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

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Abstract

Environmental toxicologists readily adopted QSARs from pharmacology to predict organic contaminant toxicity. In contrast, models relating metal ion characteristics to their bioactivity remain poorly explored and underutilized. Quantitative Ion Character-Activity Relationships (QICARs) have recently been developed to predict metal toxicity. The QICAR approach, based on metal-ligand binding tendencies, has been applied successfully to a wide range of effects, species, and media on a single metal basis. In previous single metal studies, a softness parameter and the $|\log K_{OH}|$ were among the ion qualities with the highest predictive value for toxicity.

Here, QICAR modeling is extended to predict toxicity using data from the US EPA ECOTOX database and for binary metal mixtures. Using the US EPA ECOTOX database, predictive single metal models were produced for four fish species (bluegill, carp, fathead minnow, and mummichog). Using the Microtox® bioassay, the interactions of binary mixtures of metals (Co, Cu, Mn, Ni, and Zn) were quantified using a linear model with an interaction term. A predictive relationship was developed for metal interaction between metal pairs and the difference in softness.

This study supports the hypothesis that general prediction of metal toxicity and interactions from ion characteristics is feasible. It is important that additional work with metals of different valences and sizes be done to further enhance the general accuracy of metal interaction predictions.

1 Introduction

Chemists have been interested in predicting bioactivity from chemical properties since the early 1900s. Structure-activity relationships (SARs) were developed more than a century ago to relate organic compound structure to activity. Conceptual models for qualitatively predicting the effect of both organic compounds and metals were developed during the early and middle part of the century. Models for organic compounds relied on structural similarities of classes of molecules and metal models were based on atomic size and electrode potentials. Pharmacologists and mammalian toxicologists improved SARs by developing methods to quantify effects based on particular functional groups on molecules. Medical research proceeded quickly for organic compounds, including the development of quantitative methods for designing drugs to specifically target active sites. Environmental toxicologists adopted these quantitative structure-activity relationships (QSARs) and applied them to predict bioactivity (i.e., toxicity or bioavailability) of organic compounds.

Development of methods for metals has not been as actively pursued. Like SARs and QSARs for organic compounds, ICARs (ion character-activity relationships)

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Abbreviations: List of abbreviations used in paper: SAR – Structure Activity Relationship; QSAR – Quantitative Structure Activity Relationship; ICAR – Ion Character Activity Relationship; QICAR – Quantitative Ion Character Activity Relationship; HSAB – Hard and Soft Acid Base Theory; HOMO – Highest occupied molecular orbital; LUMO – Lowest unoccupied molecular orbital; LD50 – Median lethal dose; LC50 – Median lethal concentration; r – Pauling ionic radius; χ_m – electronegativity; Z – ion charge; $\Delta\beta$ – [log of the first stability constant for the metal fluoride] – [log of the stability constant for the metal chloride]; σ_p – softness index; $|\log K_{OH}|$ – log of the first hydrolysis constant; AN/ΔIP – atomic number/ change in ionization potential; ΔE_o – electrochemical potential of the ion and its first stable reduced state; w/v – weight to volume ratio; EC50 – median effect concentration; PRESS – predicted residual sum of squares; n – number of observations

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and QICARs potentially provide a way to predict bioactivity based upon toxicant qualities. Bienvenu demonstrated the periodicity of toxic properties of metals in the early 1960s as referenced in [1]. The periodic correlation with atomic number and the existence of trends within families of elements shown in his results suggested the existence of other correlations more directly linked to metrics of elemental properties [1]. Building on this foundation, Jones and Vaughn [1] correlated metrics based on hard and soft acid and base (HSAB) theory with mouse LD50 values.

