver toxicity would be obtained by reducing the affinity of the ToxAg sites for Ag^+ (i.e., a lower log *K* value for Ag−ToxAg). Therefore, for modeling scenarios after scenario 1, the physiologically based log *K* values for ToxAg were used (see Tables 1 and 2), starting with the one derived from the inhibition of branchial Na^+/K^+ ATPase (*8*; log $K = 7.8$, see Table 1).

Modeling scenario 2 yielded both a low *r* value and slope, and in two water chemistries it was not possible to achieve a 50% predicted loading of ToxAg sites with Ag^+ (Table 4). The primary reason for the poor agreement between measured and predicted acute toxicities was that lowering the log *K* for ToxAg to 7.8 without altering the log *K* values for Na−ToxAg and Ca−ToxAg gave Na^+ and Ca^{2+} much more of an influence on reducing the predicted Ag toxicity than actually occurred. Therefore, it was necessary to lower the log *K* values for Na−ToxAg and Ca−ToxAg to reflect their true effect on measured silver toxicity. This reduction in the sensitivity of the model for the effects of Na^+ and Ca^{2+} was achieved by adjusting the log *K* values for Na−ToxAg and Ca−ToxAg downward using the data of Bury et al. (*13*) to provide a physiological basis. According to Bury et al. (*13*), both Na^+ and Ca^{2+} in the range of 1500 μM offered no protection against Ag^+-induced inhibition of gill Na^+/K^+ ATPase activity, suggesting that the log *K* values for Na−ToxAg and Ca−ToxAg must both be ≤2.9. In addition, Galvez and Wood (*15*) showed that the protective effect of Ca^{2+} on Ag^+-induced acute toxicity is about 10-fold less than that of waterborne Cl^-. Accordingly, the log *K* value for Ca−ToxAg was set at 2.3, one log unit below the log *K* value for Ag−Cl complexation. The model of Janes and Playle (*16*) demonstrated that Na^+ was more effective than Ca^{2+} in keeping Ag^+ off the gills of rainbow trout (see scenario 1 log *K* values, Table 4), so the log *K* value for Na−AgTox was tested at 2.9. This value gave waterborne Na^+ the maximum competitve effect possible within the physiological constraints, and the suitability of this and the other log *K* values was tested in modeling scenario 3.

The adjustments for the competitive effects of Na^+ and Ca^{2+} in scenario 3 greatly improved the match between predicted and measured acute toxicity (Table 4). The model now reasonably predicted the relative effects of altered water chemistry on acute silver toxicity ($r = 0.96$), but it remained overly sensitive (slope of 0.92) because calculated LC_{50} values were always less than measured, particularly for the effects of Cl^-. In subsequent modeling scenarios the Ag−ToxAg log *K* value was decreased, first to 7.6 and then to 7.3 for modeling scenarios 4 and 5, respectively. The first of these two values

