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A Mechanistic Explanation for the In(LC50) vs In(Hardness) Adjustment Equation for Metals

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I demonstrate that a combination of (a) competitive binding of transition-metal cations, hardness cations, and protons to transition-metal-binding sites on fish gills and (b) aqueous complexation of transition-metal cations by HCO_3^- and CO_3^{2-} explains why the regression slopes of $\ln(\text{LC50})$ vs $\ln(\text{hardness})$ for five divalent transition metals (Cd, Cu, Ni, Pb, and Zn) are ~ 1 , where LC50 is the median lethal concentration. For these calculations, I assumed the amount of the transition metal bound to the fish gill at 50% mortality is constant (i.e., independent of water quality). Although the slopes theoretically should vary between 0 and 2 (at extremely low and high hardness, respectively), a slope of ~ 1 is expected at midrange hardness (~ 20 – $200 \text{ mg}\cdot\text{L}^{-1}$ as CaCO_3) if alkalinity covaries with hardness—a common condition in most laboratory toxicity tests. But if alkalinity is held constant while hardness is varied, a slope of ~ 0.5 is expected at midrange hardness. Although predictions of LC50s using regressions of $\ln(\text{LC50})$ vs $\ln(\text{hardness})$ might be acceptable for regulating discharges of transition metals to waters in the midrange of hardness, extrapolations beyond this range might drastically overpredict metal toxicity.

Introduction

Toxicity of transition metals to aquatic biota varies as a function of several water-quality parameters (e.g., hardness, pH, alkalinity, concentration of dissolved and particulate organic matter) (1, 2). The U.S. Environmental Protection Agency (USEPA) water quality criteria for Cd, Cu, Ni, Pb, and Zn (3–7) specifically account for the increase in LC50 (median lethal concentration) as water hardness increases, using regression equations written in the following general form

$$\ln(\text{LC50}) = a \cdot \ln(\text{hardness}) + b \quad (1)$$

where a = slope, b = ordinate intercept, LC50 is in units of $\mu\text{g}\cdot\text{L}^{-1}$, and hardness is in units of $\text{mg}\cdot\text{L}^{-1}$ as CaCO_3 . The form of this equation has never been mechanistically justified; instead, eq 1 merely provides a convenient, empirical fit to the data. Surprisingly, though, the slopes for all five transition metals are ~ 1 (range = 0.76 – 1.27 ; Table 1).

In this paper, I propose an explanation for these apparently coincidental relationships between LC50 and water hardness. This argument is based on the concept of competitive binding of cations to fish gills (8, 9).

Model

I present the model in terms of H^+ , Ca^{2+} , and a generic, divalent transition-metal ion M^{2+} binding to fish gills.

TABLE 1. Coefficients for the Equations Predicting Acceptable Concentrations ($\mu\text{g}\cdot\text{L}^{-1}$) of Transition Metals as a Function of Water Hardness ($\text{mg}\cdot\text{L}^{-1}$ as CaCO_3) for Freshwater Biota Exposed to Cd, Cu, Ni, Pb, and Zn^c

metal	slope ^a	intercept ^b	dependent variable	ref
Cd	1.128	−3.828	criterion max. concn	(3)
Cu	0.9422	−1.464	criterion max. concn	(4)
Ni	0.76	4.02	final acute concn	(5)
Pb	1.273	−1.460	criterion max. concn	(6)
Zn	0.8473	0.8604	criterion max. concn	(7)

^a The coefficient a in eq 1. ^b The coefficient b in eq 1. ^c The general form of the predictor equation is dependent variable = $\exp\{\text{slope} \cdot \ln(\text{hardness}) + \text{intercept}\}$.

However, it also applies to Mg^{2+} instead of (or in conjunction with) Ca^{2+} as the hardness modifier of toxicity. The model also may apply to other biotic ligands such as soft tissue of invertebrates.

