

PREDICTING RELATIVE TOXICITY AND INTERACTIONS OF DIVALENT METAL IONS: MICROTOX[®] BIOLUMINESCENCE ASSAY

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Abstract—Both relative toxicity and interactions between paired metal ions were predicted with least-squares linear regression and various ion characteristics. Microtox[®] 15 min EC50s (expressed as free ion) for Ca(II), Cd(II), Cu(II), Hg(II), Mg(II), Mn(II), Ni(II), Pb(II), and Zn(II) were most effectively modeled with the constant for the first hydrolysis (K_H for $M^{n+} + H_2O \rightarrow MOH^{n-1} + H^+$) although other ion characteristics were also significant in regression models. The $|\log K_H|$ is correlated with metal ion affinity to intermediate ligands such as many biochemical functional groups with O donor atoms. Further, ordination of metals according to ion characteristics, e.g., $|\log K_H|$, facilitated prediction of paired metal interactions. Pairing metals with strong tendencies to complex with intermediate or soft ligands such as those with O or S donor atoms resulted in strong interactions.

Keywords—Metal Toxicity Bioactivity Microtox[®] Bacteria

INTRODUCTION

Quantitative structure–activity relationships (QSARs) allow prediction of organic toxicant and drug bioactivity. These relationships, first developed in pharmacology, are often based on surrogate or indirect measures of molecular qualities such as lipophilicity using K_{ow} , electrical qualities using Hammett constants, or topology using the molecular connectivity index. Most QSARs used by the Office of Toxic Substances were based on the surrogate measure, K_{ow} (Table 7.1 in Suter [1]). Measures used for QSARs can also involve more fundamental or primary characteristics, e.g., electrical qualities using ionization potentials or steric qualities using total molecular surface area. Quantitative structure–activity relationships based on such qualities provide a richer understanding of underlying processes than those using surrogate measures and, consequently, can be more effective for prediction beyond the particular compounds used to develop the QSAR.

Although seldom done, characteristics of inorganic species can similarly be used for predicting intermetal trends in bioactivity [2–9]. Like QSARs for organic compounds, properties of metal ions useful for predicting toxicity include both surrogate and more direct measures of toxicant qualities. For example, Biesinger and Christensen [3] correlated effects on aquatic biota with metal sulfide solubility, a surrogate measure thought to reflect metal tendency to combine with sulfhydryl groups of biomolecules. Based on hard and soft acid and base (HSAB) theory, Jones and Vaughn [4] and Williams and Turner [7] correlated toxic effects to mice with the softness parameter, σ_p (coordinate bond energy of the metal fluoride – coordinate bond energy of the metal iodide)/coordinate bond energy of the metal fluoride). Metal hydroxide solubility product ($\log -K_{so}$ MOH), notionally reflecting metal affinity to O-containing groups, was also correlated with inhibition of algal growth [8]. Similarly, the log of the constant for the first hydrolysis (K_H for $M^{n+} + H_2O \rightarrow MOH^{n-1} + H^+$) could be used as it also is correlated with metal ion affinity to intermediate ligands like

those with O donor atoms. (The log of K_H will increase linearly with the ion charge divided by the M–OH distance [10].)

Also found useful was the bivariate characterization of metal–ligand complexation outlined by Turner et al. [11]. In this scheme, $\Delta\beta$ (log of the stability constant for the metal fluoride – log of the stability constant for the metal chloride) and Z^2/r (Z = ion charge, r = ionic radius) were surrogate measures of covalent and ionic bond stabilities for metal–ligand complexes. The tendency to form covalent bonds with groups possessing an S donor atom (soft ligands) decreased with $\Delta\beta$. The polarizing power, Z^2/r , was a measure of the electrostatic interaction strength between a metal ion and ligand. If these two variables were used as axes to produce a complexation field diagram of cations, stability of metal–intermediate ligand complexes (e.g., ligands with an O donor atom) would increase along a line extending diagonally between the two axes [11–13].

Kaiser [6] generated an effective model from fundamental ion characteristics by combining $AN/\Delta IP$ and ΔE_0 where AN = atomic number, ΔIP = difference in ionization potentials between ion oxidation number OX and OX – 1, and ΔE_0 = the absolute difference in electrochemical potential between the ion and its first stable reduced state. The atomic number (AN) reflected the size or inertia of the ion. The ΔIP and ΔE_0 parameters reflected the effects of atomic ionization potential and the ability of the ion to change its electronic state, respectively. These parameters were used successfully to develop models of effect for three metal groupings based on electron configuration. Nieboer and Richardson [5] developed another set of variables for prediction of bioactivity based on fundamental ion characteristics (X_m^2/r and Z^2/r). Again, Z^2/r reflected the energy of an ion when interacting electrostatically with a ligand. The X_m^2/r (X_m = electronegativity and r = the Pauling ionic radius) quantified the importance of covalent interactions in the metal–ligand complexation relative to ionic interactions. (Electronegativity is correlated with the energy of an empty valence orbital and reflects the ability of the metal to accept electrons. Combining electronegativity with the Pauling ionic radius yields an index that quantifies the importance of covalent interactions relative to ionic interactions [5].)