Hard and soft acid base theory groups metal ions into three classes: hard (e.g., Be^{+2}, Al^{+3}, Fe^{+3}), soft (e.g., Cu^{+}, Ag^{+}, Hg^{+}, Pt^{+2}), and borderline (e.g., Fe^{+2}, Co^{+2}, Ni^{+2}, Zn^{+2}, Cu^{+2}) metal ions. Hard acids preferentially bind to O or N, soft acids to S, and the borderline ions form stable complexes with S, O, or N. The energy difference between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) can be related to HSAB theory. Hard species have large HOMO-LUMO differences, and soft species have small differences. The presence of low-lying unoccupied molecular orbitals capable of mixing with the ground state accounts for the polarizability of soft atoms. Such polarizability allows distortion of electron clouds to reduce repulsion [2]. The consequence of high polarizability is that the cation actually penetrates the anionic electron cloud producing a predominately covalent bond.

Hard and soft acid base concepts relate to toxicity patterns. Jones and Vaughn plotted LD50 data for 25 metals versus the "softness parameter," a measure of the hard/soft character of the ions. They found that the relationship between softness and metal LD50 values was clearer if metals were grouped as hard, soft, or borderline acids [1].

Newman and colleagues proposed that ICARs could be extended to produce useful QICARs. Their studies [3–7] assessed several ion characters, reflecting metal-ligand binding tendencies. The electronegativity (χ_m) and the Pauling ionic radius (r) were combined to produce a covalent index ($\chi_m^2 r$), quantifying the relative importance of covalent versus electrostatic interactions during metal-ligand binding. The ion charge (Z) and Pauling ionic radius were combined to form a second index, the cation polarizing power (Z^2/r), reflecting the energy of the metal ion during electrostatic interaction with a ligand. The $\Delta\beta$ ([log of the first stability constant for the metal fluoride] – [log of the stability constant for the metal chloride]) reflects covalent bond stability of the metal-ligand complex. The softness index (σ_p) used by Jones and Vaughn reflects metal ion softness, or the tendency for the outer electron shell to deform (i.e., polarizability), and the ions tendency to share electrons with ligand donor atoms. The absolute value of the log of the first hydrolysis constant ($|\log K_{OH}|$) was used as a metric of metal affinity to intermediate ligands such as those with O donor atoms. They included the approach of Kaiser [8] that used ionization potential (AN/ΔIP), and the difference between the electrochemical potential of the ion and its first stable reduced state (ΔE_o). Atomic number (AN) 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 the ability of an ion to change electronic state [3].

There were several progressive stages to their studies. The first used the marine bacterium *Vibrio fischeri* (Microtox®) and a second used the soil nematode, *Caenorhabditis elegans*. The $|\log K_{OH}|$ provided the best fit for nine divalent metals in a standard Microtox® test (2% (w/v) NaCl solution) using free ion concentrations [5]. Using a modified Microtox® test (3.02% (w/v) $NaNO_3$ solution) for twenty metals, models generated with each of these metal ion characteristics were significant (slope significantly different from 0) in both one and two factor models, except AN/ΔIP. The softness parameter was the single variable that best predicted total dissolved metal EC50 values for the Microtox® tests. Fitting the model using free metal ion concentrations produced similar results, except that those using either AN/ΔIP or Z^2/r were not statistically significant ($\alpha = 0.05$). The best one-variable model used the softness index and the best two-variable model was a combination of $|\log K_{OH}|$ and $\chi_m^2 r$. Modeling the metals by valence improved model fits, although the number of data points for mono- and trivalent metal ions were low [4].

The *C. elegans* test results were presented in a manner similar to the Microtox® tests. The ion characteristic in the best model for nine divalent metals was $|\log K_{OH}|$ [7]. Regardless of the LC50 metameter used (free ion or total), all ion characteristics except AN/ΔIP were statistically significant in the model including 18 metals. The best predictive relationships were found between LC50 values and the absolute value of the first hydrolysis constant ($|\log K_{OH}|$) and a two-variable model containing both $|\log K_{OH}|$ and $\chi_m^2 r$. Speciation did not improve model fit in the models containing 18 metals [6].

