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Use of ion characteristics to predict relative toxicity of mono-, di- and trivalent metal ions: *Caenorhabditis elegans* LC50

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Abstract

Predictive models for relative toxicity of divalent metal ions using ion characteristics have been produced with both Microtox®, a 15 min microbial bioassay, and the 24 h *Caenorhabditis elegans* bioassay. Relative toxicity of mono-, di- and trivalent metal ions has also been successfully modeled using ion characteristics with the Microtox® bioassay. This study extends this approach to include longer exposure durations (24 h) and a more complex organism (metazoan). Twenty-four-hour LC50s (expressed as total and free ion concentrations) for the free-living soil nematode, *C. elegans*, were determined for Li, Na, Mg, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, Sr, Cd, Cs, Ba, La, and Pb in an aqueous medium. Relative metal toxicity was predicted with least squares linear regression and several ion characteristics. Toxicity was most effectively predicted ($r^2 = 0.85$) with a two-variable model containing $|\log K_{OH}|$ (where K_{OH} is the first hydrolysis constant) and $\chi_m^2 r$ (the covalent index). The first hydrolysis constant reflects a metal ion's tendency to bind to intermediate ligands such as biochemical groups with O donor atoms, while $\chi_m^2 r$ reflects binding to soft ligands such as those with S donor atoms. The use of LC50s based on free ion concentrations did not significantly improve model fit. The results of this study are consistent with earlier models generated with Microtox® data, with the exception of barium, which was much more toxic to *C. elegans* than would be predicted from the model. We conclude that, with thoughtful application, ion characteristics can be used to predict the relative toxicity of metal ions that vary widely in both valence and binding tendency.

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1. Introduction

Quantitative structure–activity relationships (QSARs) have been used extensively to predict the bioactivity and toxicity of classes of organic compounds. The QSAR approach was first developed in pharmacology and was quickly adopted by environmental toxicologists. The goal of a QSAR is to relate characteristics of a compound to observed toxicity (or bioavailability), and account for variation in toxicity in a related group of chemicals. Once the relationship has been established, one can also predict the additional effects of molecular substitutions. Most often, these relationships are based on indirect measures of molecular qualities (e.g. lipophilicity using octanol–water partition coefficients, K_{ow}).

Although the QSAR is a widely used approach for organic compounds, application of predictive models to inorganic toxicants (e.g., metals) is poorly represented in the environmental toxicology literature. A mathematical equation that relates metal toxicity to ion characteristics of metals would be useful to toxicologists for predicting intermetal trends in bioactivity (Khangarot and Ray, 1989, Newman and McCloskey, 1996). The number of metals of environmental concern is small in comparison to the number of toxic organic chemicals. This, however, does not diminish the utility of predictive models for metals. The toxic response of different organisms, the chemistry of different exposure media, the effect (endpoint) measured, and the duration of exposure combine to produce thousands of situations where metals would have unique and different toxicities. Any two exposure scenarios would require different predictive models. Situations that would be particularly amenable to predictive modeling occur when few or no metal toxicity data exist for a particular organism. Using data available on the toxicity of some metals, toxicity of additional metals could be predicted from the models, thereby reducing waste and expense. If more precise estimates are required, predictive models would be a useful tool for estimation of concentration levels in a formal toxicity test. Predictive models would also help make the most of existing metal toxicity data for rare or threatened species, when additional toxicity data would be inappropiate to collect but a toxic threat still exists.

Ion characteristics have been used to predict the relative toxicity or sublethal effects of metal ions (e.g. Mathews, 1904, Jones, 1939, Shaw, 1954, 1961, Shaw and Grushkin, 1957, Biesinger and Christensen, 1972, Fisher, 1986, Jones and Vaughn, 1978, Kaiser, 1980, 1985, Williams and Turner, 1981, Babich et al., 1986, Khangarot and Ray, 1989, Magwood and George, 1996). Most ion characteristics that are useful in predictive modeling of metal toxicity reflect the binding tendencies of metals to ligands. Such tendencies are conceptually linked

to metal binding to biomolecules and consequent toxic effects (McCloskey et al., 1996). Although this approach is not a novel idea, predictive models of metal toxicity have never been fully developed, or applied to the extent that QSARs have for organic chemicals.