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Table 1. Metal ion characteristics used in regression models^a

Metal ion	Ion electron configuration	Outer shell electrons	<i>AN</i>	<i>r</i> (Å)	ΔIP (eV)	ΔE_0 (V)	$\Delta\beta$	X_m	$ \log K_H $	Log $-K_{SO}$ MOH	$X_m^2 r$	Z^2/r	$\frac{AN}{\Delta IP}$	σ_p
Mg ²⁺	{He}2s ² 2p ⁶ or {Ne}	8 ^b	12	0.72	7.39	2.38	5.76	1.31	11.61	10.50	1.236	5.56	1.62	0.167
Ca ²⁺	{Ne}3s ² 3p ⁶ or {Ar}	8 ^b	20	1.00	5.76	2.76	4.80	1.00	12.72	5.00	1.000	4.00	3.47	0.181
Mn ²⁺	{Ar}3d ⁵	5	25	0.67	8.21	1.03	0.66	1.55	10.59	12.70	1.994	5.97	3.04	0.125
Ni ²⁺	{Ar}3d ⁸	8	28	0.69	10.52	0.23	0.50	1.91	9.86	16.00	2.517	5.79	2.66	0.126
Cu ²⁺	{Ar}3d ⁹	9	29	0.73	12.57	0.16	1.12	1.90	8.00	19.80	2.635	5.48	2.31	0.104
Zn ²⁺	{Ar}3d ¹⁰	10	30	0.74	8.57	0.76	0.66	1.65	8.96	16.50	2.015	5.40	3.50	0.115
Cd ²⁺	{Kr}4d ¹⁰	10	48	0.95	7.91	0.40	-0.89	1.69	10.08	14.00	2.713	4.21	6.07	0.081
Hg ²⁺	{Xe}4f ¹⁴ 5d ¹⁰	10	80	1.02	8.32	0.91	-5.80	2.00	3.40	25.50	4.080	3.92	9.62	0.065
Pb ²⁺	{Xe}6s ² 4f ¹⁴ 5d ¹⁰	10	82	1.18	7.61	0.13	0.48	2.33	7.71	18.70	6.406	3.39	10.78	0.131

^a See Materials and Methods section for data sources.

^b Noble gas configuration.

In the present study, we assumed that the difference in application of predictive modeling for organic compound and metal ion bioactivity rests on two factors. First, the QSAR approach for organic compounds was incorporated into ecotoxicology rapidly because it had already demonstrated its worth in pharmacology. No similar body of knowledge existed for metal ions. Second, prediction is complicated by metal speciation because several potentially bioavailable forms can be present simultaneously. Some of the ambiguity associated with metal speciation can be reduced with speciation models based on thermodynamic equilibrium and the simplifying assumption that bioactivity is generally correlated with the free ion concentration. Therefore, organic compounds may have no inherent advantage over metal ions relative to developing predictive models of bioactivity. We hypothesized that both relative metal ion toxicity and interactions between paired metal ions could be predicted using least-squares linear regression and various ion characteristics. Specifically, we tested the value of surrogate and more direct measures of ion characteristics for prediction of toxicity using a simple and widely accepted microbial assay, Microtox[®]. Nine metal ions differing in electronic configurations were selected (Table 1). They included two class A metals (Mg²⁺ and Ca²⁺) [14] with hard spheres and electron configurations of noble gases. The covalent interactions of these cations with ligands were generally much weaker than those of the other seven metals. The remaining seven ranged from borderline to class B metals with different tendencies for covalent interaction with hard ligands. Both the $\Delta\beta$ and $X_m^2 r$ parameters in Table 1 reflect this increasing tendency toward covalent bonding with ligands such as those with S donor atoms [11,12].

MATERIALS AND METHODS

Microtox toxicity assay

The Microtox assay was used to determine 15-min EC50 values for the nine metals (chloride salts) listed in Table 1. A reconstituted marine bacterium (*Vibrio fischeri* Beijerinck 1889, formerly *Photobacterium phosphoreum*) was exposed at 15°C to osmotically adjusted (2% NaCl [w/v]) solutions of metals. Bioluminescence, quantified over a range of metal concentrations with the Microtox model 500 toxicity analyzer (Microbics Corp., Carlsbad, CA, USA) was used to calculate the concentration resulting in a 50% decrease in light output after 15 min of exposure.