In the third stage of their studies, the softness parameter and the first hydrolysis constant were found to work well in the development of QICARs for 19 diverse data sets taken from the literature. These data sets reported effects of seven or more metals to a variety of organisms and endpoints including enzyme inactivation, cultured cell viability, germination inhibition of fungi, bioaccumulation in a marine diatom, inhibition of bacterial bioluminescence (Microtox®), acute toxicity to soil nematodes and an array of aquatic invertebrates, and chronic effects on lethal or sublethal endpoints. Recent work by other groups has used this approach with additional ion qualities and plant [9] or mammalian species [10].

Establishment of good predictive QICARs for single metal effects provides the groundwork for the next stage of study. Models with predictive value were developed for metals singly from metal ion characteristics that reflected metal-ligand binding tendencies. The next logical steps in exploring the robustness of the QICAR approach is to expand on the single metal examples in the literature using

Table 1. 96 h LC50 values by species for each metal used from the USEPA Ecotox database system reported as number of observations, geometric mean (μM), and range (μM).

Species	Ag	Cd	Co	Cu	Fe	Hg	Ni	Pb	Zn
Common Carp	NA[a]	12, 17, 0.05 – 1900	1, 5650, 5650	3, 2.3, 1.7 – 3.6	NA	6, 1.7, 0.35 – 22	11, 140, 22 – 820	NA	7, 18, 6.9 – 34
Bluegill	NA	9, 44, 14 – 430	NA	7, 17, 11 – 34	1, 360, 360	2, 1.1, 0.59 – 2.0	6, 360, 88 – 1100	2, 490, 110 – 2100	7, 100, 64 – 150
Fathead Minnow	4, 17, 4.7 – 52	38, 9.6, 0.04 – 650	1, 370, 370	NA	1, 390, 390	3, 0.78, 0.75 – 0.83	16, 240, 53 – 850	10, 280, 3.9 – 16000	10, 87, 6.0 – 730
Mummichog	NA	15, 420, 200 – 1000	1, 4700, 4700	2, 15, 6.3 – 36	NA	10, 1.7, 0.34 – 7.5	3, 2600, 7.5 – 6000	NA	3, 490, 270 – 920

[a] NA – Data not available in EPA ECOTOX database.

available databases and to develop relationships for predicting interactions in binary metal mixtures.

2 Materials and Methods

2.1 US EPA ECOTOX database

Data were taken from the US EPA Ecotox database system (http://www.epa.gov/cgi-bin/ecotox search) for four fish, Carp (*Cyprinus carpio*), Mummichog (*Fundulus heteroclitus*), Bluegill *(Lepomis macrochirus),* and Fathead Minnow (*Pimephales promelas*), often used in toxicity testing, exposed to eight metal chloride salts (Ag, Cd, Co, Cu, Hg, Ni, Pb, and Zn). The data were those for 96 h LC50 tests only. Duplicate citations of the same study within the database were removed and the geometric mean of the remaining LC50 values was calculated (Table 1). The geometric mean was used for LC50 values to limit the influence of outlying values, of which there were a few across all data sets. Metal concentrations were converted from μg/L to molar and log transformed. Linear regression models were generated with two variables that had previously had much success in predicting toxicity (softness and $|\log K_{OH}|$) using the SAS procedure GLM (SAS® Institute, Cary, NC, USA). Values for the ion characteristics used in this study were taken from McCloskey et al. [4].

