Using Metal-Ligand Binding Characteristics to Predict Metal Toxicity: Quantitative Ion Character–Activity Relationships (QICARs)

Michael C. Newman,[1] John T. McCloskey,[2] and Christopher P. Tatara[3]

[1]College of William and Mary, Virginia Institute of Marine Science, Gloucester Point, Virginia; [2]U.S. Fish and Wildlife Service, Gloucester, Virginia; [3]University of Georgia, Savannah River Ecology Laboratory, Aiken, South Carolina

Ecological risk assessment can be enhanced with predictive models for metal toxicity. Modelings of published data were done under the simplifying assumption that intermetal trends in toxicity reflect relative metal–ligand complex stabilities. This idea has been invoked successfully since 1904 but has yet to be applied widely in quantitative ecotoxicology. Intermetal trends in toxicity were successfully modeled with ion characteristics reflecting metal binding to ligands for a wide range of effects. Most models were useful for predictive purposes based on an F-ratio criterion and cross-validation, but anomalous predictions did occur if speciation was ignored. In general, models for metals with the same valence (i.e., divalent metals) were better than those combining mono-, di-, and trivalent metals. The softness parameter (σ_p) and the absolute value of the log of the first hydrolysis constant ($|\log K_{OH}|$) were especially useful in model construction. Also, ΔE_o contributed substantially to several of the two-variable models. In contrast, quantitative attempts to predict metal interactions in binary mixtures based on metal–ligand complex stabilities were not successful. — *Environ Health Perspect* 106(Suppl 6):1419–1425 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-6/1419-1425newman/abstract.html*

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Quantitative structure–activity relationships (QSARs) are applied widely to predict bioactivity (e.g., toxicity or bioavailability) of organic compounds. In contrast, models relating metal ion characteristics to their bioactivity remain underexploited. A few models exist for human risk prediction [e.g., Williams et al. (*1*)] but quantitative models have not been fully explored for nonhuman species. This is surprising because such quantitative ion character–activity relationships (QICARs) would be extremely useful for predicting effects of untested metals during risk assessment activities. Also, qualitative ion character–activity relationships (ICARs) based on simple metal–ligand binding have been described in the literature for nearly a century. As an early example, Mathews (*2*) assumed that metals were most active in their ionic form (the ionic hypothesis) and correlated metal toxicity to characteristics of ion binding to biomolecules. Especially useful were characteristics reflecting bond stability with ligand groups possessing O, N, and S donor atoms. For the last half-century, permutations on this approach were applied successfully by Jones (*3,4*), Binet (*5*), Loeb (*6*), McGuigan (*7*), Biesinger and Christensen (*8*), Jones and Vaughn (*9*), Kaiser (*10*), Williams and Turner (*11*), Babich et al. (*12,13*), Fisher (*14*), Newman and McCloskey (*15*), McCloskey et al. (*16*), and Tatara et al. (*17,18*). Modeling was often based on hard and soft acid and base theory (*9,11,19*).

This approach has not been evaluated for its predictive usefulness despite clear indications from ICARs that QICARs were feasible. Newman and McCloskey (*15*) suggested that the contrasting extent of QSAR and QICAR development resulted from two factors. First, the QSAR approach was quickly incorporated into ecotoxicology because it had already proven its worth in pharmacology and human toxicology. In contrast, QICARs were not well established in pharmacology or human toxicology because the major focus of these disciplines was organic drugs and poisons. Second, chemical speciation complicates prediction because several metal species are present simultaneously and the bioavailability of each is ambiguous. However, some of this ambiguity can be removed by judiciously applying the free ion activity model (FIAM) (*20*). The FIAM, an extension of the ionic hypothesis, holds that the bioactivity of a dissolved metal is correlated with its free ion concentration or activity. The complication of simultaneous exposure to many species can be minimized by focusing on the free ion. Because both impediments are resolvable, no inherent obstacle impedes QICAR development to the same level of utility as that of QSARs.

This paper assesses the QICAR approach for predicting metal toxicity. This is done by reanalyzing metal effects data reported elsewhere. Models are assessed by cross-validation (PRESS method as described in "Methods") relative to their effectiveness for predicting bioactivity of untested metals. An attempt is also made to extend this approach to prediction of metal interactions in binary mixtures.

Methods

Data Sets

Nineteen published data sets were selected that report effects for an adequate number and range of metals (Table 1). To reinforce the generality of conclusions, we used data for widely differing species, metals, modes of exposure, and effects. To avoid bias in conclusions, only the most comprehensive data set was selected if very similar sets existed. Published data sets involved

Table 1. Data sets used in modeling.