Equilibrium equations for binding of Ca^{2+} and M^{2+} to sites at which M binds on a fish gill can be written as (9)

$$K_{\text{M=gillM}} = \frac{[\text{M=gillM}]}{[\text{M}^{2+}] \cdot [\text{=gillM}]} \quad (2)$$

and

$$K_{\text{Ca=gillM}} = \frac{[\text{Ca=gillM}]}{[\text{Ca}^{2+}] \cdot [\text{=gillM}]} \quad (3)$$

where $K_{\text{M=gillM}}$ = stability constant for M^{2+} binding to M-binding sites on the gill ($\text{L}\cdot\text{mol}^{-1}$), $K_{\text{Ca=gillM}}$ = stability constant for competitive binding of Ca^{2+} to M-binding sites on the gill ($\text{L}\cdot\text{mol}^{-1}$), $[\text{M}^{2+}]$ = aqueous concentration of M^{2+} ($\text{mol}\cdot\text{L}^{-1}$), $[\text{Ca}^{2+}]$ = aqueous concentration of Ca^{2+} ($\text{mol}\cdot\text{L}^{-1}$), $[\text{=gillM}]$ = concentration of unoccupied M-binding sites on the gill ($\text{mol}\cdot\text{L}^{-1}$), $[\text{M=gillM}]$ = concentration of M=gillM complexes ($\text{mol}\cdot\text{L}^{-1}$), and $[\text{Ca=gillM}]$ = concentration of Ca=gillM complexes ($\text{mol}\cdot\text{L}^{-1}$).

For simplicity, I have ignored activity coefficients in these equations. Moreover, I have expressed the concentrations of the binding sites and the M=gillM and Ca=gillM complexes as if they are uniformly distributed in the exposure water. This implies that the fish gills are in equilibrium with the entire volume of water in the exposure chamber.

Combining eqs 2 and 3, I express $[\text{M}^{2+}]$ as a function of $[\text{Ca}^{2+}]$:

$$[\text{M}^{2+}] = \frac{K_{\text{Ca=gillM}} \cdot [\text{M=gillM}]}{K_{\text{M=gillM}} \cdot [\text{Ca=gillM}]} \cdot [\text{Ca}^{2+}] \quad (4)$$

Assume $[\text{M=gillM}]$ at 50% mortality is constant (i.e., independent of hardness, as demonstrated recently for Cu and Ni (10) and first proposed as a general concept by (1) and (8)). Equation 4 can be rewritten as

$$\text{LC50}_{\text{M}^{2+}} = K' \cdot \frac{[\text{Ca}^{2+}]}{[\text{Ca=gillM}]} \quad (5)$$

where $\text{LC50}_{\text{M}^{2+}}$ is the concentration of the aquo ion of M that causes 50% mortality, and K' is a constant ($= (K_{\text{Ca=gillM}} / K_{\text{M=gillM}}) \cdot [\text{M=gillM}]_{50\% \text{ mortality}}$). Combining eq 5 with the equilibrium speciation relationships in the Appendix, the LC50 of a divalent transition metal to fish can be approximated algebraically as a function of pH, water hardness, alkalinity,

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TABLE 2. Approximations to Eq 7 at Various Alkalinity and Hardness Conditions

hardness	alkalinity	approximation to eq 7 ^a	explanation
low	constant	$\ln(\text{LC50}) \cong 0.0 \cdot \ln([\text{Ca}^{2+}]) + \ln(k)$	At low $[\text{Ca}^{2+}]$, $[\text{Ca}^{2+}]/[\text{Ca} \equiv \text{gillM}] (= K_{\text{Ca} \equiv \text{gillM}}/[\equiv \text{gillM}])$ is approximately constant because the concentration of unoccupied M-binding sites on the gill ($[\equiv \text{gillM}]$) is approximately constant (i.e., little Ca binds to the gill, causing little change in $[\text{M} \equiv \text{gillM}]$, $[\text{H} \equiv \text{gillM}]$ and $[\equiv \text{gillM}]$); thus, $\ln([\text{Ca}^{2+}]) \cong \ln([\text{Ca} \equiv \text{gillM}]) + \ln(\text{constant})$.
low	equal to hardness	$\ln(\text{LC50}) \cong 0.0 \cdot \ln([\text{Ca}^{2+}]) + \ln(k)$	Same as above, and Alk (alkalinity) approaches zero as $[\text{Ca}^{2+}]$ approaches zero.
high	constant	$\ln(\text{LC50}) \cong 1.0 \cdot \ln([\text{Ca}^{2+}]) + \ln(k)$	If $[\text{M} \equiv \text{gillM}]$ is constant at 50% mortality (independent of $[\text{Ca}^{2+}]$), $[\text{Ca} \equiv \text{gillM}]$ will approach a constant ($= [\equiv \text{gillM}]_{\text{total}} - [\text{M} \equiv \text{gillM}]$; eq A-5) as Ca fills the remaining binding sites on the gill (i.e., as H^+ is outcompeted and no unoccupied M-binding sites remain).
high	equal to hardness	$\ln(\text{LC50}) \cong 2.0 \cdot \ln([\text{Ca}^{2+}]) + \ln(k)$	Same as above, but Alk = hardness results in $\text{LC50} \propto [\text{Ca}^{2+}]^2$ (i.e., $\ln(\text{LC50}) = \ln([\text{Ca}^{2+}]) + \ln(\text{Alk}) + \ln(\text{constant}) = \ln([\text{Ca}^{2+}]) + \ln([\text{Ca}^{2+}]) + \ln(\text{constant})$).