2.2 Microfox Mixture Study

The Microtox® bacterial assay was used to provide 15-min EC10, EC20, EC30, EC40, and EC50 values and concentration-effect relationships for five divalent metals ions: Co^{+2}, Cu^{+2}, Mn^{+2}, Ni^{+2}, and Zn^{+2}. A 3.02% (w/v) $NaNO_3$ solution was prepared with reagent grade $NaNO_3$ in deionized water and the solution was filtered through a 0.45 μm filter. Nitrate salts were used in this series of tests to represent a freshwater environment rather than a marine system. We also wished to avoid complications from metal-Cl binding that would be present in a chloride salt solution. A stock metal solution was prepared daily for the toxicity testing with the nitrate salt of the metal in the $NaNO_3$ solution. Serial dilutions of the stock solution were made to create a series of seven concentrations. A reconstituted marine bacterium (*Vibrio fischeri*) was exposed at 15 °C to solutions of the metals. Bioluminescence was quantified over a range of metal concentrations using a Microtox® Model 500 toxicity analyzer (Microbics Corp., Carlsbad, CA, USA). The metal concentrations resulting in a 10, 20, 30, 40, and 50% decrease in light output after 15 min of exposure were calculated from this curve.

Single metal effect concentrations used in the mixture experiments were calculated based on the mean of three or more replicate tests using bacteria from two different lots. Effect concentrations for each individual test were calculated using the procedure PROBIT of the SAS system (SAS® Institute, Cary, NC, USA).

Binary mixtures of metals were tested using six concentrations of one metal (EC0, EC10, EC20, EC30, EC40, and EC50) combined with five concentrations of the second metal, with a blank control. The EC0 was defined as the case in which one of the metals was not included. Metal solutions were prepared as above and serial dilutions were made for each combination of exposure concentrations to be tested. Each combination was run as tandem duplicates, and then again to produce four measurements of the decrease in light output for each combination of concentrations. This experimental design was used in order to produce a response surface for each binary mixture.

Metal interactions for each binary pair of metals were calculated with a mixed model: the arcsine square root of the proportional decrease in bioluminescence as a function of day, Microtox® reagent lot, effect of metal 1, effect of metal 2, and metal interaction. The SAS procedure MIXED was used to produce the response surface containing the effect of metal 1, the effect of metal 2, and the metal interaction as fixed effects, with day of test and Microtox® bacteria lot as random terms in the model. The metal interaction is the random effect due to the *i*th level of the effect of metal 1 and the *j*th level of effect of metal 2, and is normally distributed. Day of test and bacteria lot were included to access any influence different reagent lots or day of analysis might have had on the model. Linear regression models of the metal interaction with the absolute value of the difference in ionic qualities for each of the pairs of metals were produced using the SAS GLM procedure (SAS® Institute, Cary, NC, USA).

Table 2. Correlation coefficients (r^2), F-statistics, and deviation from perfect prediction for QICAR relationships using 96 h LC50 data retrieved from the USEPA Ecotox database system. The number of metals listed is the number of data points used in the model.

Species Metals[a] Softness σ_p						$\lvert \log K_{OH} \rvert$			
(Common name)		r^2	F	p	Dev[b]	r^2	F	p	Dev[b]
Cyprinus carpio (Common Carp)	Cd, Co, Cu, Hg, Ni, Zn	0.56	5.01	0.089	18.6(15)	0.37	2.34	0.20	22.5(13)
Lepomis macrochirus (Bluegill)	Cd, Cu, Fe, Hg, Ni, Pb, Zn	0.72	12.55	0.016	9.1(12)	0.07	0.40	0.55	17.5(16)
Pimephales promelas (Fathead Minnow)	Ag, Cd, Co, Fe, Hg, Ni, Pb, Zn	0.79	22.79	0.003	7.6(7)	0.01	0.07	0.80	18.7(10)
Fundulus heteroclitus (Mummichog)	Cd, Co, Cu, Hg, Ni, Zn	0.64	7.07	0.056	15.0(17)	0.78	14.51	0.019	14.6(16)

[a] All tests used metal chloride salts in fresh water, except for *Fundulus heteroclitus* which was tested in salt water.
[b] Mean relative deviation from perfect fit expressed as a percentage, [(Observed)-(Fitted)/ (Observed)] x100, and interquartile range