Species or enzyme system	Exposure time	Effect	Ions	Reference
Carbonic anhydrase (from catfish)	–	I_{50}	Na, Ca, Mn(II), Fe(III), Co, Ni, Cu(II), Zn, Ag, Cd, Hg, Pb	Christensen (21)
Lactic dehydrogenase (from white sucker)	–	I_{20}	Mn(II), Fe(III), Co, Cu(II), Zn, Ag, Cd, Hg	Christensen and Tucker (22)
Glutamic oxalacetic transaminase (from white sucker)	–	I_{20}	Ca, Fe(III), Ni, Cu(II), Zn, Ag, Cd, Hg	Christensen and Tucker (22)
Turbot (TF) cells	96 hr	NR_{50}	Mn(II), Co, Ni, Cu(II), Zn, Cd, Pb	Magwood and George (23)
Bluegill (BF-2) cells	96 hr	NR_{50}	Cr(III), Mn(II), Co, Ni, Cu(II), Zn, Ag, Hg, Pb	Babich et al. (12,13)
Hamster cells	7 days	CE_{50}	Mg, Mn(II), Co, Ni, Cu(II), Zn, Sr, Ag, Cd, Hg	Hsie et al. (24)
Vibrio fischeri (Microtox) (bioluminescence)	15 min	EC_{50}	Li, Na, Mg, K, Ca, Cr(III), Mn(II), Fe(III), Co, Ni, Cu(II), Zn, Sr, Ag, Cd, Cs, La, Hg, Pb	McCloskey et al. (16)
Diatom	–	VCF	Mg, Ca, Cr(III), Mn(II), Fe(III), Co, Ni, Cu(II), Zn, Ag, Cd, Cs, Hg, Pb	Fisher (14)
Fungi (Alternaria tenuis) (germination)	18 hr	ED_{50}	Li, Na, Mg, K, Ca, Cr(III), Mn(II), Co, Ni, Cu(II), Zn, Sr, Ag, Hg, Pb	Somers (25)
Fungi (Botrytis fabae) (germination)	18 hr	ED_{50}	Li, Na, Mg, K, Ca, Cr(III), Mn(II), Co, Ni, Cu(II), Zn, Sr, Ag, Hg, Pb	Somers (25)
Nematode (Caenorhabditis elegans)	24 hr	LC_{50}	Li, Na, Mg, K, Ca, Cr(III), Mn(II), Fe(III), Co, Ni, Cu(II), Zn, Sr, Cd, Cs, La, Pb	Tatara et al. (18)
Planaria (Polycelis nigra)	48 hr	TC	Na, Mg, K, Ca, Cr(III), Mn(II), Co, Ni, Cu(II), Zn, Sr, Ag, Cd, Hg, Pb	Jones (4)
Daphnia magna	48 hr	LC_{50}	Na, Mg, K, Ca, Cr(III), Mn(II), Fe(III), Co, Ni, Cu(II), Zn, Sr, Cd, Hg, Pb	Khangarot and Ray (26)
Daphnia magna (reproduction)	3 weeks	EC_{16}	Na, Mg, K, Ca, Cr(III), Mn(II), Fe(III), Co, Ni, Cu(II), Zn, Sr, Cd, Hg, Pb	Biesinger and Christensen (8)
Daphnia magna	3 weeks	LC_{50}	Na, Mg, K, Ca, Cr(III), Mn(II), Fe(III), Co, Ni, Cu(II), Zn, Sr, Cd, Hg, Pb	Biesinger and Christensen (8)
Fruit fly (Drosophila melanogaster)	4 days	LC_{50}	Mg, Cr(III), Mn(II), Co, Ni, Cu(II), Zn, Sr, Ag, Cd, Hg	Williams et al. (1)
Amphipod (Cranogonyx pseudogracillis)	96 hr	LC_{50}	Cr(III), Mn(II), Fe(III), Co, Ni, Cu(II), Zn, Ag, Cd, Hg, Pb	Martin and Holdich (27)
Three-spined stickleback (Gasterosteus aculeatus)	10 days	TC	Na, Mg, K, Ca, Cr(III), Mn(II), Co, Ni, Cu(II), Zn, Sr, Ag, Cd, Hg, Pb	Jones (3)
Mouse	14 days	LD_{50}	Mg, Cr (III), Mn (II), Fe (III), Co, Ni, Cu(II), Zn, Sr, Ag, Cd, Hg, Pb	Williams et al. (1)

Abbreviations: CE_{50}, 50% effect concentration on ability to form colonies; EC_{16}, effective concentration for 16% response; ED_{50}, effective dose for 50% response; I_{20}, inhibition at the 20% level; I_{50}, inhibition at the 50% level; LC_{50}, concentration killing 50% of exposed individuals; NR_{50}, neutral red response at the 50% level; TC, threshold concentration; VCF, volume concentration factor.

enzyme inactivation (21,22), viability of cultured metazoan cells including cells from two fish (12,23) and a mammal (24), germination inhibition of two fungi (25), bioaccumulation in a marine diatom (14), inhibition of bacterial bioluminescence (Microtox assay, Microbics Corporation, Carlsbad, California) (16), and acute toxicity to soil nematodes (17,18). Acute toxicity was also examined for diverse aquatic invertebrates including a planarian (4), cladoceran (26), insect (1), and amphipod (27). Several data sets involved chronic exposures with lethal (1,3,8) or sublethal (8) end points. For all studies, barium toxicity was excluded from models because of its very specific interference with K^+ flux in excitable tissues of metazoans (28–31).

Ion Characteristics

One- and two-explanatory variable models were constructed from six ion qualities. The electronegativity (χ_m) and Pauling ionic radius (r) were combined to produce a covalent index ($\chi_m^2 r$) reflecting the relative importance of covalent versus electrostatic interactions during metal–ligand binding (32). The ion charge (Z) and Pauling ionic radius were combined to generate a second index, the cation polarizing power (Z^2/r), reflecting the energy of the metal ion during electrostatic interaction with a ligand (32). However, no models using Z^2/r are reported here because this index did not contribute to the best candidate model for any data set. A softness index (σ_p) (9,11) was produced by dividing the difference between the coordinate bond energies of the metal fluoride and iodide by the coordinate bond energy of the metal fluoride. This index reflected metal ion softness, the relative tendency for the outer electron shell to deform (polarizability), and the ion's tendency to share electrons with ligands. Metal affinity to intermediate ligands such as those with O donor atoms was estimated