Final EC50 values were expressed in terms of specific metal species concentrations. Concentrations of species, including those of the free (aquated) ion, were predicted with PC MIN-

TEQA2 version 3.10 [15]. The concentrations of Na (342.3 mM/L), Cl (342.3 mM/L), pH (5.51), and total alkalinity (22.98 μ eq/L) of the osmotically adjusted medium plus the dissolved metal and Cl from the added metal salt were used in speciation calculations. Assumptions of a fixed pH, closed system, and no precipitation of solid phases were made during computations.

Ion characteristics

Ion characteristics were obtained from a variety of sources and are summarized in Table 1. Ionic radii were taken from Shannon and Prewitt [16,17] using "IR" values and the CRC Handbook of Chemistry [18]. The ΔIP values were calculated from ionization potentials in the CRC Handbook of Chemistry [18]. Those for ΔE_0 came from Kaiser [6] and were checked against more current tables of electrochemical series [18]. Most $\Delta\beta$ values were calculated with stability constants from Smith and Martell [19]. Those for the weak and consequently difficult to quantify complexes came from Lindsay [20]. Average electronegativity values (X_m) were from Allred [21]. The first hydrolysis constants, expressed in Table 1 as the absolute value of the log of K_H ($|\log K_H|$), were taken from Baes and Mesmer [10], Turner et al. [11], and Brown and Allison [15]. Values for the metal hydroxide solubility ($\log -K_{SO}$ MOH) came directly from Fisher [8] and those of the softness parameter (σ_p) were extracted from Pearson and Mawby [22].

Covalent ($X_m^2 r$) and ionic (Z^2/r) indices [5] were estimated from the parameters in Table 1. The covalent index reflected the "importance of covalent interactions relative to ionic interactions" or, more succinctly, "the electron attracting capability of an atom in a molecule" [5]. The ionic index "correlates successfully with interactions that are known to be highly ionic such as the hydration of cations . . ." [5]. The combined use of $AN/\Delta IP$ and ΔE_0 incorporated ion inertia or size (AN), atomic ionization potential (ΔIP), and electronic configuration (ΔE_0) during model development [6].

Relative metal toxicities

Initially, both total and estimated free metal ion concentrations (Ca²⁺, Cd²⁺, Cu²⁺, Hg²⁺, Mg²⁺, Mn²⁺, Ni²⁺, Pb²⁺, and Zn²⁺ in μ M/L) were used in model development. Models including free metal ion plus neutral chloro complexes were also examined because Simkiss and coworkers [23,24] suggested that chloro complexes may be more bioactive in marine systems than previously suspected. For example, the Hg(Cl)₀ concentration was considered in addition to that of Hg²⁺ because Hg(Cl)₀ may be bioavailable due to its lipophilicity [23,24], and it has an estimated membrane pen-

Table 2. Unspeciated concentrations ($\mu\text{M/L}$) of metals used in inter-metal interaction experiment

Metal	Interacting metal							
	Ca	Cd	Hg	Mg	Mn	Ni	Pb	Zn
Cu								
1.57	49,900	89.0	0.095	20,600	455.0	85.2	1.21	15.3
2.36	(100/83)	(6/86)	(<0.1/86)	(100/84)	(72/86)	(73/86)	(20/86)	(83/86)
3.15	99,800	177.9	0.189	41,120	910.1	170.3	2.41	30.6
4.72	(100/79)	(6/86)	(<0.1/86)	(100/83)	(72/86)	(73/86)	(20/86)	(83/86)
6.30	149,700	266.9	0.284	61,680	1,365	255.5	3.62	45.9
9.44	(100/74)	(6/86)	(<0.1/86)	(100/82)	(72/86)	(73/86)	(20/86)	(83/86)
	199,600	355.8	0.374	82,240	1,820	340.7	4.83	61.2
	(100/70)	(6/86)	(<0.1/86)	(100/80)	(72/86)	(73/86)	(20/86)	(83/86)
Mg								
41.1	49,900	0.89	0.189		1.82	1.70	1.21	76.5
61.7	(100/100)	(3/100)	(<0.1/100)		(58/100)	(56/100)	(10/100)	(69/100)
82.2	99,800	1.78	0.374		3.64	3.41	2.41	152.9
123.4	(100/100)	(3/100)	(<0.1/100)		(58/100)	(56/100)	(10/100)	(69/100)
164.5	149,700	2.67	0.563		5.46	5.11	3.62	229.4
246.7	(100/100)	(3/100)	(<0.1/100)		(58/100)	(56/100)	(10/100)	(69/100)
	199,600	3.56	0.748		7.28	6.81	4.83	305.9
	(100/100)	(3/100)	(<0.1/100)		(58/100)	(55/100)	(10/100)	(69/100)