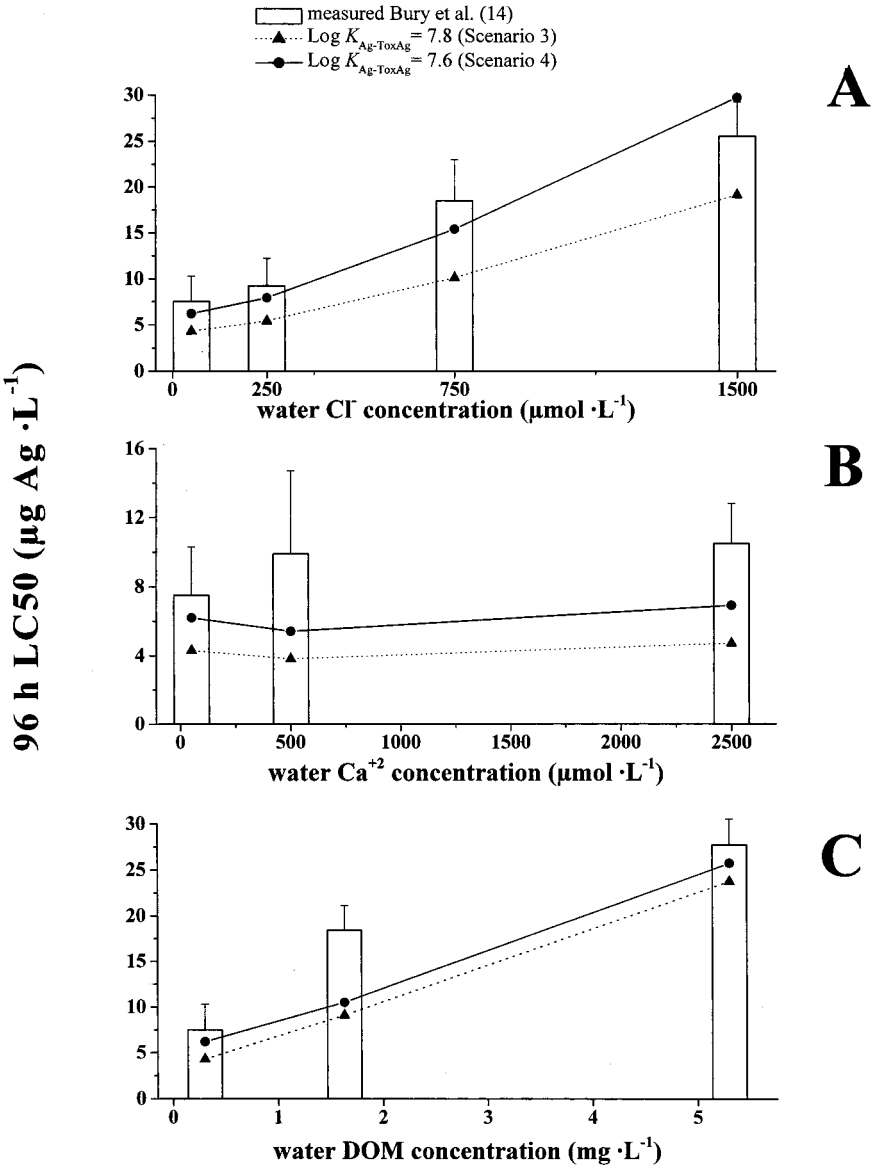

FIGURE 2. Measured ± SEM (open bars) and calculated acute toxicity (lines and symbols) 96-h LC_{50} values for silver (μg L^{-1}) in juvenile rainbow trout under various concentrations of waterborne Cl^- (A), Ca^{2+} (B), or DOM (C). The two modeling scenarios, 3 (dotted line and triangles) and 4 (final acute model, solid line and circles), illustrate the effect of the adjustments in log *K* value for Ag−ToxAg (Table 4). The measured data were from Bury et al. (*14*; see Table 3).

represents the IC_{85} for the effect of Ag^+ on gill Na^+/K^+ ATPase activity (*10*; see Table 2) as well as the Ag^+ LC_{50} reported by Hogstrand et al. (*9*). The Ag−ToxAg log *K* value of 7.3 was expected to provide the best fit as it was based on Ag^+ concentrations at the LC_{50} of the data set used for testing (*14*).

Reduction in the strength of the Ag−ToxAg interaction in modeling scenario 4 improved the match between predicted and measured acute silver toxicity, yielding a highly significant correlation ($r = 0.94$) plus a higher slope (1.13) and less undercalculation of the LC_{50} values (Table 4). A comparison of scenarios 3 and 4 against the Bury et al. (*14*) data is given in Figure 2. A further decrease in log $K_{Ag-ToxAg}$ to 7.3 (scenario 5) did not improve the model, which now overpredicted the protective effect of waterborne Cl^- (Table 4). In fact, for the highest Cl^- concentration, an acute toxicity value could not be calculated as cerargyrite formation was induced before 50% loading of the ToxAg sites occurred (Table 4).

The results of scenarios 1−5 indicated that, among the available log $K_{Ag-ToxAg}$ values listed in Tables 1 and 2, log $K_{Ag-ToxAg} = 7.6$ provides the best match with the measured acute toxicity data set (*14*). Therefore, scenario 4 was adopted as the final acute toxicity model (Table 5 and Figure 3).