Table 3. Single Metal Microtox® Results ± SD (μM)

EC	Mn (n = 3)*	Co (n = 4)	Ni (n = 4)	Zn (n = 3)	Cu (n = 5)
10	48.5 ± 57.0	90.0 ± 45.8	86.1 ± 59.1	8.1 ± 1.8	1.2 ± 0.1
20	210 ± 204	258.1 ± 67.5	197.3 ± 97.8	15.9 ± 3.7	1.6 ± 0.1
30	619 ± 496	568.1 ± 91.2	365.2 ± 130.3	25.9 ± 6.2	1.8 ± 0.2
40	1583 ± 1022	1137 ± 258	626.6 ± 158.2	39.3 ± 9.5	2.2 ± 0.2
50	3868 ± 1937	2208 ± 767	1049 ± 208	58.1 ± 14.3	2.5 ± 0.3

* n = number of experiments

The mean relative deviation from perfect fit was expressed as a percentage, [(Observed interaction)-(Fitted interaction)/ (Observed interaction)] × 100.

3 Results

3.1 US EPA ECOTOX database

Toxicity data for six or more metal chlorides were found for the four fish species in the US EPA ECOTOX database. Metal ion characteristics (softness and $\lvert \log K_{OH} \rvert$) were then used to develop predictive models. Both softness and $\lvert \log K_{OH} \rvert$ produced good relationships with the data available (Table 2). Softness produced the best model for the freshwater exposures (carp, bluegill, and fathead minnow), and $\lvert \log K_{OH} \rvert$ produced the best model for the saltwater exposure (mummichog) based upon r^2 values, F-statistics, and mean relative deviation from fit.

3.2 Microtox® mixtures

The 15-min EC10, 20, 30, 40, and 50 values (±standard deviation) of the total metal ion are provided in Table 3. The EC50 values ranged from 2.5 μM for Cu to 3868 μM for Mn.

The effect concentrations from Table 3 were used in binary combinations in the acute Microtox® test to measure the interactions of each pair. Each of the metal concentrations (the concentrations that caused an EC10, 20, 30, 40, and 50) was used alone and in the binary metal mixture during each set of tests. The metal interaction for each of the ten metal combinations reported in Table 4 were calculated based on the measured effect concentrations of the single metal exposures (the EC0 series), not the calculated ones from the single metal test. For example, if the concentration that was calculated to cause an EC10 effect actually caused a 12% effect, then 0.12 was used in the model calculations. All metal interactions were significant ($p < 0.001$) in their respective mixed models, except that for the Cu and Ni mixture ($p = 0.259$). Day and Microtox® reagent lot variables were not significant in any model ($\alpha = 0.05$).

Table 4. Metal Interaction Coefficients for Microtox® ± Standard Error

	Cu	Zn	Ni	Co
Mn	− 1.56 (0.25)	− 2.54 (0.29)	− 2.61 (0.25)	− 2.06 (0.24)
Co	− 1.07 (0.06)	− 1.41 (0.24)	− 2.12 (0.31)	
Ni	− 0.39 (0.34)*	− 1.75 (0.18)		
Zn	− 2.20 (0.32)			

* $p = 0.259$, All other interaction coefficients were significant at $p < 0.001$.

The difference in softness between paired metal ions ($\Delta\sigma_p$) produced the best relationship with metal interactions based on r^2 values, F-statistics, and deviation from perfect prediction (Table 5). The differences in the other seven chemical properties examined produced poorer regression relationships with the metal interactions.

4 Discussion

Quantitative ion character-activity relationships can be derived from toxicity databases lacking sufficient information with which to include speciation calculations, (e.g., to

Table 5. Metal interaction models fitted for eight ion characteristics.