with another index ($|\log K_{OH}|$) based on the first hydrolysis constant of the ion, i.e., K_{OH} for $M^{n+} + H_2O \rightarrow MOH^{n-1} + H^+$ (15). Following the approach of Kaiser (10), $AN/\Delta IP$ and ΔE_o were also explored in model development. Log $AN/\Delta IP$ did not improve models, as suggested by Kaiser (10); $AN/\Delta IP$ was used instead. Atomic number (AN), notionally reflecting ion inertia or size, was combined with ΔIP (the difference in ionization potentials for the ion oxidation number OX and OX-1), which reflected ionization potential. The absolute difference between the electrochemical potential of the ion and its first stable reduced state (ΔE_o) reflected an ion's ability to change electronic state. Values for these ion characteristics used in this study are tabulated by McCloskey et al. (16).

Model Assessment

Linear regression models were generated with these six variables ($\chi_m^2 r$, Z^2/r, σ_p,

$|\log K_{OH}|$, $AN/\Delta IP$, ΔE_o), and the SAS Procedure GLM general linearized model (33). Models including Z^2/r were not reported for reasons already stated. Three levels of model selection followed model generation. This procedure was applied to models including divalent metals alone or all metals regardless of valence. First, the contribution of a variable to each model was tested for statistical significance (F statistic from Type III sum of squares, $\alpha = 0.05$). Only models in which all variables contributed significantly were considered further. Second, the predictive potential of models was estimated with an F-ratio approach because usefulness for prediction is not reflected accurately by a model's statistical significance. More rigorous criteria must be applied. A ratio of the observed F statistic (regression sum of squares divided by the residual sum of squares) to the critical F statistic ($\alpha = 0.05$) greater than 4 to 5 is one accepted, albeit arbitrary, threshold for acceptable predictive utility (34). The most stringent $F_{observed}/F_{critical}$ of ≥ 5 was adopted here. Finally, if more than one useful model existed for a data set, the best was selected by minimum Akaike's information criterion estimation (MAICE) (35). With MAICE, models that differ in complexity (i.e., one- vs two-explanatory variables) can be compared. An Akaike's information criterion (AIC) was calculated with the log likelihood function of each model [details can be found in Yamaoka et al. (35) and Newman and McCloskey (15)]: AIC = −2(log likelihood) + 2P, where P is the number of estimated parameters in the candidate model. The model with the smallest AIC was judged to contain the most information. With this three-step procedure, the best model was selected from among those that were potentially useful for prediction.

Cross-validation was performed on the best divalent metal models to estimate the magnitude of deviations in effect prediction for unknown metals. For each of the 13 divalent metal data sets producing potentially useful models, a series of models was generated after omitting one metal at a time. Each time this was done, the ion characteristics of the omitted metal were placed into the model to predict an effect for the omitted metal. This cross-validation (36) was done with the option PRESS in SAS Procedure REG (33). The deviation from perfect prediction was expressed as the percentage [(observed effect$_{metal\ i}$ − predicted effect$_{model\ without}$

metal $_i$)/observed effect$_{metal\ i}$] × 100. Median and interquartile ranges for these percentages summarize the general deviations from perfect prediction.

Interactions in Binary Mixtures

Bacterial bioluminescence data for binary mixtures of metals (15) were examined statistically to assess the hypothesis that metals with strong and similar covalent binding to ligands will interact strongly. The qualitative conclusions of Newman and McCloskey (15) were tested statistically for two series of mixtures: Cu in combination with Ca, Cd, Hg, Mg, Mn, Ni, Pb, or Zn; and Mg in combination with Ca, Cd, Hg, Mn, Ni, Pb, or Zn. Interactions were assessed statistically using the SAS Procedure MIXTURE (33) with an interaction term (i.e., metal$_1$ × metal$_2$). Statistical significance and magnitude of the interaction terms were used to assess interactions between paired metals. If the above hypothesis was correct, the intensity of interaction would be greatest between Cu and similar metals (e.g., Hg or Cd) and lowest for Cu and dissimilar metals (e.g., Mg or Ca). In the second series of mixtures there would be little interaction between Mg and other metals.