Recently, studies have been conducted in an attempt to define the mechanisms that underlie the prediction of metal toxicity (Newman and McCloskey, 1996, McCloskey et al., 1996, Tatara et al., 1997). Ion characteristics used in earlier studies were critically reviewed and selected for these studies on the basis of their ability to reflect a metal ion's affinity for different types of biochemical ligands. The following ion characteristics were used in this study. The absolute value of the log of the first hydrolysis constant, $|\log K_{OH}|$ (K_{OH} for $M^{n+} + H_2O \rightarrow MOH^{n-1} + H^+$), reflects a metal ion's affinity to intermediate ligands (e.g. those ligands with O donor atoms) (Baes and Mesmer, 1976). Several ion characteristics reflect binding to soft ligands (e.g. those ligands with S donor atoms), including the softness parameter (σ_p), and the covalent index ($\chi_m^2 r$). The softness parameter is defined as: [(Coordinate bond energy of the metal fluoride) − (Coordinate bond energy of the metal iodide)]/(Coordinate bond energy of the metal fluoride) (Jones and Vaughn, 1978, Williams and Turner, 1981). The covalent index ($\chi_m^2 r$) is composed of two fundamental ionic qualities: electronegativity, χ_m; and Pauling ionic radius, r. Electronegativity 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 (Nieboer and Richardson, 1980). Ionic binding of metal ions to ligands was quantified with Z^2/r (Z is ion charge) (Nieboer and Richardson, 1980, Turner et al., 1981). Also chosen were AN/ΔIP and ΔE_0 (AN is atomic number, ΔIP is the difference in ionization potential between oxidation number OX and OX − 1, ΔE_0 is the absolute difference in electrochemical potential between the ion and its first stable reduced state). AN/ΔIP and ΔE_0 reflect qualities affecting interactions with ligands. The atomic number reflects the size of the ion, while ΔIP and ΔE_0 reflect the effects of atomic ionization potential and the ability of the ion to change electronic state, respectively (Kaiser, 1980).

Newman and McCloskey (1996) developed predictive models of relative toxicity using the divalent metal ions Ca^{2+}, Cd^{2+}, Cu^{2+}, Hg^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+}, and the ion characteristics mentioned above. Predictive models were based on 15 min EC50s using the Microtox® bioassay, with the endpoint being a reduction in light output by a bioluminescent bacterium. Several useful models were generated using this simple microbial assay. Tatara et al. (1997) successfully increased exposure duration to 24 h, using a more complex test organism (*Caenorhabditis elegans*, a metazoan) for nine divalent metals. McCloskey et al. (1996) successfully extended the Microtox® bioassay models to include 20 mono-, di- and trivalent metals. The present study expands the scope of predictive modeling of relative toxicity for the free-living soil nematode *C. elegans* to include 18 mono-, di- and trivalent metals that vary widely in binding tendency.

2. Materials and methods

2.1. Test organism

Caenorhabditis elegans, a free-living soil nematode, was chosen as the test organism for several reasons: *C. elegans* is found in soils on water films, so aqueous exposures would be considered relevant; methods for testing *C. elegans* in an aquatic medium had previously been developed (Williams and Dusenbery, 1990, Donkin and Williams, 1995, Cressman and Williams, 1997); and large numbers (60 animals per concentration) can be tested in a small volume of solution (6 ml). A 24 h exposure period covers a significant percentage of the nematode's typical 10 day life span. Use of *C. elegans* as a test organism retains many elements of a simple model system. *C. elegans* can be tested in a simple medium consisting of deionized water, NaCl, and KCl, thus reducing interaction (i.e. precipitation) of metal ions with ligands in the testing matrix. Additionally, *C. elegans* has a high tolerance range for a wide variety of water quality parameters (e.g. pH, salinity, and hardness; Khanna et al., 1997).

2.2. Maintenance and synchronization of nematode culture

A wild-type (N2) strain of *C. elegans* was maintained as dauer larvae stocks in M9 buffer, replenished monthly (Cox et al., 1981). The dauer larva is an alternate state in the life cycle of *C. elegans* when, in the absence of a food source, the worm experiences arrested growth (Brenner, 1974, Cassada and Russell, 1975). Dauers were used to obtain adult worms that provide the eggs needed to produce a synchronized culture of adult worms. A synchronized culture was accomplished by transferring dauers onto a Petri dish containing K-agar (Williams and Dusenbery, 1988) inoculated with OP50 (a uracil-deficient strain of *Escherichia coli*) to produce a bacterial lawn that served as a food source (Brenner, 1974). The dauers were incubated at 20°C for 2 days, and agar plates with a high density of eggs were chosen to produce synchronized adult worms for toxicity tests. Eggs were isolated from adult worms by rinsing the Petri dishes to remove the eggs and adult worms, and treating the mixture with a mild bleaching solution of 10.5 g l^{-1} NaClO and 10 g l^{-1} NaOH (Emmons et al., 1979), to which the eggs are resistant. Eggs were finally isolated by centrifuging the mixture at 2500 rpm for 3 min followed by three rinse cycles with K-medium (2.36 g KCl + 3.0 g NaCl per liter deionized water) (Williams and Dusenbery, 1990). A synchronized adult worm culture was produced by transferring the eggs to K-agar plates with an established lawn of OP50, and subsequent incubation at 20°C for 3 days.