Metal Interaction = f(x) deviation from fit†	r^2	PRESS*	Model**	Mean relative
$\Delta(\sigma)$	0.69	2.1	68.5x – 2.63	14.2
$\Delta(Z^2/r)$	0.26	4.4	– 1.23x – 1.25	19.8
$\Delta(\text{Log } K_{OH})$	0.22	5.3	0.45x – 2.32	24.8
$\Delta(X_m)$	0.21	5.6	– 2.25x – 1.33	30.2
$\Delta(\Delta E_o)$	0.18	6.0	– 0.95x – 1.34	30.9
$\Delta(X^2r)$	0.07	6.5	– 0.67x – 1.51	26.7
$\Delta(\Delta IP)$	0.04	5.8	0.098x – 1.98	25.6
$\Delta(AN/\Delta IP)$	0.00	6.4	– 0.013x – 1.76	22.4

* Predicted Residual Sum of Squares
** Model referred to here is metal interaction
† Expressed as a percentage: [(Obs – Fitted)/Obs]*100

estimate free, aquated ion concentrations). Data were retrieved for four fish (bluegill, carp, fathead minnow, and mummichog) and were used to develop predictive relationships based on metal-ligand binding characteristics. This is an assessment of the robustness of the QICAR approach. It reflects the case where speciation calculations cannot be performed to improve the QICAR models because of lack of data being reported. The water chemistry probably varied significantly among the tests for the different metals but was not reported. The toxicity for the three freshwater fish was predicted with the softness parameter. This is consistent with previous studies using Microtox® in freshwater [4], *Daphnia magna*, *Planaria*, and mice [3]. The toxicity for the mummichog in saltwater was predicted with $|\log K_{OH}|$. Microtox® effects [5], Nematode toxicity [6, 7] and enzyme inhibition [3] in marine solutions were predicted with $|\log K_{OH}|$.

Models predicting intermetal trends in toxicity have been underexplored. Environmental toxicologists have adopted quantitative structure activity relationships for organic compounds, but similar development of such methods for metal toxicity has not occurred. Single metal quantitative ion character activity relationships (QICARs) are possible for a range of metals (mono-, di-, and trivalent), and organisms (bacteria, nematodes, algae, amphipods, and mice) based on metal-ligand binding theory [3]. The softness parameter (σ_p) and the log of the first hydrolysis constant ($|\log K_{OH}|$) were the characteristics that best predicted single metal effect on microbial bioluminescence [4, 5] and nematode toxicity [6,7].

The interactions of metals in mixture have typically been addressed in a descriptive manner in the literature (e.g., [11, 12]). This study addressed metal interactions based on fundamental chemistry principles. It is the connection of QICARs to mechanism that allows scientific explanation of activity variations for tested metals and provides the basis for the quantitative prediction of activity for untested metals. The interaction of metal mixtures can be predicted by ion characteristics. Relationships between ion characteristics and metal interactions were estimated for ten metal pair Microtox® tests. Similar to the single metal Microtox® tests, prediction of metal interactions was best made by the difference in softness for Microtox® interactions. This model