Results

Models for Divalent Metals

High correlation coefficients were associated with many one- and two-variable models for divalent metal effects (Figure 1 and Table 2). Based on a stringent criterion of an F ratio ≥ 5, 13 of the 19 data sets had at least one model of predictive utility. Data sets failing to produce useful models involved cultured cell viability (three studies), *in vitro* inactivation of carbonic anhydrase, and inhibition of fungal germination. Fruit fly mortality data also failed to produce a useful model according to our stringent criterion but nevertheless had a high F ratio of 4.5. One- or two-variable models of most predictive promise included $|\log K_{OH}|$ or σ_p. Several two-variable models, especially those including ΔE_o, were also among those with predictive promise. The covalent index ($\chi_m^2 r$) alone or combined with another variable never produced the best model for any data set. Five of the thirteen most informative models (MAICE) were single-variable models. The median r^2 for these best and predictively useful models was 0.90 (range 0.78 to 0.97). Approximately 90% of the variation in metal effect could be explained by the models.
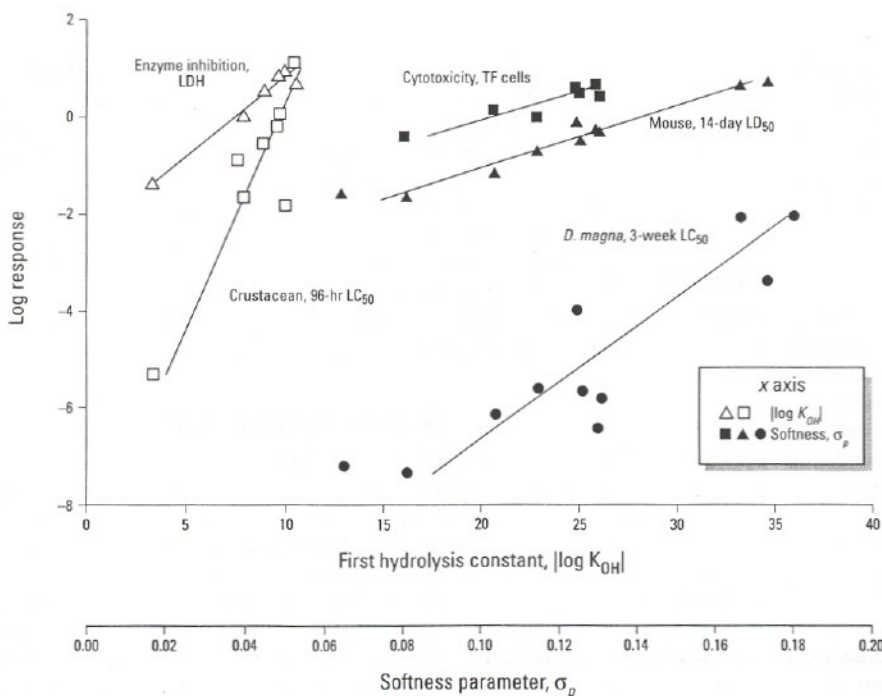

Figure 1. Representative data illustrating the relationship between metal effects and metal ion characteristics. Responses range widely from enzyme inhibition (lactic dehydrogenase, LDH) (22) to toxicity of cultured turbot cells (23) to acute lethality of a crustacean (amphipod) (27) to chronic toxicity of mice (1) and *Daphnia magna* (8).

Table 2. Correlation coefficients (r^2) for models with divalent metals.[a]

Study	One-variable models			Two-variable models				
	$\chi_m^2 r$	σ_p	$|\log K_{OH}|$	$(\chi_m^2 r, |\log K_{OH}|)$	$(AN/\Delta IP, \Delta E_0)$	$(\sigma_p, |\log K_{OH}|)$	$(\sigma_p, \Delta E_0)$	$(|\log K_{OH}|, \Delta E_0)$
Carbonic anhydrase	0.09 N	0.42 N	0.51 S	0.55 N, S	0.22 N, N	0.54 N, N	0.56 S, N	0.58 S, N
Lactic dehydrogenase	0.71 S	0.45 N	**0.97**[b] S	0.97 N, S	0.50 N, N	0.97 N, S	0.51 N, N	0.97 S, N
Glutamic oxalacetic transaminase	0.76 S	0.70 S	**0.91**[b] S	0.91 N, N	0.75 N, N	0.92 S, S	0.85 S, N	0.92 N, N
Turbot (TF) cells	0.01 N	0.85 S	0.01 N	0.06 N, N	0.02 N, N	0.89 S, N	0.85 S, N	0.01 N, N
Bluegill (BF-2) cells	<0.01 N	0.81 S	0.39 N	0.54 N, N	0.17 N, N	0.82 S, N	0.82 S, N	0.41 N, N
Hamster cells	0.49 S	0.73 S	0.24 N	0.64 S, N	0.84 N, S	0.89 S, S	0.85 N, N	0.73 N, S
Bacteria (*V. fischeri*)	0.63 S	0.72 S	**0.77** S	0.82 N, S	0.81 S, S	0.82 N, N	0.81 N, N	**0.89**[b] S, S
Diatom	0.48 S	0.57 S	0.63 S	0.68 N, N	0.79 N, N	0.67 N, N	0.79 N, N	**0.90**[b] S, S
Fungi (*Altemaria tennis*)	0.64 S	0.60 S	0.72 S	0.80 N, N	0.71 N, S	0.73 N, N	0.66 N, N	0.78 N, N
Fungi (*Botrytis fabae*)	0.58 S	0.72 S	0.76 S	0.80 N, N	0.85 N, S	0.77 N, N	0.85 N, N	**0.91**[b] S, S
Nematode (*Caenorhabditis elegans*)	0.73 S	0.34 S	**0.79**[b] S	0.86 N, S	0.73 N, S	0.80 N, S	0.52 N, N	0.83 N, S
Planaria (*Polycelis nigra*)	0.20 N	**0.84**[b] S	0.70 S	0.75 N, S	0.43 N, N	**0.86** S, N	0.85 S, N	0.72 N, N
Daphnia magna (48-hr LC_{50})	0.37 S	**0.83** S	0.86 S	0.86 **N, S**	0.62 N, S	**0.93**[b] S, S	0.83 S, N	**0.88** S, N
Daphnia magna (reproduction)	0.43 S	**0.78**[b] S	0.50 S	0.54 N, N	0.82 N, S	0.78 S, N	0.86 S, N	0.77 N, S
Daphnia magna (3-week LC_{50})	0.38 S	**0.80** S	0.54 S	0.56 N, N	0.81 N, S	0.80 N, N	**0.88**[b] S, S	0.79 N, S
Fruit fly (*Drosophila melanogaster*)	0.55 S	0.60 S	0.26 N	0.75 S, N	**0.89**[b] S, S	0.66 S, N	0.61 N, N	0.45 N, N
Amphipod (*Cranogonyx pseudogracilis*)	0.12 N	0.77 S	0.81 S	0.83 N, S	0.37 N, N	**0.97**[b] S, S	0.79 S, N	0.81 S, N
Three-spined stickleback	0.55 S	**0.78** S	**0.79** S	0.81 N, S	0.79 N, S	0.86 N, N	0.83 S, N	**0.89**[b] S, S
Mouse	0.16 N	**0.95**[b] S	0.52 S	0.53 N, S	0.53 N, S	0.96 S, N	0.96 S, N	0.65 N, N