Average MINTEQ-predicted percentages of the total concentrations of metal present as free ions for the paired metals are indicated in parentheses (e.g., % competing metal ion/% Cu^{2+} or % interacting metal ion/% Mg^{2+}).

etration rate 10^6 times faster than charged Hg complexes [24]. Further, estimated Hg^{2+} concentrations were more than 10^5 times lower than $\text{Hg}(\text{Cl})_2^0$ concentrations in the saline exposure solution. However, for this paper, models including neutral chloro complexes were abandoned after they demonstrated no clear superiority to the total or free ion-based models.

Regression models of 15-min EC50 values versus candidate ion characteristics were generated with PROC GLM of the SAS package [25]. Models involving only one independent variable used ΔE_0 , X_m^2/r , $\Delta\beta$, $\log -K_{so}$ MOH, $|\log K_H|$, or σ_p . Models with two independent variables included $(AN/\Delta IP, \Delta E_0)$, $(\log[AN/\Delta IP], \Delta E_0)$, $(X_m^2/r, Z^2/r)$, or $(\Delta\beta, Z^2/r)$, and were consistent with the work described in the Introduction. Both $(AN/\Delta IP, \Delta E_0)$ and $(\log[AN/\Delta IP], \Delta E_0)$ were used because, contrary to the original study of Kaiser [6], there was no apparent advantage to using the log transformation of $AN/\Delta IP$ during our model development.

Model selection was based on the principle of parsimony: models with lowest dimensionality were favored. The principle of parsimony was formally applied to model selection by minimum Akaike's information criterion estimation (MAICE). Akaike's information criterion (AIC) was calculated for each model using the estimated log likelihood ($\log L$). The AIC quantifies the fit of the model to the data after adjusting the $\log L$ for any differences in model complexity, i.e., different numbers of model parameters. If two models with identical fits but different numbers of estimated parameters were compared with MAICE, the model with the lowest number of parameters would be favored. Neter et al. [26] and Newman [27] provide the following formulae for the log likelihood function and AIC, respectively.

$$\log L = -\frac{n}{2} \log_e 2\pi - \frac{n}{2} \log_e \sigma^2 - \frac{1}{2\sigma^2} \sum_{i=1}^n (Y_i - b_0 - b_1 X_{1i})^2$$

$$\text{AIC} = -2(\log L) + 2P$$

where

- n = the number of observations,
- σ^2 = the model variance (estimated by the model mean square error, MSE),
- Y_i = the i th Y value,

x_{1i} = the i th x_1 value,

b_0 = the estimated intercept,

b_1 = the estimated slope, and

P = number of parameters estimated in the model.

If two independent variables (x_1, x_2) were used, an additional term, $-b_2 X_{2i}$, was added to the squared term at the end of the above equation to estimate the log likelihood. The squared term is the square of the difference in the observed and predicted values from the model. The model with the smallest AIC was judged to have the most information.

Metal interactions

We assumed that metal ion interactions result primarily from competition for ligand groups on biomolecules. This simplifying assumption has been applied successfully in previous modeling efforts [3,8,28–32]. Metal pairs were added simultaneously to the bacterial suspension to determine the interaction between metals on bacterial bioluminescence. Metals were paired based on the rankings of contrasting characteristics defined above for the nine metal ions. Combined metal concentrations producing significant but incomplete inhibition were used. Copper was paired with Ca, Cd, Hg, Mg, Mn, Ni, Pb, and Zn, and Mg was paired with Ca, Cd, Hg, Mn, Ni, Pb, and Zn (Table 2). In this manner, the metals were matched with two metals displaying contrasting interactions with ligands (i.e., Cu with primarily covalent interactions with affinities for donor atoms of $S > N > O$, and Mg with primarily electrostatic interactions and relative affinities of $O > N > S$ [5,8]).