Worms were prepared for toxicity testing by washing the adult worms from the Petri dishes into a centrifuge tube. Worms were allowed to settle by gravity to the bottom of the tube. The supernatant was decanted and the worms were rinsed with K-medium. The rinse procedure was repeated three times, and on the final rinse the worms were transferred to a glass Petri dish for loading into test wells.

2.3. Test media, food source, and solution preparation.

All tests were conducted in K-medium. A uracil-deficient strain of *E. coli*, OP50, was cultured in L-Broth (3 g beef extract, 5 g peptone and 5 g lactose in 1 liter of deionized water) as a food source. A volume of saturated OP50 equal to that of the test solution was centrifuged, and the pellet resuspended in K-medium. This wash procedure was repeated three times. On the final centrifugation, the pellet was resuspended in the metal solution to be tested (Donkin and Williams, 1995). Use of a food source for the 24 h exposure greatly reduced control mortality. Metal solutions were prepared with metal nitrate salts using K-medium as the diluent. The temperature and pH of the test solutions were recorded at the beginning of the test and were stable throughout the duration of the test. Samples for metal analyses were collected at the beginning and end of each test in 20 ml polyethylene bottles. Samples were acidified to pH < 2.0 with concentrated nitric acid and stored at room temperature until analysis was performed.

2.4. Experimental design and test procedure

Nematodes were tested in Costar-3512 12-well tissue culture plates (Corning Costar, Kennebunk, ME) containing 1 ml of test solution per well. Test solutions consisted of six metal concentrations and a control with each replicated six times. Using a dissecting microscope, 9–11 (average ten) nematodes were transferred into each test well with a 10 µl pipette. Worms were incubated at 20°C for 24 ± 1 h, and the number of dead was determined by visual inspection and probing the worms with a platinum wire under a dissecting microscope. This concentration–response experiment was repeated twice for each of the following metals (nitrate salts): Li^+, Na^+, Mg^{2+}, K^+, Ca^{2+}, Cr^{3+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Sr^{2+}, Cd^{2+}, Cs^+, Ba^{2+}, La^{3+}, Pb^{2+}. It was our intention to test Ag^+ and Hg^{2+}, but these metals formed visible precipitates in the test media; consequently, they were not used in the study.

2.5. Metal analysis and speciation

Analyses of initial and final concentrations were done for all metals using a Perkin-Elmer Model 5100 PC atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, CT). Metal concentrations were stable during the test. Free ion concentrations were predicted with PC MINTEQA2 Version 3.10 (Allison et al., 1991). The concentrations of Na^+ (51.3 mM), Cl^- (83.0 mM), K^+ (31.7 mM), pH (varied with metal), and total alkalinity (0.001 mM as $CaCO_3$) of the K-medium plus the total metal and NO_3^- concentrations from the added metal salt were used in speciation calculations. Assumptions made during computations were fixed pH, a closed system, and no precipitation of solid phases. As many variables of the exposures solutions as possible were accounted for in the speciation calculations, but a perfect description of the exposure solutions is impossible to accommodate in a predictive model (e.g. presence of food and test organisms). It is logical to assume that minor errors in the metal speciation may be present.

2.6. Statistical analysis

Two independent toxicity tests were conducted for each metal, and one LC50 was calculated for each toxicity test using the probit procedure of Toxstat®3.4 (WEST, Inc. and Gulley, 1994). The probit procedure required log transformation of the initial measured metal concentrations (determined by atomic absorption spectroscopy). The two independent LC50s were averaged, and the averaged LC50 values were then converted to percent free ion with values estimated by MINTEQA2 Version 3.10. The logarithms of the averaged LC50s were used in linear regression analyses. Simple and multiple linear regression of log LC50 against the ion characteristics was performed with the PROC GLM of the SAS package (SAS Institute, 1988). Ion characteristics were obtained from Table 1 of McCloskey et al. (1996). Akaike's Information Criterion (AIC) was calculated for each model using the estimated log likelihood to determine which variable(s) gave the best fit. The formulas used to calculate log likelihood and AIC were obtained from Neter et al. (1990) and Newman (1995), respectively. The model with the smallest AIC was judged to be the most informative, regardless of the number of variables used in the model.