Model Testing. The final acute toxicity model was developed from the toxicity experiments of Bury et al. (*14*), which were run with $AgNO_3$ at eight different water chemistries. To test the predictive capabilities of the final version of the acute toxicity model (scenario 4, Table 5), water chemistries from 31 separate toxicity measurements from 10 studies (Table 3) were applied to the model. Results of this model validation are shown in Figure 4 where the predictive capability of the acute toxicity model over a wide range of water chemistries is shown. With all data included, the calculated LC_{50} values fall within the reported error of the measured values for 55% of the data points (SEM or 95% CI). When the nine data points from the Karen et al. (*25*) study are excluded (see below), 68% of calculated values fall within the reported error of measured LC_{50} values (Figure 4). Of these remaining seven predicted values outside the error range of measured values, in all but two cases the calculated

TABLE 5. Selected Stability Constants (*K*) in the Final Model (Physiological BLM Values)[a]

complex	Morel and Hering (*34*)	Paquin et al. (*18*)	physiological BLM value	source for physiological BLM value
Ag−ToxAg		7.3	7.6	current model calculations
Na−ToxAg		2.3	2.9	current model calculations
H−ToxAg		4.3	5.9	Janes and Playle (*16*)
Ca−ToxAg		2.3	2.3	current model calculations
Ag−DOM		NA[b]	9.0	Janes and Playle (*16*)
H−DOM		NA	4.0	Janes and Playle (*16*)
$AgS_2O_3^-$	8.8	NP[c]	8.80	MINEQL$^+$ (*17*)
AgCl	3.3	3.31[d]	3.27	MINEQL$^+$ (*17*)
$AgCl_2^-$	5.3	5.25[d]	5.27	MINEQL$^+$ (*17*)
$AgCl_3^{2-}$	6.4	5.20[d]	5.29	MINEQL$^+$ (*17*)
$AgCl_4^{3-}$		5.51[d]	5.51	MINEQL$^+$ (*17*)
$AgNO_3$		−0.10[d]	−0.29	MINEQL$^+$ (*17*)
$CaCO_3$	3.2	3.22[e]	3.15	MINEQL$^+$ (*17*)
$CaHCO_3^+$	11.59	11.44[e]	11.33	MINEQL$^+$ (*17*)
HCO_3^-	10.33	10.33[e]	10.33	MINEQL$^+$ (*17*)
H_2CO_3	16.68	16.68[e]	16.68	MINEQL$^+$ (*17*)

[a] Values include those added to the model as well as a selection of values from the database within the MINEQL$^+$ computer program (version 4.0). For comparison the constants given in Paquin et al. (*18*) and Morel and Hering (*34*) are also shown. [b] NA, a comparative value is not available as the Paquin et al. (*18*) model considers DOM differently (see Discussion). [c] NP, a comparable value for thiosulfate is not provided. [d] Values taken from the NIST database (*35*). [e] Values taken from Tipping (*36*).

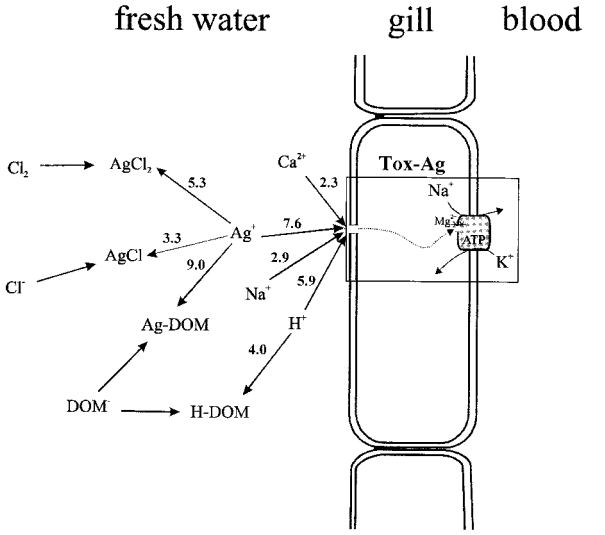

FIGURE 3. Schematic diagram of the final acute toxicity model for predicting the acute toxicity of silver to rainbow trout in freshwater. The numbers represent log conditional equilibrium binding constants (*K*) for Ag^+ and other cations binding at the ToxAg site on the gill as well as Ag^+ binding to the complexing agents such as dissolved organic matter (DOM) and Cl^- in the water column.