The bioluminescence of *V. fischeri* was measured at 1-min intervals for a period of 5 min using all Cu- and Mg-metal pairs. Each metal pair was assayed three or four times. After preliminary trials indicated apparent first-order kinetics for inactivation, data were analyzed by fitting the natural log of light output against time. The absolute values of the slopes of these lines, as determined using least-squares linear regression, were estimates of the first-order rate constant (K) for inactivation. The exposure concentration of Cu or Mg was then plotted against the probit of K to produce four lines, one for each concentration

Table 3. Total EC50s, free ion-based EC50s, and free plus neutral chloro complex-based EC50s (mean \pm SD) in μ M/L for nine metals (added as chloride salts) using Microtox[®]

Metal	<i>n</i>	Total EC50	Proportion free ion ^a	Free ion-based EC50	Proportion free + neutral chloro complexes ^a	Free ion + neutral chloro complexes EC50
Ca	3	226,508 \pm 23,069	1.0	226,508 \pm 23,069	1.0	226,508 \pm 23,069
Cd	4	195.6 \pm 18.8	0.061	11.94 \pm 1.15	0.408	79.81 \pm 7.71
Cu	3	2.78 \pm 0.52	0.858	2.39 \pm 0.44	0.868	2.41 \pm 0.45
Hg	3	0.4574 \pm 0.0345	1.07E-12	4.89E-13 \pm 3.69E-14	0.107	0.0494 \pm 0.0037
Mg	3	209,301 \pm 2,844	1.0	209,301 \pm 2,844	1.0	209,301 \pm 2,844
Mn	3	1,352 \pm 62	0.717	969.4 \pm 44.7	0.729	985.6 \pm 45.3
Ni	3	336.6 \pm 67.6	0.729	245.4 \pm 49.3	0.831	279.7 \pm 56.2
Pb	3	0.8555 \pm 0.0200	0.197	0.1685 \pm 0.0039	0.375	0.3208 \pm 0.0075
Zn	4	18.28 \pm 2.15	0.834	15.24 \pm 1.79	0.856	15.65 \pm 1.84

^a Estimated using MINTEQA2 version 3.10.

of the potentially interacting metal (Interacting metal, Table 2). Probit transformations of rate constants allowed linearization of the sigmoidal curve of exposure concentration versus *K*. The probit metameter was used assuming the sigmoidal curve could be described as a cumulative normal distribution. Additional attempts to linearize these data with two metameters (logistic and Weibull transformations) frequently used to analyze dose-response data did not improve fit.

RESULTS

Relative metal toxicities

The exposure concentrations were expressed as either total metal, free ion, or free ion plus neutral chloro complex concentrations during initial model development. The predicted free ion concentrations were examined under the assumption that these concentrations more accurately reflected bioactive concentrations than total metal concentrations. As discussed in the Introduction, the neutral chloro complexes were also considered based on their lipophilicity [23,24]. The 15-min EC50 values (\pm standard deviation) expressed in these three concentration metameters are provided in Table 3.

If models were fit using total dissolved metal concentrations, all variables except Z^2/r and $AN/\Delta IP$ were statistically significant ($\alpha = 0.05$) in the regression models (Table 4). Although several (e.g., those based on $\log -K_{SO}OH$ or $|\log K_H|$) provided adequate fit, use of calculated free ion concentrations (Table 5) provided the best fitting model. That using free ion concentration and $|\log K_H|$ had a high r^2 of 0.93 and the lowest AIC (Fig.

1). (Because AIC depends on the magnitude of the concentration metameter in the models, it cannot be used to compare relative fit of models based on total versus free ion concentrations.) Those using $\Delta\beta$ also provided adequate fit. Using MAICE, the best fitting, two-independent variable model ($r^2 = 0.85$, AIC = 44.64) was $\log EC50$ (free ion) = $f(\Delta\beta, Z^2/r)$. But $\Delta\beta$ alone accounted for 82% of the variation in EC50 values indicating that Z^2/r contributed little to the model fit.

Metal interactions

When Cu was paired with the other eight metals, interactions conformed to expectations based on ligand-binding tendencies alone. The $|\log K_H|$ values for the various metals were used here to reflect relevant differences in ligand-binding tendencies although, as indicated above, other variables such as $\Delta\beta$ could also have been used for this purpose. Nonparallel (intersecting) lines of metal concentration versus probit of *K* at different competing metal concentrations were produced after combining metal ions with similar $|\log K_H|$ values (e.g., Cu^{2+} and Pb^{2+} in Fig. 2A), indicating metal interaction. In contrast, when metal pairs with dissimilar $|\log K_H|$ values were combined (e.g., Cu^{2+} and Mg^{2+}), the resulting lines of Cu^{2+} concentration versus probit of *K* at different Mg^{2+} concentrations tended to be parallel, indicating little interaction between metals (Fig. 2B).