Cross-validation was performed on all the models reported in Tables 2 and 3 to estimate the magnitude of deviations in effect prediction for unknown metals. A series of models was generated after omitting one metal at a time. Each time this

Table 1
Total metal 24h LC50s and free ion based 24 h LC50s (mean ± S.D., $n = 2$) for 18 metals (nitrate salts) for *C. elegans*

Metal	Total LC50 (mM)	Proportion free ion[a]	Free ion based LC50 (mM)
Li	215 ± 13	0.99	213 ± 13
Na	398 ± 9	0.97	386 ± 9
Mg	250 ± 2	0.68	170 ± 1
K	479 ± 15	0.88	422 ± 13
Ca	173 ± 1	0.71	123 ± 1
Cr	18.6 ± 2.2	0.98	18.2 ± 2.2
Mn	132 ± 2	0.82	108 ± 2
Fe	0.32 ± 0.05	0.18	0.06 ± 0.01
Co	21.6 ± 0.2	0.95	20.5 ± 0.2
Ni	68.8 ± 1.2	0.85	58.5 ± 1.0
Cu	1.71 ± 0.08	0.92	1.57 ± 0.07
Zn	6.50 ± 0.96	0.92	5.98 ± 0.88
Sr	181 ± 2	0.65	118 ± 1
Cd	11.63 ± 0.11	0.22	2.56 ± 0.02
Cs	323 ± 21	0.87	281 ± 18
Ba[b]	2.80 ± 0.30	0.96	2.69 ± 0.29
La	9.73 ± 0.47	0.98	9.54 ± 0.46
Pb	0.26 ± 0.01	0.41	0.106 ± 0.004

[a] Estimated using MINTEQA2 Version 3.10.
[b] Barium was not included in the models.

Table 2
Results from the regression analysis of the log LC50 based on total metal concentration ($n = 17$) and several ion characteristics

Log LC50 = $f(x)$	r^2	Model (log LC50 =)	MSE[b]	AIC
ΔE_0^a	0.53	$0.53 + 0.63(\Delta E_0)$	0.555	38.22
$\chi_m^2 r^a$	0.55	$2.63 - 0.58(\chi_m^2 r)$	0.527	37.35
σ_p^a	0.49	$-0.75 + 14.63(\sigma_p)$	0.598	39.49
$\lvert\log K_{OH}\rvert^a$	0.69	$-1.00 + 0.24(\lvert\log K_{OH}\rvert)$	0.366	31.15
AN/ΔIP; ΔE_0^a	0.56	$0.73 - 0.05(AN/\Delta IP) + 0.65(\Delta E_0)$	0.551	39.10
$\chi_m^2 r^a$; Z^2/r^a	0.77	$3.19 - 0.53(\chi_m^2 r) - 0.12(Z^2/r)$	0.284	27.84
$\chi_m^2 r^a$; $\lvert\log K_{OH}\rvert^a$	0.85	$0.33 - 0.36(\chi_m^2 r) + 0.18(\lvert\log K_{OH}\rvert)$	0.187	20.72
ΔE_0; $\chi_m^2 r^a$	0.63	$1.66 + 0.34(\Delta E_0) - 0.35(\chi_m^2 r)$	0.473	36.52
ΔE_0; $\lvert\log K_{OH}\rvert^a$	0.72	$-0.78 + 0.22(\Delta E_0) + 0.19(\lvert\log K_{OH}\rvert)$	0.355	31.62
σ_p; $\lvert\log K_{OH}\rvert^a$	0.70	$-1.13 + 3.05(\sigma_p) + 0.21(\lvert\log K_{OH}\rvert)$	0.382	32.88

The model with the smallest Akaike's Information Criterion (AIC) was judged to have the most information regardless of the number of independent variables.
[a] Variable had a significant effect on log LC50 ($\alpha = 0.05$).
[b] MSE, mean squared error of the model.

was done, the ion characteristics of the omitted metal were placed into the model to predict an effect. This cross-validation (Neter et al., 1990) was done with the option PRESS in the SAS procedure REG (SAS Institute, 1988). The deviation from perfect prediction was expressed as the percentage, [(Observed $\text{effect}_{\text{metal I}}$) − (Predicted $\text{effect}_{\text{model without metal I}}$)/(Observed $\text{Effect}_{\text{metal I}}$)] × 100. Median and interquartile ranges for these percentages summarize the general deviations from perfect prediction. The models in Tables 2 and 3 were also cross-validated only when the LC50s for divalent metals were modeled.