[a] Correlation coefficients are bold for models with all significant covariates and an F ratio ≥ 5.0. The specific ions fit to each models are listed in Table 1. Statistical significance is noted by an S or N, e.g., N, S for the $(\sigma_p, |\log K_{OH}|)$ model indicates that σ_p did not, but $|\log K_{OH}|$ did, contribute significantly to the model. [b] Characteristic(s) providing the best model (MAICE).

Cross-validation of the best divalent metal models (Table 3) indicated that the median deviations between observed and predicted effects were small. The median deviations were less than 22%; most were closer to 10%. For comparison, a well-known QSAR model for bioconcentration of eight organic compounds in fish (*37*) had a median difference of –7% and an interquartile range of –13 to 14%. However, many models poorly predicted effects for specific metals. These metals tended to be extreme class a (e.g., Mg), class b metals that undergo considerable speciation in solution (e.g., Hg, Pb), or metals with the tendency to precipitate from solution (e.g., Mn). Under the assumption that speciation contributed to some of these poor predictions by models built from total metal concentrations, concentrations of free metal ion were estimated with the MINTEQA2 Version 3.10 program (*38*) for two data sets involving bacterial bioluminescence (*15,16*). These assays were conducted in contrasting media having speciation similar to marine (*15*) or freshwater (*16*) environments. Except for

Hg in media having speciation similar to marine systems, EC_{50} values of metals were expressed as the free ion concentration. The EC_{50} for Hg was expressed in terms of the free ion plus neutral chloro complex concentration because neutral chloro complexes of Hg can also be bioavailable (*39*). In both media the extremely discordant predictions were greatly diminished or eliminated if EC_{50} values were expressed in terms of the speciated metal concentration (Table 3).

Models Including All Metals

Although correlation coefficients were lower than those for the divalent metal models (median 0.80, range 0.67 to 0.87), useful models including all metals (mono-, di-, and trivalent) were generated for 13 of the 19 data sets (Table 4). Approximately 80% of the variation in effect for metals could be attributed to the explanatory variables. Eight of thirteen data sets producing useful models had the best (MAICE) model involving only one explanatory variable. Again, the $|\log K_{OH}|$ or σ_p indices contributed to many of the best one-variable

models. As with the divalent metal models, data sets failing to produce useful models tended to be those for *in vitro* enzyme inactivation or cultured cell viability. Data for bioaccumulation of metals in a marine diatom and crustacean toxicity also failed to produce useful models.

Metal Mixtures

Although there were qualitative indications of concentration-dependent interactions between metals with similar and high covalent binding tendencies, no such trends were noted in this formal analysis. The only significant trends in the intensity of the interaction term for both series of mixtures was a consequence of increasing LC_{50} values with decreasing covalent interactions; this trend was an artifact of the data structure. Regardless of whether Cu or Mg was combined with metals, there was a upward trend in the interaction term, with increasing tendency of the competing metal to interact covalently with ligands. Such a trend for the Mg series of binary mixtures was inconsistent with predictions from the initial hypothesis. The results did not support the

Table 3. Cross-validation of the best models for divalent metals.[a]

Data set	Median, %	Interquartile range (Q_{25} to Q_{75}), %	Extreme predictions, % deviation $\geq 100\%$
Lactic dehydrogenase	13	−53 to 21	−1484 (Cu)
Glutamic oxalacetic transaminase	4	−15 to 27	
Bacteria, freshwater (V. fischeri)	−9	−445 to 13	−9806 (Hg)
			−1510 (Pb)
			−445 (Cu)
Bacteria, freshwater (V. fischeri) speciated	6	−17 to 22	156 (Pb)
			−389 (Cu)
			−105 (Cd)
Bacteria, marine (V. fischeri)	7	−55 to 15	−1027 (Hg)
			1194 (Pb)
Bacteria, marine (V. fischeri) speciated	12	−28 to 52	−149 (Hg)
			129 (Pb)
Diatom	1	−30 to 13	−325 (Mg)
			135 (Ca)
Fungi (Botrytis fabae)	21	−47 to 57	786 (Zn)
Nematode (Caenorhabditis elegans)	12	−24 to 30	182 (Pb)
Planaria (Polycelis nigra)	−6	−46 to 105	132 (Mn)
			105 (Pb)
Daphnia magna (48-hr LC$_{50}$)	−6	−40 to 21	101 (Mg)
Daphnia magna (reproduction)	22	−154 to 68	250 (Mg)
			154 (Mn)
Daphnia magna (3-week LC$_{50}$)	−1	−9 to 7	
Amphipod (Cranogonyx pseudogracilis)	19	−31 to 70	287 (Co)
			−241 (Ni)
Three-spined stickleback	−7	−83 to 23	−708 (Mn)
			−200 (Co)
Mouse	8	−8 to 17	−258 (Mn)

[a]Deviations are expressed as percentages, [(observed effect$_{metal\ i}$ − predicted effect$_{model\ without\ metal\ i}$)/observed effect$_{metal\ i}$] × 100.

initial hypothesis that mixture interactions could be predicted from the tendency to covalently bind with ligands.