All metals paired with Cu (Ca, Cd, Hg, Mn, Ni, Pb, or Zn) except Mg produced intersecting lines. The point of intersection was approximated visually and the mean point of intersection from three to four replicates was calculated for each pair. There

Table 4. Results from the regression of total log EC50 and several ion characteristics^a

Log EC50 = $f(x)$	r^2	Model (log EC50=)	MSE	AIC
ΔE_0^b	0.67	0.46 + 1.80(ΔE_0)	1.304	32.06
$X_m^{2,r,b}$	0.61	4.96 - 1.01($X_m^2 r$)	1.429	33.74
$\Delta\beta^b$	0.63	1.80 + 0.51($\Delta\beta$)	1.377	33.06
$\log -K_{SO}MOH^b$	0.86	7.39 - 0.336($\log -K_{SO}MOH$)	0.861	25.37
$ \log K_H ^b$	0.78	-4.17 + 0.69($ \log K_H $)	1.074	28.68
Softness index (σ_p) ^b	0.61	-3.24 + 44.85(σ_p)	1.428	33.74
$AN/\Delta IP, \Delta E_0^b$	0.82	2.02 - 0.26($AN/\Delta IP$) + 1.48(ΔE_0)	1.055	29.34
$\log AN/\Delta IP, \Delta E_0^b$	0.80	2.51 - 2.91($\log AN/\Delta IP$) + 1.48(ΔE_0)	1.108	30.12
$X_m^{2,r,b}, Z^2/r$	0.65	8.46 - 1.22($X_m^2 r$) - 0.60(Z^2/r)	1.448	34.56
$\Delta\beta^*, Z^2/r$	0.64	1.09 + 0.50($\Delta\beta$) + 0.15(Z^2/r)	1.480	34.95

^a Those models with the smallest Akaike's information criterion (AIC) were judged to have the most information regardless of the number of independent variables.

^b Variable had a significant effect on log EC50 ($\alpha = 0.05$).

Table 5. Results from the regression of free ion-based log EC50 and several ion characteristics^a

Log EC50 = f(x)	r ²	Model (log EC50=)	MSE	AIC
ΔE_0	0.15	$-1.42 + 2.10(\Delta E_0)$	5.206	71.83
X_m^2/r	0.34	$5.71 - 1.86(X_m^2/r)$	4.595	66.44
$\Delta\beta^b$	0.82	$-0.55 + 1.44(\Delta\beta)$	2.403	45.26
$\log -K_{so}MOH^b$	0.75	$12.59 - 0.78(\log -K_{so}MOH)$	2.845	49.87
$ \log K_H ^b$	0.93	$-16.65 + 1.87(\log K_H)$	1.534	35.13
Softness index (σ_p) ^b	0.60	$-12.87 + 110.9(\sigma_p)$	3.554	56.84
$AN/\Delta IP, \Delta E_0$	0.52	$4.79 - 1.04(AN/\Delta IP) + 0.83(\Delta E_0)$	4.214	60.77
$\log AN/\Delta IP, \Delta E_0$	0.49	$7.03 - 11.99(\log AN/\Delta IP) + 0.78(\Delta E_0)$	4.338	61.78
$X_m^2/r, Z^2/r$	0.35	$1.70 - 1.62(X_m^2/r) + 0.690(Z^2/r)$	4.926	66.42
$\Delta\beta^b, Z^2/r$	0.85	$-5.22 + 1.36(\Delta\beta) + 0.98(Z^2/r)$	2.381	44.64

^a Those models with the smallest Akaike's information criterion (AIC) were judged to have the most information regardless of the number of independent variables.

^b Variable had a significant effect on log EC50 ($\alpha = 0.05$).

was a clear positive relationship if $|\log K_H|$ values were plotted against these mean points of intersection. There was a general increase in the point of intersection (Fig. 3) as $|\log K_H|$ increased, suggesting diminishing interactions between paired metal ions.

Strong metal interactions were not predicted for Mg with other metals based on the very weak metal-ligand covalent interactions for Mg. Indeed, if the metal pairs with the most similar but high values of $|\log K_H|$ (Mg and Ca) were combined, the lines of Mg^{2+} concentration versus probit K at different Ca^{2+} concentrations were parallel, suggesting little interaction between metals (Fig. 2C). For Mg paired with other metals (Cd, Hg, Mn, Ni, Pb, or Zn), the lines of Mg^{2+} concentration versus probit K generated for the different competing ion concentrations were also parallel.

DISCUSSION

Relative toxicities of metal ions were predictable with linear regression using several measures of ion characteristics. The best model involved $|\log K_H|$, and accounted for 93% of the variation in EC50 values (free ion) for the nine metals. This implied that the differences in ion affinities for intermediate ligands such as many biochemical functional groups with O donor atoms strongly influenced bioactivity of the nine metals. The lack of significance for Z^2/r was expected as the tested divalent metal ions had relatively similar Z^2/r values. It is important to remember that the precision of values for the ion characteristics vary and likely contributed to the relative value of each in fitting the toxicity data also.