Table 3
Results from the regression analysis of log LC_{50} based on percent free ion ($n = 17$) and several ion characteristics

Log $LC_{50} = f(x)$	r^2	Model (log LC_{50} =)	MSE[b]	AIC
ΔE_0^a	0.49	$0.29 + 0.69(\Delta E_0)$	0.759	43.58
$\chi_m^2 r^a$	0.54	$2.59 - 0.64(\chi_m^2 r)$	0.694	42.04
σ_p^a	0.50	$-1.23 + 16.72(\sigma_p)$	0.743	40.18
$\lvert\log K_{OH}\rvert^a$	0.67	$-1.43 + 0.27(\lvert\log K_{OH}\rvert)$	0.501	36.50
AN/ΔIP; ΔE_0^a	0.53	$0.53 - 0.06(AN/\Delta IP) + 0.72(\Delta E_0)$	0.752	44.40
$\chi_m^2 r^a$; Z^2/r^a	0.75	$3.22 - 0.60(\chi_m^2 r) - 0.14(Z_2/r)$	0.397	33.32
$\chi_m^2 r^a$; $\lvert\log K_{OH}\rvert^a$	0.83	$0.05 - 0.39(\chi_m^2 r) + 0.20(\lvert\log K_{OH}\rvert)$	0.280	27.64
ΔE_0; $\chi_m^2 r$	0.60	$1.61 + 0.35(\Delta E_0) - 0.41(\chi_m^2 r)$	0.647	41.86
ΔE_0; $\lvert\log K_{OH}\rvert^a$	0.69	$-1.21 + 0.22(\Delta E_0) + 0.22(\lvert\log K_{OH}\rvert)$	0.500	37.44
σ_p; $\lvert\log K_{OH}\rvert^a$	0.68	$-1.63 + 4.58(\sigma_p) + 0.22(\lvert\log K_{OH}\rvert)$	0.512	37.88

The model with the smallest Akaike's Information Criterion (AIC) was judged to have the most information regardless of the number of independent variables.
[a] Variable had a significant effect on log LC_{50} ($\alpha = 0.05$).
[b] MSE, mean squared error of the model.