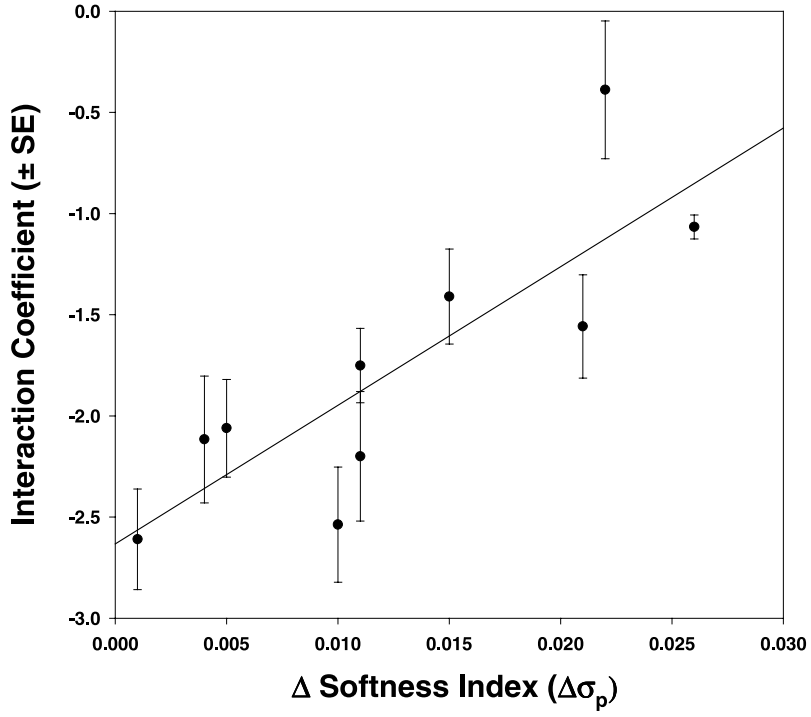

Figure 1. Difference in softness versus calculated Microtox® metal interaction coefficients (±standard error) produced the regression equation: metal interaction = $68.5(\Delta\sigma_p) - 2.63$; $r^2 = 0.69$.

accounted for 69% of the variation in metal interaction for the ten tested binary mixtures (pairs of Co, Cu, Mn, Ni, and Zn). Other models accounted for less than 30% of the variation. This suggests that the similarity between two metal ions in their tendencies to accept an electron during interaction with a ligand was important in predicting interactions in the Microtox® system.

The effect measured in the Microtox® system was a decrease in bioluminescence. Toxicants were thought to interfere with the production of light by interrupting electron flow or binding to active sites on enzymes. As the difference in the ability of a metal ion to accept an electron increased (i.e., $\Delta\sigma_p$ increased), the interaction of the metals approached negative one (Figure 1). The metal interaction approaches -1 as the metal's actions become independent. As the system deviates from independent action, the metal interaction decreases from -1. This study supports the hypothesis that σ_p, or preference to bind to a particular type of ligand is important not only in prediction of single metal toxicity, but also in the prediction of metal interactions.

Application of the QICAR approach to predicting the interaction of metals was done by Newman and McCloskey [5]. They found qualitative trends based on estimating the intersection of lines calculated from the probit of first order rate constants for a series of potentially competing metal ions. Strong interactions were noted between Cu^{+2} and Pb^{+2}, but no apparent interactions were noted for Cu^{+2} and Mg^{+2} nor Ca^{+2} and Mg^{+2} metal ion pairs. Metals combined with Mg^{+2}, a metal with weak covalent interactions with N, S, and O containing ligands, showed little evidence of interaction in the Microtox^R test. 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. The hypothesis that interactions reflected competition for ligand sites of biomolecules was supported for their qualitative testing. They proposed that refinement of their semiquantitative methods was needed.

Newman et al. [3] reanalyzed the data from the Newman and McCloskey [5] paper with a more formal analysis that included using the SAS procedure MIXTURE with an interaction term (i.e., $metal_1$ concentration x $metal_2$ concentration). They concluded that although there were qualitative indications of concentration-dependent interactions between metals with similar and high covalent binding tendencies, no statistically significant trends were evident in their formal analysis. In addition, the only significant trends in the intensity of the interaction term for both of their series of mixtures was a consequence of increasing EC50 values with decreasing covalent interactions that they concluded was a scaling artifact in the data analysis.

The scaling artifact was avoided in the work presented in this paper by using proportions of effect in the analysis rather than concentration. Concentrations in the earlier tests ranged over several orders of magnitude and caused a scaling effect on the data analysis. The use of proportions avoided the confounding scaling effect present in earlier work [3, 5] and a revised statistical design of the experiment facilitated the development of predictive relationships for binary interactions using ion characteristics with the simple Microtox® test system. Future research for QICAR mixture models could include a more complex organism, matrix (i.e., salt water, soil, or sediment), or combinations of these factors.

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