values are lower than measured values, illustrating the slightly conservative nature of our model. The regression of predicted and measured LC_{50} values, for the full data set, gave a linear relationship of

$$\text{calculated } LC_{50} = 0.95 \times [\text{measured } LC_{50}] + 0.84$$
$$(r = 0.85,\ n = 31,\ \text{dotted line in Figure 4})$$

When the data of Karen et al. (*25*) were separated (see discussion below), the linear relationship for the remaining 22 studies was

$$\text{calculated } LC_{50} = 1.01 \times [\text{measured } LC_{50}] - 1.4$$
$$(r = 0.90;\ \text{solid line in Figure 4})$$

The regression of predicted on measured just for the data of Karen et al. (*25*) yielded the relationship

$$\text{calculated } LC_{50} = 1.06 \times [\text{measured } LC_{50}] + 3.5$$
$$(r = 0.90,\ n = 9,\ P < 0.001)$$

showing that the effect of water chemistry in this study was consistent with that of the model (slope = 1.06) but that the trout in this study were more sensitive to silver (*y* intercept = $+3.5\mu g\ L^{-1}$).

Karen et al. (*25*) and Hogstrand et al. (*9*) measured 96-h LC_{50} values that were particularly low (i.e., high Ag toxicity) in relation to calculated values and to other studies. The reason for the high Ag toxicity from Karen et al. (*25*) may be that very young (~20 d) rainbow trout were used, there was only minimal holding (48 h) after transport to the testing laboratory, and there was no acclimation to the ion-depleted test waters before silver exposure. These factors, plus the elevated exposure pH (>9.0) and the apparent zero Na^+ content of the test waters may have stressed the fish enough to make them overly sensitive to $AgNO_3$. It is also possible that very young trout are more sensitive to the acute toxic effects of silver. The reasons for the poor predictive capabilities of the model for the study of Hogstrand et al. (*9*) are unknown, but our estimated DOM values may not be accurate. It is interesting to note that a recent study by Galvez and Wood (unpublished data) in the same laboratory with similar water chemistry and source of trout produced 96-h LC_{50} values that better matched predicted values (Figure 4; Table 3).

General Assessment. Our acute toxicity model for rainbow trout accurately calculates the acute toxicity of silver in a variety of water chemistries and stocks of juvenile rainbow trout. Our model incorporates the best available information on the effects of water chemistry on silver toxicity in rainbow trout as well as a log *K* for Ag−ToxAg that is derived from physiological data on toxic Ag−gill interactions and the mechanism of toxicity (*10*, *11*). It is important to note that the model represents the biotic ligand in a very simplified form. For example Ag^+ toxicity occurs via binding to basolaterally located Na^+/K^+ ATPase sites, whereas cationic competition for Ag^+ uptake to the gill presumably occurs apically. However in the model cationic competition and Ag^+ toxicity are presented as a single entity (Figure 3). Although the model is a simplification of reality and may not accurately reflect the intricacies of the biological processes, it does accurately reflect acute silver toxicity (Figure 4).