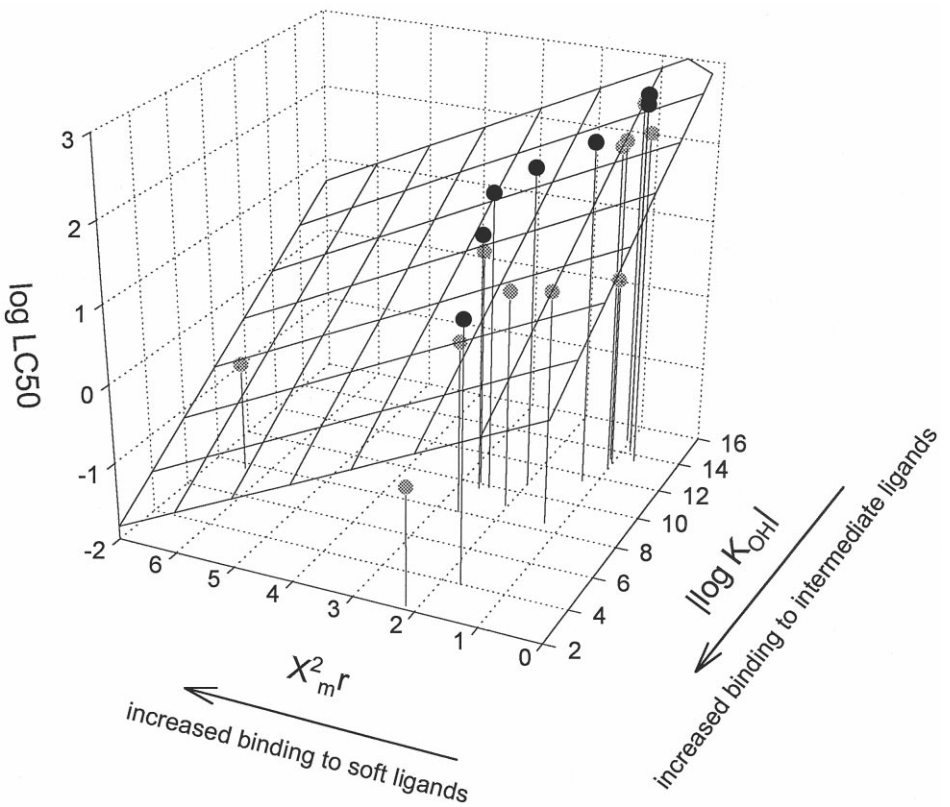

Fig. 1. Log 24 h LC_{50}s for *C. elegans* plotted against $|\log K_{OH}|$ and the covalent index ($\chi^2_m r$). The plane represents the model predicted LC_{50}. Points are the observed LC_{50}s for 18 metals. The points above the plane are solid, while points below the plane are shaded.

3. Results

Twenty-four-hour LC50 values ($\pm$ standard deviation) for total metal and free ion are provided in Table 1. These two concentration metameters were considered during initial model development. Free ion concentrations were examined because it was assumed that free ion concentrations more accurately reflect bioreactive concentrations than total metal concentrations. Neutral chloro-complexes were not considered in this study, because none of the metals tested are thought to form appreciable concentrations of lipophilic neutral metal–chloro complexes.

The results of the regression analysis based on total metal concentration and free ion concentration for several ion characteristics are presented in Tables 2 and 3, respectively. Regardless of the LC50 metameter used (free ion versus total), all ion characteristics except AN/ΔIP were statistically significant ($\alpha = 0.05$) (Tables 2 and 3).

The observed toxicity of barium (2.80 mM) was greater than the model predicted (226 mM; Figs. 1 and 2). For this reason, barium was treated as an outlier, and excluded from the model. When barium was excluded, the fits of the models improved dramatically.

Single-variable (ion characteristic) models were not as effective as two-variable models in predicting toxicity (Tables 2 and 3). The best two-variable model (using total metal concentrations and excluding barium) was a combination of $\chi^2_m r$ and $|\log K_{OH}|$ ($r^2 = 0.85$; AIC = 20.72) (Table 2 and Fig. 2). Use of free ion based LC50s did not improve model fit (Tables 2 and 3).

Cross-validation of the models indicated that the median deviation between observed and predicted effects was reasonable. The median deviations for models based on total LC50s were less than 17%, with most below 11%. However, many models poorly predicted effects for specific metals (e.g., Cu, Pb, and Fe). These metals tended to be class b or borderline class b metals that tend to undergo considerable speciation in solution. When cross-validation was performed on these same models based on free ion LC50s, the median deviations from perfect prediction did not improve. Also, Cd as well as Cu, Pb and Fe provided poor predictions with the free ion based models.

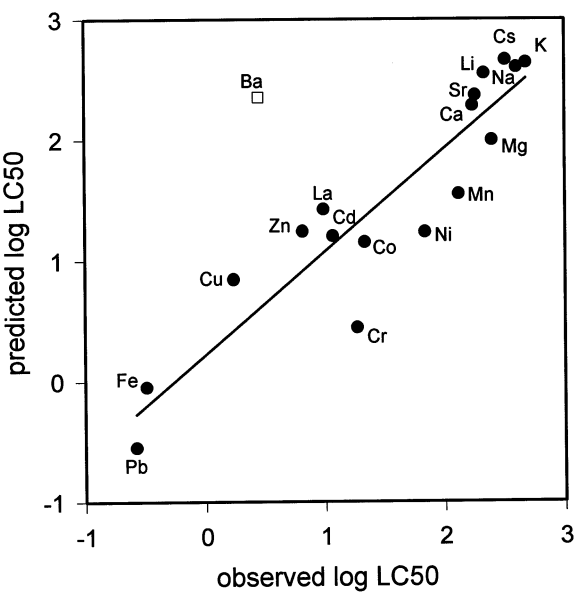

Fig. 2. Observed log LC50 vs. predicted log LC50 for the model: $\log LC50 = 0.33 - 0.36(\chi^2_m r) + 0.18(|\log K_{OH}|)$; $r^2 = 0.85$.