Conclusion

Quantitative ion character–activity relationships can be developed for a range of effects based on metal–ligand binding theory. Estimations of speciation and application of the FIAM were not required to develop useful QICARs for some metals in the data sets. Our work with QICAR development for microbial bioluminescence (15,16) and nematode toxicity (17,18) supports this observation. However, there are clear indications that calculation of free ion concentrations or activities will greatly improve modeling, i.e., eliminate or reduce the magnitude of anomalous predictions for some class b metals.

The results for the relatively simple *in vitro* enzyme inactivation and cultured cell viability studies illustrate the difficulties associated with using models based on total metal concentration. These data sets involved buffered or complex media, i.e., the enzyme inactivation in a buffered phosphate solution and cell culture experiments in complex media containing components such as 10% fetal calf serum. Another data set failing to produce a useful model was associated with a high ionic strength media, i.e., bioaccumulation in a marine diatom.

Table 4. Correlation coefficients (r^2) for models with all metals (mono-, di- and trivalent metals).[a]

Study	One-variable models			Two-variable models				
	$\chi_m^2 r$	σ_p	$\lvert \log K_{OH} \rvert$	($\chi_m^2 r$, $\lvert \log K_{OH} \rvert$)	(AN/ΔIP, ΔE$_o$)	(σ_p, $\lvert \log K_{OH} \rvert$)	(σ_p, ΔE$_o$)	($\lvert \log K_{OH} \rvert$, ΔE$_o$)
Carbonic anhydrase	0.23 N	0.58 S	0.25 N	0.35 N, N	0.28 N, N	0.59 S, N	0.69 S, N	0.26 N, N
Lactic dehydrogenase	0.79 S	0.49 N	<0.01 N	0.79 S, N	0.57 N, N	0.50 N, N	0.52 N, N	0.08 N, N
Glutamic oxalacetic transaminase	**0.84**[b] S	0.60 S	<0.01 N	0.85 S, N	0.60 S, N	0.67 S, N	0.75 S, N	0.05 N, N
Turbot (TF) cells	0.01 N	0.85 S	0.01 N	0.06 N, N	0.02 N, N	0.89 S, N	0.85 S, N	0.01 N, N
Bluegill (BF-2) cells	0.10 N	0.60 S	0.05 N	0.15 N, N	0.31 N, N	0.76 S, N	0.60 S, N	0.18 N, N
Hamster cells	0.55 S	**0.77**[b] S	0.11 N	0.55 S, N	0.82 S, S	0.83 S, N	0.86 S, N	0.67 N, N
Bacteria (V. fischeri)	**0.70** S	**0.80** S	0.49 S	**0.83**[b] S, S	0.80 N, S	0.81 S, N	0.84 S, N	0.78 N, S
Diatom	0.44 S	0.60 S	0.44 S	0.68 S, S	0.73 N, S	0.65 S, N	0.73 N, S	0.73 N, S
Fungi (Alternaria tennis)	0.59 S	**0.67**[b] S	0.56 S	0.74 S, S	0.67 N, N	0.71 S, N	0.71 N, N	0.67 N, N
Fungi (Botrytis fabae)	0.58 S	**0.69**[b] S	0.56 S	0.74 S, S	0.76 N, S	0.72 S, N	0.77 N, N	0.75 N, S
Nematode (Caenorhabditis elegans)	0.55 S	0.49 N	**0.69** S	**0.85**[b] S, S	0.56 N, S	0.70 N, S	0.54 N, N	0.72 S, N
Planaria (Polycelis nigra)	0.35 S	**0.70**[b] S	0.29 S	0.43 N, N	0.47 N, N	0.71 S, N	0.70 S, N	0.41 N, N
Daphnia magna (48-hr LC$_{50}$)	0.52 S	**0.80**[b] S	0.58 S	0.66 N, N	0.67 N, S	0.82 S, N	0.80 S, N	0.66 N, N
Daphnia magna (reproduction)	0.50 S	0.68 S	0.28 S	0.54 S, N	**0.84**[b] S, S	0.74 S, N	0.76 N, N	0.71 N, S
Daphnia magna (3-week LC$_{50}$)	0.47 S	**0.70** S	0.30 S	0.52 S, N	**0.83**[b] S, S	0.75 S, N	0.77 N, N	0.71 N, S
Fruit fly (Drosophila melanogaster)	0.43 S	0.56 S	0.13 N	0.46 N, N	**0.87**[b] S, S	0.57 S, N	0.59 N, N	0.39 N, N
Amphipod (Cranogonyx pseudogracilis)	0.27 N	0.68 S	<0.01 N	0.27 N, N	0.46 S, N	0.71 S, N	0.69 S, N	0.05 N, N
Three-spined stickleback	0.63 S	**0.71**[b] S	0.42 S	0.71 S, N	0.76 S, S	0.71 S, N	0.74 S, N	0.63 N, S
Mouse	0.20 N	**0.71**[b] S	0.05 N	0.23 N, N	0.50 N, S	0.76 S, N	0.73 S, N	0.39 N, S

[a]Correlation coefficients are bold for models with all significant covariates and an F ratio ≥ 5.0. The specific ions fit to each models are listed in Table 1. Statistical significance is noted by a S or N, e.g., N, S for the (σ_p, $\lvert \log K_{OH} \rvert$) model indicates that σ_p did not, but $\lvert \log K_{OH} \rvert$ did, contribute significantly to the model. [b]Characteristic(s) providing the best model (MAICE).