^a k is a composite constant that, in each equation, represents the products of the individual k 's and all other terms (or ratios of terms) in eq 7 that are approximately constant when hardness is extremely low or high.

the amount of Ca^{2+} bound to the transition-metal-binding sites on the fish gill, and the amount of unoccupied metal-binding sites, as follows (see derivation of eq A-4 in the Appendix)

$$\text{LC50}_{\text{M}_{\text{total}}} \cong k \cdot \frac{[\text{Ca}^{2+}]}{[\text{Ca} \equiv \text{gillM}]} \left(1 + \frac{k''}{10^{-\text{pH}}} + k''' \cdot \text{Alk} \right) \quad (6)$$

where $\text{LC50}_{\text{M}_{\text{total}}}$ is the total dissolved metal concentration at 50% mortality, Alk is the alkalinity of the exposure water (eq·L⁻¹), and k'' and k''' are constants. Taking the natural logarithm of both sides results in a relationship that begins to resemble eq 1:

$$\ln(\text{LC50}_{\text{M}_{\text{total}}}) \cong \ln([\text{Ca}^{2+}]) - \ln([\text{Ca} \equiv \text{gillM}]) + \ln \left(1 + \frac{k''}{10^{-\text{pH}}} + k''' \cdot \text{Alk} \right) + \ln(k) \quad (7)$$

Because $k''' \cdot \text{Alk} \ll 1 + k''/10^{-\text{pH}}$ at low alkalinity and $k''' \cdot \text{Alk} \gg 1 + k''/10^{-\text{pH}}$ at high alkalinity, eq 7 simplifies and attains the same form as eq 1 when hardness is very low (e.g., $< \sim 1$ mg·L⁻¹ as CaCO_3) or very high (e.g., $> \sim 1000$ mg·L⁻¹ as CaCO_3). At low hardness (either when alkalinity is constant or when alkalinity equals hardness), the slope approaches 0 (i.e., $\ln(\text{LC50}) \cong 0.0 \cdot \ln([\text{Ca}^{2+}]) + \ln(k)$, where k is a composite constant; Table 2); at high hardness the slope approaches either 1 or 2, depending on whether alkalinity is held constant (slope approaches 1; i.e., $\ln(\text{LC50}) \cong 1.0 \cdot \ln([\text{Ca}^{2+}]) + \ln(k)$; Table 2) or alkalinity equals hardness (slope approaches 2; i.e., $\ln(\text{LC50}) \cong 2.0 \cdot \ln([\text{Ca}^{2+}]) + \ln(k)$; Table 2).

Example

To demonstrate the effects of hardness, alkalinity and pH on transition-metal toxicity, I present results of simulations for Cu binding to fathead minnow (FHM; *Pimephales promelas*) gills. These calculations incorporate all of the Cu complexes and activity coefficients ignored for heuristic purposes in the previous section and in the Appendix. Although it would be more mechanistically appropriate to express LC50s of Cu in units of μM and hardness and alkalinity in units of meq·L⁻¹, I have retained the more traditional units of $\mu\text{g} \cdot \text{L}^{-1}$ for LC50s and mg·L⁻¹ as CaCO_3 for hardness and alkalinity to allow easier comparison to the historic database on metals toxicity.