4. Discussion

The relative toxicity of metal ions to *C. elegans* was predictable with linear regression using ion characteristics. Several models provided adequate fits, indicating that predictive models can be developed for metals that vary widely in both valence and binding tendency, and that these models are successful for extended durations with complex organisms. The best model used a combination of $\chi^2_m r$ and $|\log K_{OH}|$ to predict the *C. elegans* 24 h LC50 ($r^2 = 0.85$; Fig. 2). The correlation with $\chi^2_m r$ suggests that binding of metals to soft ligands (e.g. those with sulfur donor atoms) on biomolecules plays an important role in intermetal trends in metal toxicity. The correlation with $|\log K_{OH}|$ suggests that covalent binding of metals to intermediate ligands (e.g. those with O or N donor atoms) on biomolecules also plays an important role in metal toxicity. These results agreed with the findings of McCloskey et al. (1996), where the best predictive model ($r^2 = 0.84$) used a combination of $\chi^2_m r$ and $|\log K_{OH}|$ to predict reduction in luminescence in a photobacterium (Microtox®).

Models based on only one ion characteristic were not as good as models based on two ion characteristics. The best one-variable model ($r^2 = 0.69$) used $|\log K_{OH}|$ as the independent variable to predict total LC50 (Table 2). This is in contrast to the results from McCloskey et al. (1996), who found that σ_p produced the best one-variable model ($r^2 = 0.81$). However, the results of the present study are still in agreement with McCloskey et al. (1996), because one-variable models were generally inferior than two-variable models for the prediction of metals that vary in valence. When modeling effort was restricted to divalent metals, one-variable models were generally superior (Newman and McCloskey, 1996, Tatara et al., 1997). Two-variable models were expected to perform better when modeling metals that vary in valence because more information is required (in the form of ion characteristics) to represent the metals' tendencies to bind to biological ligands.

The use of LC50s based on the free ion concentration did not improve the model fit, even though the free ion is often assumed to be the major bioactive form for metals. This result is in agreement with the findings from our previous studies (Newman and McCloskey, 1996, McCloskey et al., 1996, Tatara et al., 1997). It is possible that the free ion LC50s did not yield superior performance because they were estimated (with MINTEQA2) in these studies. The estimation of the free ion concentrations may not be accurate, as assumptions are required for the models used to obtain these estimates. Perhaps the free ion based LC50s would produce better fitting models if the free ion concentrations were measured instead of being estimated. However, measuring the free ion concentration with specific ion electrodes, or determining metal speciation using anodic stripping voltometry, would involve considerable expense and expertise. Like most people who conduct these types of experiments, we chose speciation techniques that were based on calculated estimates. In the practical application of these predictive models most researchers would also choose the calculated estimates.

The investigation of these 'bioactive' LC50 metameters has provided information that will facilitate the application of predictive models for metals. If predictive

models needed to be developed for metal toxicity using data from the literature, LC50s would likely be reported as the total metal concentration. If the free ion concentration was wanted, the detailed water quality data necessary for estimation would probably not be reported. The results of this and the above studies suggest that LC50s reported as total metal concentration can be used in model development without a reduction in model quality.

The results of the cross-validation also suggested that free ion based LC50s did not help to reduce the magnitude of spurious predictions for metals that undergo considerable speciation. This is in contrast to the findings of Newman et al. (submitted), who found that large deviations from perfect prediction were reduced when metal speciation was accounted for in the Microtox® EC50. The lack of improvement in these poor predictions by these predictive models may be related to unique metal speciation in the K-medium used in the exposures. Conducting the concentration–response experiments in a more realistic media (reconstituted water) might result in the better performance of free ion based LC50s. Regardless, cross-validation using free ion based LC50s holds promise for improving the accuracy of model predictions for certain metals.

In the present study, the observed LC50 for Ba^{2+} was much lower than the predicted LC50 (Fig. 2). This was not observed in a previous study using Microtox® (McCloskey et al., 1996). There are several possible explanations. Barium is known to interfere in ion regulation, particularly with K^+ (K^+ channels and Na^+/K^+-ATPase), and to a lesser extent with Ca^{2+} (calmodulin complex) regulation (Das et al., 1988, Delfino et al., 1988, Taglialatela et al., 1993, Bradberry and Vale, 1995). The atomic radius of the barium ion is approximately equal to the atomic radius of the potassium ion (Ba^{2+} 1.36 Å, K^+ 1.38 Å), but blocks rather than permeates the ion conducting pore of K^+ channels (Taglialatela et al., 1993). It appears likely that, due to the close similarity in the crystal diameters of K^+ (0.266 nm) and Ba^{2+} (0.270 nm), Ba^{2+} may bind to structures normally used for K^+ binding to establish high selectivity for this monovalent cation (Taglialatela et al., 1993). Using site-directed mutagenesis, Taglialatela et al. (1993) identified channel mutations in delayed rectifier K^+ channels (DRK1) that were able to affect blockage by internal Ba^{2+}. A valine residue in position 374 seemed to play a critical role in the blocking of the channel by Ba^{2+}. When this valine residue was replaced with amino acids with polar R groups (threonine and serine), the dissociation speed of the Ba^{2+} from its site(s) of action was decreased more than ten-fold. In contrast, no change was detected when the valine was substituted by another nonpolar amino acid, isoleucine. This indicates that increased ionic bonding of Ba^{2+} with polar R-groups was responsible for the decreased rate of Ba^{2+} dissociation from its internal site of action. Also noted were several sites external to the channel that were subject to Ba^{2+} blockade.