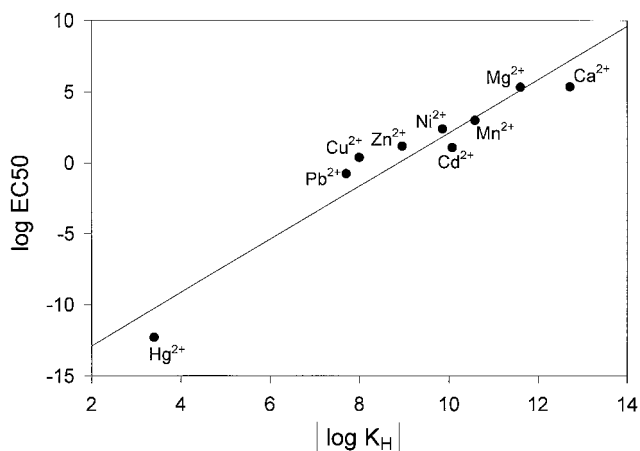


Fig. 1. The model for log EC50 (free ion) and $|\log K_H|$ for nine metals.

Data sets from the literature were fit using these same procedures and good models resulted even without speciation estimation. For growth inhibition of marine algae by $Ca(II)$, $Mg(II)$, $Mn(II)$, $Zn(II)$, $Cd(II)$, $Cu(II)$, $Pb(II)$, and $Hg(II)$ [8], the best model included $|\log K_H|$ ($r^2 = 0.87$), $\log -K_{so} MOH$ ($r^2 = 0.91$), or both X_m^2/r and Z^2/r ($r^2 = 0.87$). *Daphnia magna* reproductive impairment (3 week EC16) [6] was modeled with $AN/\Delta IP$ and ΔE_0 values for 17 metals ($Ca(II)$, $Mg(II)$, $Mn(II)$, $Ni(II)$, $Zn(II)$, $Cd(II)$, $Cu(II)$, $Pb(II)$, $Hg(II)$, $Na(I)$, $K(I)$, $Sr(II)$, $Ba(II)$, $Fe(III)$, $Al(III)$, $Sn(II)$, and $Co(II)$) and accounted for 76% of the variation among EC16 values. Similarly, 48 h LC50 of *D. magna* [6] for $Ca(II)$, $Mg(II)$, $Mn(II)$, $Ni(II)$, $Zn(II)$, $Cd(II)$,

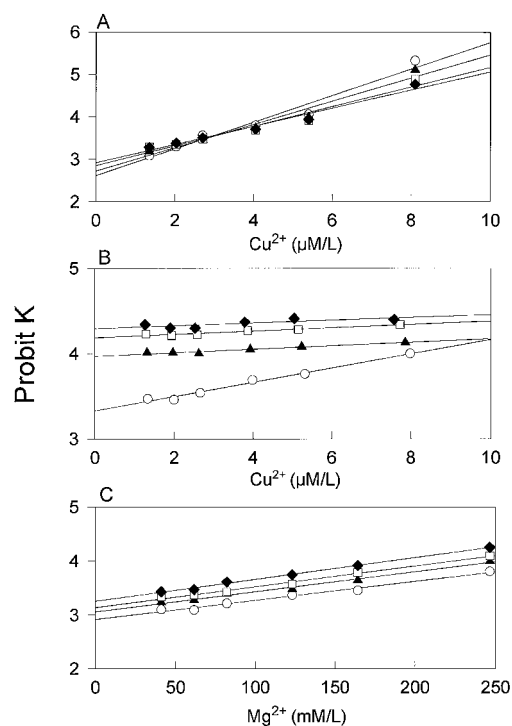


Fig. 2. Probit of first order rate constants (K) versus metal ion concentrations at four different concentrations of potentially competing metal ions. Strong interactions were noted between Cu^{2+} and Pb^{2+} (intersecting lines in A) but no apparent interactions (parallel lines) were noted for the Cu^{2+} and Mg^{2+} (B) and Ca^{2+} and Mg^{2+} (C) metal ion pairs. Respectively, the symbols \circ , \blacktriangle , \square , and \blacklozenge designate Pb^{2+} concentrations in A of 0.238, 0.475, 0.713, and 0.951 $\mu M/L$, Mg^{2+} concentrations in B of 20.6, 41.1, 61.7, and 82.2 mM/L, and Ca^{2+} concentrations in C of 49.9, 99.8, 149.7, and 199.6 mM/L.