Using toxicity data reported for FHM larvae by Erickson et al. (11), I first calculated the amount of Cu bound to the FHM gills at 50% mortality using the geochemical speciation program MINTEQA2 (12). For this and the subsequent

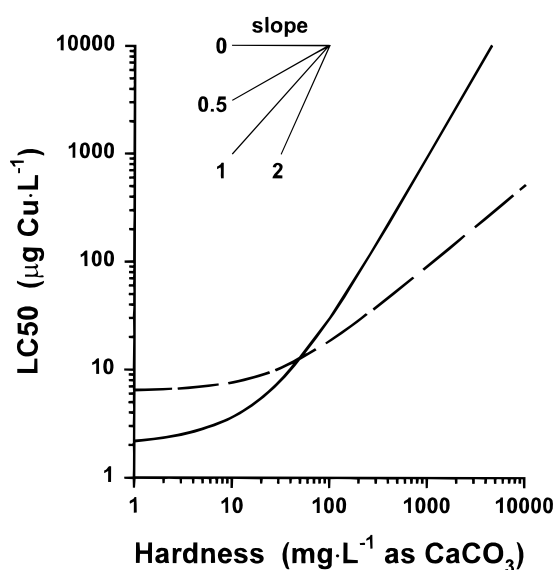


FIGURE 1. Predicted LC50s of Cu_{total} to fathead minnows as hardness varies at pH 7, assuming that the amount of Cu bound to the gills is constant at 50% mortality (i.e., independent of water quality). Cu bound to the gills was held constant at 36.5% of the 10^{-9} M Cu-binding sites. The solid curve is predicted LC50s when alkalinity equals hardness; the dashed curve is predicted LC50s when alkalinity is held constant at 50 mg·L⁻¹ as CaCO_3 .

calculations in this example, I assumed the density of Cu-binding sites was 10^{-9} M (i.e., ~ 1 g of fish per L of exposure water). Although this choice of binding-site density is arbitrary, the calculations were insensitive to changes in binding-site density at $\leq 10^{-9}$ M. Based on Figure 1 in Erickson et al. (11), I assumed the 96-h LC50 of Cu in flow-through exposures is $0.2 \mu\text{M}$ ($12.7 \mu\text{g} \cdot \text{L}^{-1}$) at pH 7.0 and alkalinity and hardness equal to ~ 50 mg·L⁻¹ as CaCO_3 . Additionally, I replaced the default stability constants in MINTEQA2 with the values in Version 5.0 of the National Institute of Standards and Technology's electronic database (13) (but with log K values adjusted to zero ionic strength using the Davies equation (14)) and used conditional stability constants reported for binding of Cu^{2+} , Ca^{2+} , and H^+ to FHM gills (log $K = 7.4$, 3.4 , and 5.4 , respectively (9)). Because (a) those gill-binding constants are not precise estimates and (b) the ionic strength of the exposure solutions in which they were determined was only ~ 0.0001 M (i.e., using the Davies equation (14), $< 4\%$ correction would be needed to adjust for nonzero ionic strength), I did not correct the gill-binding

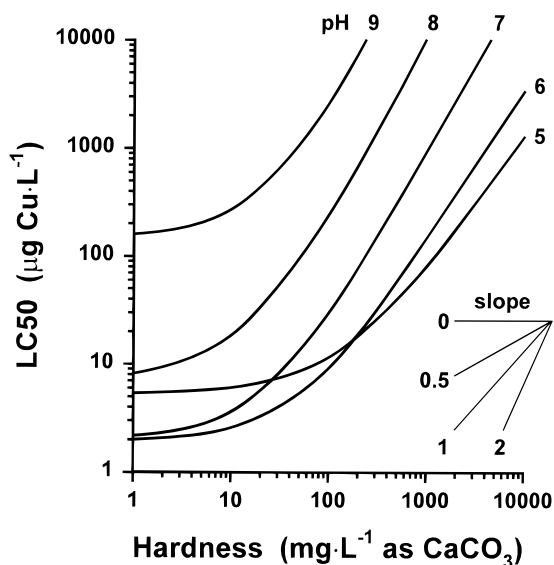


FIGURE 2. Predicted LC50s of Cu_{total} to fathead minnows as pH varies from 5 to 9, based on the assumptions that (a) the amount of Cu bound to the gills at 50% mortality is 36.5% of the 10^{-9} M Cu-binding sites and (b) alkalinity equals hardness.

constants to zero ionic strength. Under these conditions at 25 °C, 36.5% of the Cu-binding sites on the gill were occupied by Cu at the LC50. The remaining sites were occupied by Ca (32.1%) or H (0.7%) or were unoccupied (30.7%).