This association of model failure with complex exposure media suggests that speciation calculation would improve modeling because speciation is most extensive under these conditions. This is further supported by the diminution of deviations from perfect prediction during cross-validation of models considering speciation. If speciation is ignored, predictions of effect should be done cautiously for class b and some intermediate metals characterized by extensive speciation in solution. Published trends for metal speciation in marine (40) and freshwater (40,41) systems can be used to identify those metals for which speciation should be considered during QICAR development.

The ion characteristics of most general value in constructing QICARs were |log K_{OH}| and σ_p, although other variables such as ΔE_o were also important in several models. The |log K_{OH}| reflects the tendency for a metal ion to form a stable complex with intermediate ligands. Intermediate ligands on biomolecules would include groups with O donor atoms (e.g., carboxyl groups). This suggested that binding with such functional groups is important in determining the relative bioactivity of metals. The softness index (σ_p) quantifies the ability of a metal ion to accept an electron during interaction with a ligand. It reflects the importance of covalent interactions relative to electrostatic interactions (32) in determining intermetal trends in bioactivity.

The results also suggest that QICARs based on the characteristics used in this study are best developed for metals of similar charge. Although models based on variables such as σ_p did produce viable models, Ahrland (42) and Williams and Turner (11) argue against the application of σ_p for metals differing in charge. Instead, variables adjusting for differences in charge, such as σ_k (11), may be required.

Effective application of the QICAR approach may also be improved by careful examination of the values used to generate the explanatory variables. Considerable judgment is required when selecting among published estimates. More involved analysis of these data for application to QICAR generation is currently required. Regardless, QICARs are now feasible, especially if they were produced with speciation concentrations for metals of similar charge.

Ecological risk assessment would be enhanced by reliable models for predicting effects of untested metals from known effects of tested metals. In the absence of complete information on the effect of all metals of concern on each important species under a variety of conditions, the ability to interpolate from existing data to predict effects for untested metals would improve the accuracy of assessments. QICARs would be particularly useful in preliminary screening and in situations analogous to those in which QSARs are currently applied. Our results suggest that the QICAR approach would be extremely useful for this purpose. However, several resolvable issues require attention before the QICAR approach has the same general usefulness as the QSAR approach. These issues include exploration of more explanatory variables, careful evaluation of ionic qualities used to calculate explanatory variables, examination of models capable of predicting effects for widely differing metals (e.g., metals of different valence states), effective inclusion of chemical speciation, examination of more effects, and assessment of the applicability of QICARs to phases such as sediments, soils, and food.

REFERENCES AND NOTES

1. Williams MW, Hoeschelle JD, Turner JE, Jacobson KB, Christie NT, Paton CL, Smith LH, Witschi HR, Lee EH. Chemical softness and acute toxicity in mice and *Drosophila*. Toxicol Appl Pharmacol 63:461–469 (1982).
2. Mathews AP. The relation between solution tension, atomic volume, and the physiological action of the elements. Am J Physiol 10:290–323 (1904).
3. Jones JRE. The relation between the electrolytic solution pressures of the metals and their toxicity to the stickleback (*Gasterosteus aculeatus* L.). J Exp Biol 16:425–437 (1939).
4. Jones JRE. A further study of the relation between toxicity and solution pressure, with *Polycelis nigra* as test animal. J Exp Biol 17:408–415 (1940).
5. Binet MP. Sur la toxicité comparée des métaux alcalins et alcalino-terreux. CR Acad Sci Paris 115:251–253 (1940).
6. Loeb J. Studies on the physiological effects of the valency and possibility the electrical charges of ions. I: The toxic and antitoxic effects of ions as a function of their valency and possibly their electrical charge. Am J Physiol 6:411–433 (1940).
7. McGuigan H. The relation between the decomposition-tension of salts and their antifermentative properties. Am J Physiol 10:444–451 (1954).
8. Biesinger KE, Christensen GM. Effects of various metals on survival, growth, reproduction, and metabolism of *Daphnia magna*. Can J Fish Aquat Sci 29:1691–1700 (1972).
9. Jones MR, Vaughn WK. HSAB theory and acute metal ion toxicity and detoxification processes. J Inorg Nucl Chem 40:2081–2088 (1978).
10. Kaiser KLE. Correlation and prediction of metal toxicity to aquatic biota. Can J Fish Aquat Sci 37:211–218 (1980).
11. Williams MW, Turner JE. Comments on softness parameters and metal ion toxicity. J Inorg Nucl Chem 43:1689–1691 (1981).
12. Babich H, Puerner JA, Borenfreund E. *In vitro* cytotoxicity of metals to bluegill (BF-2) cells. Arch Environ Contam Toxicol 15:31–37 (1986).
13. Babich H, Shopsis C, Borenfreund E. *In vitro* cytotoxicity testing of aquatic pollutants (cadmium, copper, zinc, nickel) using established fish cell lines. Ecotoxicol Environ Saf 11:91–99 (1986).
14. Fisher NS. On the reactivity of metals for marine phytoplankton. Limnol Oceanogr 31:443–449 (1986).
15. Newman MC, McCloskey JT. Predicting relative toxicity and interactions of divalent metal ions: Microtox® bioluminescence assay. Environ Toxicol Chem 15:275–281 (1996).
16. McCloskey JT, Newman MC, Clark SB. Predicting the relative toxicity of metal ions using ion characteristics: Microtox® bioluminescence assay. Environ Toxicol Chem 15:1730–1737 (1996).
17. Tatara CP, Newman MC, McCloskey JT, Williams PL. Predicting relative metal toxicity with ion characteristics: *Caenorhabditis elegans* LC$_{50}$. Aquat Toxicol 39:1–12 (1997).
18. Tatara CP, Newman MC, McCloskey JT, Williams PL. Use of ion characteristics to predict relative toxicity of mono-, di-, and trivalent metal ions: *Caenorhabditis elegans* LC$_{50}$. Aquat Toxicol 42:255–269 (1998).
19. Pearson RG. Hard and soft acids and bases. J Am Chem Soc 85:3533–3539 (1963).
20. Campbell PGC, Tessier A. Ecotoxicology of metals in the aquatic environment: geochemical aspects. In: Ecotoxicology. A Hierarchical Treatment (Newman MC, Jagoe CH). Boca Raton, FL:CRC Press, 1996;11–58.
21. Christensen GM. Effects of metal cations and other chemicals upon the *in vitro* activity of two enzymes in the blood plasma of the white sucker, *Catostomus commersoni* (Lacépède). Chem Biol Interact 4:351–361 (1971/1972).