Within the tested range of water chemistries, output from the final model given in Table 5 matched toxicity data over a 4-fold range of measured 96-h LC_{50} values (Figure 4). DOM has the greatest effect on silver toxicity, followed by Cl^-, in accord with their log *K* values (Table 5) and with experimental

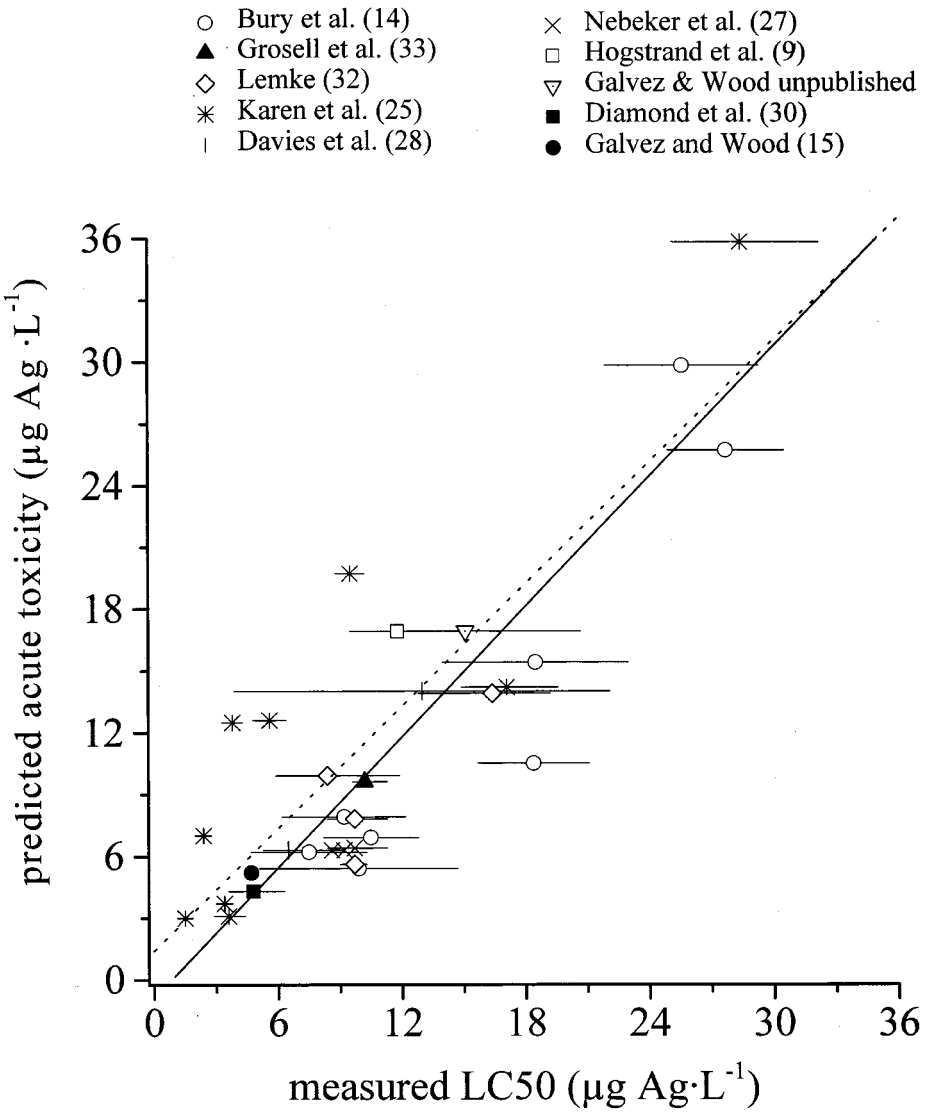

FIGURE 4. Relationship between measured 96-h LC_{50} values for Ag in rainbow trout and acute Ag toxicity as predicted by the final acute toxicity model. Horizontal lines indicate errors associated with the measured LC_{50} values (either ±1 SEM or 95% CI). The dotted line is the regression of measured and calculated LC_{50} values using all data ($Y = 0.95[X] + 0.84$, $r = 0.85$, $n = 31$, $P < 0.001$). The solid line shows the same relationship but with the data of Karen et al. (*25*) excluded ($Y = 1.01[X] - 1.4$, $r = 0.90$, $n = 22$, $P < 0.001$).

data (Table 3). DOM and Cl^- both act by complexing Ag^+ in the water column (*16*). Ag−DOM complexes do not appear to accumulate on the gill, while $AgCl_{aq}$ complexes appear to accumulate but do not cause acute toxicity (*9*, *11*, *13*, *15*). The next important modifiers of Ag toxicity are Na^+ and Ca^{2+}, with log *K* values of 2.9 and 2.3, respectively.