Then I calculated the total aqueous concentration of Cu (Cu_{total}) needed to achieve the same amount of Cu binding at other hardnesses and alkalinities but still at pH 7.0. If the amount of Cu bound to fish gills is constant at 50% mortality, this Cu_{total} is the predicted LC50. I used two scenarios for these simulations: (a) with alkalinity equal to hardness and (b) with alkalinity held constant at 50 $\text{mg}\cdot\text{L}^{-1}$ as CaCO_3 . However, because the stability constants I used for gill binding are conditional (i.e., not true thermodynamic constants because they will tend to vary as a function of pH, hardness, etc.), my calculations of percentage occupancy of gill binding sites and, hence, LC50s of Cu_{total} are only approximations.

As shown theoretically in the previous section, the predicted LC50 of Cu_{total} increased as hardness increased (Figure 1). The slope of $\ln(\text{LC50})$ vs $\ln(\text{hardness})$ approached zero at low hardness for both scenarios (alkalinity equal to hardness or alkalinity constant); whereas the slope approached 1.0 at high hardness when alkalinity was held constant, and the slope approached 2.0 at high hardness when alkalinity equaled hardness. When pH was varied, the predicted LC50 at a specified hardness decreased considerably as pH decreased from 9 to 6 (Figure 2). However, between pH 5 and 6 the predicted LC50 decreased only slightly at high hardness, and it increased at low hardness.

Discussion

Based on the assumptions that (a) the amount of a divalent transition-metal cation bound to a fish gill at 50% mortality is constant (i.e., independent of water quality) and (b) protons and hardness cations compete with the transition-metal cation for binding at these sites, it is not surprising that all of the slopes of the $\ln(\text{LC50})$ vs $\ln(\text{hardness})$ regressions for Cd, Cu, Ni, Pb, and Zn reported in the USEPA water quality criteria documents (3–7) are ~ 1 . Although the slopes theoretically could range from 0 to 2 (at extremely low and high hardness, respectively; Figures 1 and 2), the following rules-of-thumb apply:

1. If hardness and alkalinity are extremely low (e.g., < 1 $\text{mg}\cdot\text{L}^{-1}$ as CaCO_3), metal complexation with HCO_3^- and CO_3^{2-} is negligible and competition by Ca^{2+} for M-binding sites is

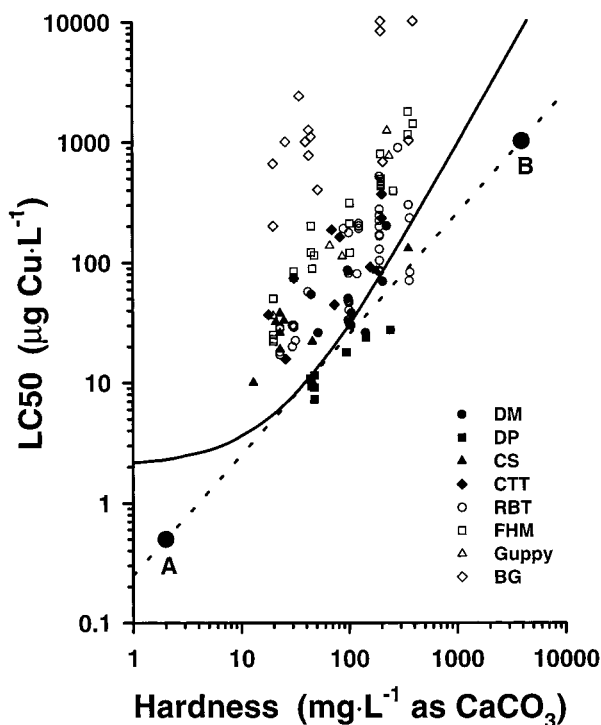


FIGURE 3. Data used in (4) to generate the slope of 0.94 for the regression of $\ln(\text{LC50})$ on $\ln(\text{hardness})$ for Cu. The solid curve represents the simulated LC50s calculated in this paper; the dashed line is a slope of 1. Species abbreviations are DM = *Daphnia magna*, DP = *Daphnia pulicaria*, CS = chinook salmon, CTT = cutthroat trout, RBT = rainbow trout, FHM = fathead minnow, and BG = bluegill.

minimal because of the large percentage of unoccupied binding sites on the gill. Thus, an incremental increase in hardness and alkalinity has almost no effect on metal binding to the fish gill, and the slope of $\ln(\text{LC50})$ vs $\ln(\text{hardness})$ is ~ 0 .