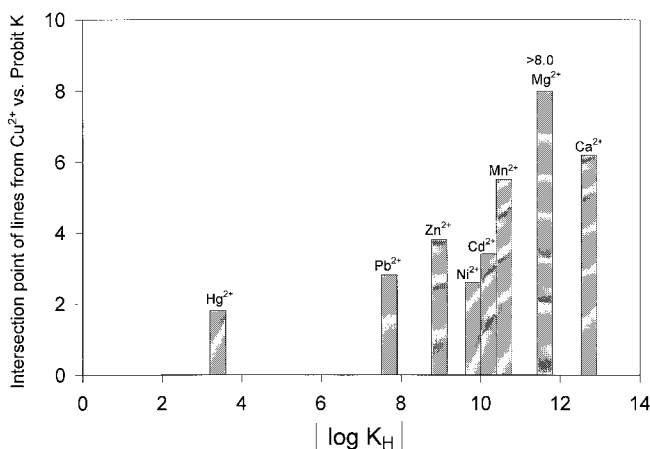


Fig. 3. The degree of metal interaction, measured as the point of intersection in Cu^{2+} versus probit of K plots, for nine metals ranked by $|\log K_H|$. A similar ranking for metals paired with Mg^{2+} showed no such trend.

Cu(II) , Hg(II) , Na(I) , K(I) , Sr(II) , Ba(II) , Al(III) , and Co(II) were effectively modeled with these two parameters ($r^2 = 0.86$). The r^2 values for the reproductive impairment and acute toxic effect data were 0.67 and 0.82, respectively, if $X_m^2 r$ alone was used to generate the regression models. Kaiser [6], using $\log AN/\Delta IP$ instead of $AN/\Delta IP$, obtained much higher correlation coefficients for these last two data sets by developing separate models for metals with noble gas electron configurations, those with partially or completely filled d orbitals, and those with filled s and d orbitals but incomplete p orbitals. It is our intention to expand the present range of metals so that separate models can be assessed for subsets of metal ions with similar electron configurations.

The reader should note that applying these models for prediction of lethal effect may require correction of a backtransformation bias [33] regardless of the final model selected. This bias arises from the use of the log of effect, e.g., $\log \text{EC}_{50}$ or $\log \text{LC}_{50}$. The mean predicted log of effect (e.g., $\log \text{EC}_{50}$) from the original regression model ($\log Y = b_0 + b_1 X + \epsilon$ where ϵ is the model error term) is unbiased, but the predicted value of the effect (e.g., EC_{50}) from the backtransformed linear model ($Y = 10^{b_0} 10^{b_1 X}$) is no longer an unbiased prediction of mean response because the term 10^ϵ is not included in the backtransformed model. The factor, $10^{\ln 10(\text{MSE}^2)}$ can be used to correct for this bias if, as is the case with the models described herein, the residuals appear to be normally distributed. The required MSE values are provided in Tables 4 and 5 for this purpose.

Based on methods for assessing competitive inhibition of enzymes [34] and the conceptual model of Voyer and Heltshe [35], the methods described here allowed visualization and semiquantitative ranking of paired metal interactions. This technique for metal ions has several advantages. First, standard methods for assessing interactions rely on descriptive statistical [35] or mathematical [36–41] models with no direct linkage to mechanisms of toxic action. Their present use is based on the assumption that each toxicant acts nonspecifically as a Selyean stressor because this satisfies the requirement of similarity of mechanism for effect [36,42]. This convenient assumption is often inappropriate. Further, there are also clear indications that resulting additive, synergistic, or antagonistic combinations may be influenced by exposure concentrations in addition to the particular pair of toxicants [42,43]. As can be seen from Figure

2A, concentrations combined near or very distant from the point of intersection would have prompted very different conclusions if the standard approach, i.e., one or few paired concentrations, were used.

As predicted under the simplifying assumption that interactions reflected competition for ligands of biomolecules, those metals with only weak covalent binding showed little interaction if combined. Any metal combined with Mg, a metal with only weak covalent interactions with pertinent ligands, showed little evidence of interaction. In contrast, pairing metals with strong tendencies to complex with intermediate or soft ligands such as those with O or S donor atoms resulted in strong interactions. Consequently, the assumption that interactions reflected competition for ligands of biomolecules was supported for this limited number of divalent metal ions. More work with a wider range of metals including those with more divergent Z^2/r values is required. Refinement of the semiquantitative methods used here is also essential during any extension of this approach. Indeed, results shown here may only be pertinent to the range of combined concentrations tested.

Regardless of the limitations of these data and their interpretations, the hypotheses were supported that trends in relative toxicities and metal ion interactions could be predicted from ion characteristics reflecting differences in metal–ligand interactions. Successful development of *D. magna* acute toxicity and reproductive impairment models and an algal growth inhibition model indicate the potential for this parsimonious conceptual model being extended beyond this simple microbial assay.

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