As validation of the acute toxicity model (Table 5), we tested its predictive capabilities against measured LC_{50} data. With the exclusion of the study by Karen et al. (*25*), the majority of the calculated LC_{50} values are within the reported (95% CI or ± 1 SEM) of measured values (Figure 4), although it must be recognized that water chemistry has been estimated in some cases, particularly for DOM. Calculated LC_{50} values that do not fit within the reported error of measured values are generally less than the measured LC_{50} values, showing the slightly conservative nature of the model.

The log *K* values in the final version of the model that quantify the competition and complexation reactions for Ag^+ binding to toxic sites on the gill (Table 5) are very different than those of the original model of Janes and Playle (*16*), the starting point of our modeling exercise. This is particularly true for the complexation of Ag^+ with the biotic ligand (Ag−ToxAg in our model). However, the change from a log *K* value of 10.0 to a value of 7.6 (2.4 log units = 250-fold change) has a theoretical, methodological, and physiological basis.

Relative to silver toxicity and the modeling in our work, the important features of the Janes and Playle (*16*) gill loading model were that it described all gill−silver interactions and the separate and independent effects of cationic competition. Because those log *K* values were based on the absence (100% effect level) of loading of silver onto the gill, it was possible to determine the effects of each cation, independent of the effects of other complexation and competition reactions. As well, the Janes and Playle (*16*) model assumed that all gill−Ag interactions produced toxicity while, in fact, both toxic and nontoxic binding can occur (*8*). The other Ag−ToxAg log *K* values that were available for use in our new model (Table 2) either represent the 50% effect level (or in one case 85%) for the physiological mechanism of Ag^+ toxicity or the level of Ag^+ at the measured acute toxicity value. Therefore, the log *K* values we tested are based on Ag^+ loading rather than the absence of silver loading as in Janes and Playle (*16*). Furthermore, the log $K_{Ag-GillAg}$ value was derived from measurements of fast (3 h) binding of Ag to the gills, whereas the log $K_{Ag-ToxAg}$ value represents interactions of Ag^+ at the gills that culminate in 50% inhibition of Na^+/K^+ ATPase

activity or in 50% toxicity over 96 h. In a simple calculation, which ignores Na^+ competition, with a 0.17 μM solution of $AgNO_3$ it took 4600 times more Cl^- than silver to keep alive 50% of the trout for 96 h (782 μM Cl^-, SW + 750 Cl, Table 3). The log of 4600 is approximately 3.7, which when added to 3.3 (the log *K* of AgCl, Table 5) is 7.0, only 4-fold from the final log $K_{Ag-ToxAg}$ value of 7.6.

Moving the log $K_{Ag-ToxAg}$ from 10.0 to a value of 7.8, as presented in scenario 2 (Table 4), dramatically improved the predictive accuracy of the model in very dilute waters (e.g., 12.5 μg L^{-1} as compared to a measured value of 7.5 μg L^{-1}; soft water treatment, Table 4). Scenario 2 also highlighted the potential problem of models that incorporate log *K* values from different sources. Decreasing the log *K* value of Ag−ToxAg while leaving other log *K* values unchanged resulted in a model that was overly sensitive to cationic competition. While it would be simple enough to scale down all other log *K* values that influence the loading of the ToxAg variable (Na−ToxAg, Ca−ToxAg, and H−ToxAg) by an equivalent 2.2 log units, we opted to use previously published data to derive new values for two of these variables. This approach provided an approximation of the final log *K* values that provided the best fit to measured data. Thus, reductions in the Na−ToxAg and Ca−ToxAg log *K* values were based on experimentally derived data from interactions between Ag^+ and either Na^+ or Ca^{2+} on gill Na^+/K^+ ATPase activity. The H−ToxAg log *K* as well as those for Ag−DOM and H−DOM were not changed from the original Janes and Playle (*16*) model for two reasons. First, there was no experimental evidence to justify alterations; second, alterations were not needed to improve the model (e.g., the calculated LC_{50} for 1.5 and 5 mg L^{-1} DOM agree very well with the measured values, Table 4).