2. But if hardness and alkalinity are extremely high (e.g., > 1000 $\text{mg}\cdot\text{L}^{-1}$ as CaCO_3), a large percentage of the metal in solution is complexed with HCO_3^- or CO_3^{2-} , and the M-binding sites on the gill are almost completely occupied by Ca^{2+} and M^{2+} . Thus, an incremental increase in hardness and alkalinity requires a large increase in aqueous metal concentration to overcome (a) the additional complexation of M^{2+} with HCO_3^- and CO_3^{2-} and (b) the tendency for additional Ca^{2+} or Mg^{2+} to displace the metal from the gill. Under these conditions, the slope of $\ln(\text{LC50})$ vs $\ln(\text{hardness})$ is ~ 2 .

3. At intermediate hardness and alkalinity (~ 20 – 200 $\text{mg}\cdot\text{L}^{-1}$ as CaCO_3)—the range in which most laboratory toxicity tests are performed (see tabulations of hardnesses and LC50s in (3–7))—these effects are intermediate and the slope of $\ln(\text{LC50})$ vs $\ln(\text{hardness})$ is ~ 1 .

LC50 data used to calculate the slope of 0.94 in the Cu criteria document (4) demonstrate this coincidence (Figure 3). All of the hardnesses in that dataset were between 10 and 400 $\text{mg}\cdot\text{L}^{-1}$ as CaCO_3 ; thus, the theoretical slopes should have been between ~ 1 and ~ 1.5 (the calculated slopes for the eight species ranged from 0.61 to 1.36 (4: p 47)). The pattern for each species approximately parallels the LC50 curve I simulated for FHM larvae, although the data for less sensitive species (e.g., bluegills) and the less sensitive adult life stage of FHM lie well above the curve. Variability within the datasets for most of the species obscures a tighter fit to the theoretical curvilinear relationship, in part because (a) a variety of strains within a species probably were tested, (b) the toxicity tests were conducted at a variety of temperatures,

pHs and alkalinities, and (c) the range of hardnesses is too narrow to clearly demonstrate the curvilinear relationship.

Although hardness and alkalinity are approximately equal in many surface waters (including those used in many toxicity tests), some transition-metal toxicity tests are conducted at constant alkalinity while hardness is varied (or vice versa) to demonstrate the effect of hardness (or alkalinity) on metal toxicity. In the midrange of hardnesses, the slope of the $\ln(\text{LC50})$ vs $\ln(\text{hardness})$ regression for divalent transition metals should be ~ 0.5 when alkalinity is maintained constant. Supporting this prediction, I found a slope of 0.50 ($r^2 = 0.85$, $P = 0.0002$) when I regressed $\ln(\text{LC50})$ on $\ln(\text{hardness})$ for the 10 data points in Figure 3 of Erickson et al. (11) (96-h LC50s for FHM larvae exposed to Cu; hardness ranged from 37 to 134 $\text{mg}\cdot\text{L}^{-1}$ as CaCO_3).

A similar effect of alkalinity on transition-metal toxicity should occur as hardness is held constant. Thus, it is not surprising that Erickson et al. (11) (their Figure 1) only observed ~ 30 – 40% increases in LC50 of Cu_{total} when they increased alkalinity from ~ 45 to ~ 150 $\text{mg}\cdot\text{L}^{-1}$ as CaCO_3 at pH between 7 and 9 and a hardness of ~ 45 $\text{mg}\cdot\text{L}^{-1}$ as CaCO_3 (15). Such small effects of alkalinity on LC50 would be predicted if the slope of the $\ln(\text{LC50})$ vs $\ln(\text{alkalinity})$ regression in this alkalinity range was ~ 0.3 —a realistic value because hardness was held constant. At higher alkalinities, Erickson et al. (11) in theory would have seen a greater percentage increase in LC50 for the same percentage change in alkalinity, whereas at low alkalinities, they would have seen almost no effect of alkalinity on the LC50. Knowledge of alkalinity and hardness is crucial to accurately predict the LC50s of transition metals using $\ln(\text{LC50})$ vs $\ln(\text{hardness})$ regressions.