22. Christensen GM, Tucker JH. Effects of selected water toxicants on the *in vitro* activity of fish carbonic anhydrase. Chem Biol Interact 13:181–192 (1976).

23. Magwood S, George S. *In vitro* alternatives to whole animal testing. Comparative cytotoxicity studies of divalent metals in established cell lines derived from tropical and temperate water fish species in a neutral red assay. Mar Environ Res 42:37–40 (1996).

24. Hsie AW, Schenley RL, Tan E-L, Perdue SW, Williams MW, Hayden TL, Turner JE. The toxicity of sixteen metallic compounds in Chinese hamster ovary cells: a comparison with mice and *Drosophila*. In: Alternative Methods in Toxicology Vol. 2: Acute Toxicity Testing: Alternative Approaches (Goldberg AM, ed). Libert, NY:Mary Ann Liebert, 1984;117–125.

25. Somers E. The fungitoxicity of metal ions. Ann Appl Biol 49:246–253 (1961).

26. Khangarot BS, Ray PK. Investigation of correlation between physicochemical properties of metals and their toxicity to the water flea *Daphnia magna* Straus. Ecotoxicol Environ Saf 18:109–120 (1989).

27. Martin TR, Holdich DM. The acute lethal toxicity of heavy metals to peracarid crustaceans (with particular reference to freshwater asellids and gammarids). Water Res 20:1137–1147 (1986).

28. Delfino G, Amerini S, Mugelli A. Barium cardiotoxicity: relationship between ultrastructural damage and mechanical effects. Toxicol in Vitro 2:49–55 (1988).

29. Das T, Sharma A, Talukder G. Effects of barium on cellular systems: a review. Nucleus 31:41–68 (1988).

30. Taglialatela M, Drewe JA, Brown AM. Barium blockade of a clonal potassium channel and its regulation by a critical pore residue. Mol Pharmacol 44:180–190 (1993).

31. Bradberry SM, Vale JA. Disturbances of potassium homeostasis in poisoning. Clin Toxicol 33:295–310 (1995).

32. Nieboer E, Richardson DHS. The replacement of the non-descript term 'heavy metals' by a biologically and chemically significant classification of metal ions. Environ Pollut Ser B1: 3–26 (1980).

33. SAS Institute. SAS/STAT User's Guide. Version 6.03. Cary, NC:SAS Institute, 1988;890.

34. Draper NR, Smith H. Applied Regression Analysis. New York:John Wiley & Sons, 1981;709.

35. Yamaoka K, Nakagawa T, Uno T. Application of Akaike's Information Criterian (AIC) in the evaluation of linear pharmacokinetic equations. J Pharmacokinet Biopharmacol 6:165–175 (1978).

36. Neter J, Wasserman W, Kutner MH. Applied Linear Statistical Models. Boston:Richard D. Irwin, 1990;1181.

37. Neely W, Branson DR, Blau GE. Partition coefficient to measure bioconcentration potential of organic chemicals in fish. Environ Sci Technol 8:1113–1115 (1974).

38. Brown DS, Allison J. MINTEQA1, An Equilibrium Metal Speciation Model: User's Manual. EPA/600/23–87/012. Athens, GA:U.S. Environmental Protection Agency, 1987.

39. Mason RP, Reinfelder JR, Morel FMM. Uptake, toxicity, and trophic transfer of mercury in a coastal diatom. Environ Sci Technol 30:1835–1845 (1996).

40. Turner DR, Whitifeld M, Dickson AG. The equilibrium speciation of dissolved components in freshwater and seawater at 25° C and 1 atm pressure. Geochim Cosmochim Acta 45:855–881 (1981).

41. Brezonik PL, King SO, Mach CE. The influence of water chemistry on trace metal bioavailability and toxicity to aquatic organisms. In: Metal Ecotoxicology. Concepts and Applications (Newman MC, McIntish AW, eds). Chelsea, MI:Lewis Publishers, 1991;1–31.

42. Ahrland S. Thermodynamics of complex formation between hard and soft acceptors and donors. Struct Bonding 5:118–149 (1968).