The biotic ligand model we developed is similar in concept to the model of Paquin et al. (*18*), which is also being developed to predict acute toxicity of silver. A comparison of gill interactions and silver complexation reactions within each of the models is given in Table 5, which also provides a comparison of silver conditional stability constants from a variety of sources. The main differences between our model and that of Paquin et al. (*18*) are the values used for gill−silver interactions plus the methodology for incorporating DOM into the modeling framework. Our model development showed that physiologically based conditional equilibrium constants provided a good description of biotic ligand interactions in rainbow trout. This physiologically based mechanistic approach differs from that of Paquin et al. (*18*) because that model, although recognizing the physiological mechanisms of toxicity, was calibrated to silver toxicity. However, the Paquin et al. (*18*) model also accounts for the possibility of neutral inorganic species such as AgCl(aq) causing toxicity, broadening the scope of their model to include toxicity data on the fathead minnow (*Pimephales promelas*). While we did not develop this feature into the final model, preferring to restrict our model to rainbow trout, it could be modified to include AgCl interactions if necessary. However, given the modeling software currently available, this inclusion would be at the expense of simplicity and usability.

The approach to DOM−silver−gill interactions is also different between the two models. On the basis of published experimental gill binding data (*16*) as well as the recent work of Van Ginneken and Blust (*37*), we were confident that a one ligand system describing strong Ag−DOM interactions would be successful. Our approach differs from that of the Paquin et al. (*18*) model, which used a multi-binding site model to describe metal DOM interactions (*36*). On the basis of the results of these two models, both approaches can be used to describe silver toxicity. It is important to note that a full understanding of Ag−DOM interactions, how these interactions influence Ag^+ toxicity, and how to accurately describe these influences within a validated modeling framework requires further study and development.

The primary contribution of our present work was to adjust the original gill receptor loading model (*16*), which was based on total silver loading, to an acute toxicity model that reflects only toxic loading of Ag^+. As such, in our final model (Table 5) we did not find it necessary to redefine or alter any conditional stability constants other than those for binding of Ag^+ and other cations at the biotic ligand, as discussed above. This reflects the fact that complexation of silver by waterborne DOM and Cl^- affects only the toxic fraction of dissolved silver, which is ionic Ag^+. Our results also support the conclusion that cationic competition (Na^+ and Ca^{2+}) is involved in protecting against Ag^+ toxicity; indeed the fact that using the log $K_{Ag-ToxAg}$ value of 7.3 (scenario 5, Table 4) did not produce the best predictive model was because of cationic competition. We originally expected this apparent log *K* value (7.3) to provide the best predictions as it and the test data were from the same work (i.e. circular modeling). This did not occur, primarily because deriving the log *K* using the Ag^+ concentration at the 96-h LC_{50} does not incorporate competitive ligand binding effects.

Thus, the original Janes and Playle (*16*) model proved to be very robust and required only some relatively simple adjustments for its transformation to a silver acute toxicity model. One of the primary strengths of our modeling is that all adjustments in log *K* values had a physiological basis. The model we present is mechanistically based and accounts for the effects of concentration, complexation, and competition. A vigorous verification of this model is now required with additional data to further understand the effects of aquatic geochemistry; this verification is necessary before a model such as this can be considered for regulatory purposes. Future adjustments to the model may be necessary and could include additional complexation or competition reactions between waterborne ligands and Ag^+. Of particular focus should be interactions of Ag^+ with Ca^{2+} and Mg^{2+} (effects of alkalinity) and DOM. As well, applicability of this model could be expanded beyond the acute effects on rainbow trout to other organisms as information on the toxic effects of species other than Ag^+, mechanisms of silver toxicity, and the binding of Ag (e.g., log *K* values and site densities) in other aquatic species becomes available. This physiologically based mechanistic approach to modeling acute silver toxicity may also serve as the basis for the development of chronic silver toxicity models.

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