Erickson et al. (11) (their Figure 1) also showed that LC50 of Cu_{total} increased as pH increased between 6 and 9. This agrees with the gill-binding theory (Figure 2), because competitive binding of protons to the gill at $\text{pH} \geq 6$ is minimal; thus, the only effect of increasing pH is to increase the CO_3^{2-} concentration (at constant alkalinity) and the amount of Cu complexed with that ligand. However, at $\text{pH} < 6$ proton binding to the gill can be significant because $[\text{H}^+]$ becomes high enough ($> 10^{-6}$ M) for protons to compete with Ca^{2+} and Cu^{2+} (i.e., $[\text{H}^+]\cdot K_{\text{H=gillCu}}$ approaches $[\text{Ca}^{2+}]\cdot K_{\text{Ca=gillCu}}$ and $[\text{Cu}^{2+}]\cdot K_{\text{Cu=gillCu}}$). Therefore, at low alkalinity and hardness, the LC50 of Cu_{total} at pH 5 theoretically should be higher than at $\text{pH} \geq 6$ (Figure 2), but at high alkalinity and hardness, the reduction in complexation of Cu by HCO_3^- and CO_3^{2-} caused by the lower pH should help offset the increase in the LC50 of Cu_{total} that otherwise might occur. This prediction does not take into account the potential onset of acid toxicity at $\text{pH} < 6$ that will be caused by accumulation of protons on the gill, a process that would tend to decrease the Cu_{total} concentration at which 50% mortality occurs when the toxicants act jointly.

I have presented examples for Cu because of the extensive LC50 and gill-binding data available. However, similar hardness-related trends should occur with Cd, Ni, Pb, and Zn. In general, I predict the slopes of $\ln(\text{LC50})$ vs $\ln(\text{hardness})$ for these four transition metals will tend to be lower than they are for Cu in the same hardness range (if alkalinity covaries with hardness), because Cd, Ni, Pb, and Zn have lower affinities for CO_3^{2-} (13). But if alkalinity is held constant, I predict the slopes for all five transition metals will be approximately the same in the same hardness range. The pH-related trends for toxicity of Cd, Ni, Pb, and Zn to FHM are less pronounced (Pb) or are the opposite (Cd, Ni, and Zn) of the trend for Cu (16), in part because the chemical speciation of Cd, Ni, Pb, and Zn is much less affected by changes in pH than is the speciation of Cu (1).

In conclusion, the LC50 of a transition metal at a specified hardness can be predicted with relatively minor error in the

hardness range 20–200 $\text{mg}\cdot\text{L}^{-1}$ as CaCO_3 by knowing the LC50 at a different hardness and assuming (a) a slope of ~ 1 for the relationship between $\ln(\text{LC50})$ and $\ln(\text{hardness})$ and (b) alkalinity covaries with hardness. Such predictions might be acceptable for regulating discharges of transition metals to surface waters. But extrapolations to hardnesses outside this range might drastically overpredict the toxicity of the metal (e.g., extrapolating from an LC50 determined at a hardness of ~ 30 $\text{mg}\cdot\text{L}^{-1}$ as CaCO_3 to point A or B in Figure 3). Conversely, starting with an LC50 determined at a relatively low or high hardness, extrapolations to midrange hardnesses might drastically underpredict the toxicity of the metal. Such errors might be magnified if the alkalinity does not change proportional to the hardness in these extrapolations. Rather than relying on a mechanistic $\ln(\text{LC50})$ vs $\ln(\text{hardness})$ regression equations, a better approach might be to calculate uptake of transition metals using a biotic-ligand model and then predict toxicity from an empirical relationship between mortality and the amount of accumulated metal.

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Appendix

Mass Balance on Metal in Solution. To construct a tractable mass balance for a divalent transition-metal cation to which fish are exposed in a toxicity test, I make the following three assumptions:

1. No particles or organic ligands are present.
2. Complexes of divalent transition-metal cations with more than one HCO_3^- , CO_3^{2-} , or OH^- (e.g., $\text{Cu}(\text{HCO}_3)_2^0$, $\text{Cu}(\text{CO}_3)_2^{2-}$, $\text{Cu}(\text{OH})_2^0$, or $\text{Cu}(\text{OH})_3^-$) are negligible. Although this is not correct at high pH and alkalinity, the complexes can be ignored for heuristic purposes.
3. At acutely lethal concentrations under realistic biomass loadings (e.g., < 10 g fish $\cdot\text{L}^{-1}$), the amount of the transition metal that complexes with the fish gills will be a negligible percentage of the total amount of dissolved metal remaining in the water. For example, MacRae et al. (17) estimated 0.03 μmol Cu-binding sites $\cdot\text{g}^{-1}$ wet weight of rainbow trout (*Oncorhynchus mykiss*) gill. If the gill constitutes $< 5\%$ of the wet weight of a rainbow trout, then the < 10 g fish $\cdot\text{L}^{-1}$ will be able to bind < 1 μg Cu $\cdot\text{L}^{-1}$. Because not all of the dissolved Cu will be present as Cu^{2+} (the species that presumably is in equilibrium with binding sites on the gill) except at $\text{pH} \leq 6$ and because a large excess of Cu would be needed to saturate the Cu-binding sites on a fish gill ($\log K_{\text{Cu=gillCu}} = 7.25$ – 7.5 (9, 17)), the amount of Cu bound to fish gills at acutely lethal concentrations (LC50s usually $\gg 1$ $\mu\text{g}\cdot\text{L}^{-1}$) will be a relatively small percentage of the total Cu in the exposure water.