The interference of Ba^{2+} with K^+ regulation has been reported in several invertebrate and vertebrate species (Das et al., 1988, Delfino et al., 1988, Taglialatela et al., 1993, Bradberry and Vale, 1995). Barium interference with K^+ regulation has serious consequences that are manifested as hyperkalemia, hypokalemia, cardiotoxicity, neurotoxicity, hypertension, and disturbance of muscle

function. Data on Ba^{2+} toxicity to bacteria suggests that Ba^{2+} is not as toxic to prokaryotes as it is to multicellular organisms. In fact, the bacteria *Desulfovibrio vulgaris* was capable of using precipitated $BaSO_4$ as a sulfur source (McReddy and Krouse, 1980). Other studies with microorganisms show that Ba^{2+} substitutes for other divalent cations (Mn^{2+} and Ca^{2+}), but do not indicate toxic effects (Davidson and Knaff, 1981, Nordlund and Noren, 1984, Vertiev and Ezepchuk, 1981). It is possible that Ba^{2+} was not as toxic to these microorganisms and the bacteria used in Microtox® because these unicellular organisms do not have complex organ systems that rely on a K^+ gradient to accomplish work (e.g. muscles, and neurons). In the case of the Microtox® study (McCloskey et al., 1996), it is possible that the short duration (15 min) was not long enough to allow barium sufficient time to exert its toxic effect. In any case, one should take care in extrapolating relative toxicity of metals between highly divergent types of organisms.

It is important to note that some thought should be used in the development and application of predictive models for metal toxicity. It was noted previously that Ba^{2+} behaved as an outlier in this study. Obviously, the ion characteristics used do not represent the impermeability of K^+ channels to Ba^{2+} ions. Another metal that requires special consideration is Hg^{2+}. Although Hg^{2+} was not included in this study because it formed a precipitate, it has been investigated in other studies (Newman and McCloskey, 1996, McCloskey et al., 1996, Tatara et al., 1997) where it also behaved as an outlier. Investigation into the speciation of Hg indicated that very little Hg existed in the free ion form, while a large proportion existed in the form of a neutral chloro-complex ($HgCl_2^0$) that was lipophilic and consequently bioavailable (Gutknecht, 1981, Simkiss, 1983, Bienvenue et al., 1984, Simkiss and Taylor, 1989, Delnomdedieu et al., 1992, Girault et al., 1995, Mason et al., 1996). This suggested the importance of including $HgCl_2^0$ when considering the bioreactive forms of inorganic mercury, and was accomplished by using an LC50 for Hg that was based on free-ion + neutral chloro-complex concentrations as calculated with MINTEQA2.

Although predictive models of relative metal toxicity are not new, they have not been developed or used to the extent that QSARs have been used for organic toxicants. These predictive models for metals could prove useful in areas where data on metal toxicity or sublethal effects are lacking or incomplete. Once a model has been developed with representative metals for a particular organism, the relative effect of additional metals could be predicted. This type of information could prove extremely useful in ecological risk assessment, where it may not be feasible to collect data on all metals for different species, durations, effects, and exposure media.

5. Conclusion

This study supports the hypothesis that general prediction of relative toxicity from ion characteristics is possible for increased exposure durations using complex organisms, and that accurate predictive models can be developed for metal ions that

vary widely in both valence and binding tendencies. This study shows that the use of free ion concentrations does not necessarily improve model fit, but suggests that LC50 metameters that incorporate speciation could aid in the improvement of poor predictions for certain metals. Finally, the unique toxicity of barium and mercury illustrate that thought and care should be exercised in the development and application of predictive models of relative metal toxicity using ion characteristics.

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