Thus, an approximate mass balance on the total amount of the transition metal in the exposure system is

$$[\text{M}_{\text{total}}] = [\text{M}^{2+}] + [\text{MOH}^+] + [\text{MHCO}_3^+] + [\text{MCO}_3^0] \quad (\text{A-1})$$

Ignoring activity coefficients, the governing equilibrium equations (with stability constants for the metal–ligand complexes listed in (13)) are

$$K_w = [H^+][OH^-] = 10^{-14}$$

$$K_{HCO_3^-} = \frac{[HCO_3^-]}{[H^+][CO_3^{2-}]} = 10^{10.33}$$

$$K_{MOH^+} = \frac{[MOH^+]}{[M^{2+}][OH^-]}$$

$$K_{MHCO_3^+} = \frac{[MHCO_3^+]}{[M^{2+}][HCO_3^-]}$$

$$K_{MCO_3^0} = \frac{[MCO_3^0]}{[M^{2+}][CO_3^{2-}]}$$

Additionally, assuming the aqueous system is buffered only by the hydroxyl and carbonate/bicarbonate systems, the alkalinity (Alk, expressed as eq·L⁻¹) is (18):

$$Alk = [OH^-] + [HCO_3^-] + 2 \cdot [CO_3^{2-}] - [H^+]$$

Substituting these relationships into eq A-1 produces

$$[M_{total}] = [M^{2+}] \cdot \left(1 + \frac{K_{MOH^+} \cdot K_w}{10^{-pH}} + (K_{MHCO_3^+} \cdot K_{HCO_3^-} \cdot 10^{-pH} + K_{MCO_3^0}) \cdot \left(\frac{Alk + 10^{-pH} - K_w \cdot 10^{pH}}{K_{HCO_3^-} \cdot 10^{-pH} + 2} \right) \right) \quad (A-2)$$

By combining eqs 5 (which expresses the LC50 of M²⁺ as a function of [Ca²⁺] and [Ca≡gillM]) and A-2, the LC50 of the total dissolved metal (LC50_{Mtotal}) can be expressed as a function of (a) the competitive binding of cations at the gill surface and (b) the equilibrium of the free-metal ion with the aqueous inorganic ligands, as follows:

$$LC50_{M_{total}} = K' \cdot \frac{[Ca^{2+}]}{[Ca \equiv gillM]} \cdot \left(1 + \frac{K_{MOH^+} \cdot K_w}{10^{-pH}} + (K_{MHCO_3^+} \cdot K_{HCO_3^-} \cdot 10^{-pH} + K_{MCO_3^0}) \cdot \left(\frac{Alk + 10^{-pH} - K_w \cdot 10^{pH}}{K_{HCO_3^-} \cdot 10^{-pH} + 2} \right) \right) \quad (A-3)$$

Equation A-3 can be simplified for heuristic purposes to the following approximation

$$LC50_{M_{total}} \cong K' \cdot \frac{[Ca^{2+}]}{[Ca \equiv gillM]} \cdot \left(1 + \frac{k''}{10^{-pH}} + k''' \cdot Alk \right) \quad (A-4)$$

where k'' and k''' are constants.

Mass Balance on Gill Binding Sites. Assuming H⁺, Ca²⁺, and M²⁺ are the only cations of importance that bind to the M-binding sites on a fish gill (although Mg²⁺ could be

substituted for or act in combination with Ca²⁺), an approximate mass balance for the M-binding sites on the gill is

$$[\equiv gillM_{total}] = [M \equiv gillM] + [Ca \equiv gillM] + [H \equiv gillM] + [\equiv gillM]$$

or

$$[Ca \equiv gillM] = [\equiv gillM_{total}] - ([M \equiv gillM] + [H \equiv gillM] + [\equiv gillM]) \quad (A-5)$$

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