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Predicting relative metal toxicity with ion characteristics: *Caenorhabditis elegans* LC50

Christopher P. Tatara[a,b,*], Michael C. Newman[a,b], John T. McCloskey[b], Phillip L. Williams[c]

[a] *Institute of Ecology, University of Georgia, Athens, GA 30602-2202, USA*
[b] *University of Georgia, Savannah River Ecology Laboratory, P.O. Drawer E, Aiken, SC 29802 USA*
[c] *Environmental Health Science Program, University of Georgia, Athens, GA 30602 USA*

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Abstract

Quantitative Structure Activity Relationships (QSAR) predict relative toxicity of a family of chemicals from fundamental and surrogate molecular qualities. Most QSARs are developed for organic toxicants, with inorganic toxicants (metals) being under-represented. Successful predictive models for relative toxicity of divalent metal ions using ion characteristics have been produced using Microtox®, a 15 min microbial bioassay. The present study extends this approach to longer exposure durations (24 h), and a more complex organism (metazoan). Twenty-four hour LC50s (expressed as total metal concentration) for the free-living soil nematode, *C. elegans* were determined for Ca, Cd, Cu, Hg, Mg, Mn, Ni, Pb, and Zn in an aqueous medium. Relative metal toxicity was predicted with least squares linear regression and several ion characteristics. Toxicity was most effectively predicted ($r^2 = 0.89$) with | log K_{OH} | (where K_{OH} is the first hydrolysis constant), which reflects a metal ion's tendency to bind to intermediate ligands such as biochemical functional groups with O donor atoms. The best fitting model was obtained using LC50 metameters based on total metal concentration, indicating that the identification of the bioactive species of metals can be ambiguous, and does not necessarily aid in the prediction of relative metal toxicity with ion characteristics. The modelling of relative metal toxicity using ion characteristics was successful for 24 h exposure durations using this more complex organism. Published by Elsevier Science B.V.

Keywords: *Caenorhabditis elegans*; Metal; Prediction; Toxicity; Nematode

1. Introduction

Quantitative structure activity relationships (QSAR) are commonly used to pre-

*Corresponding author. Savannah River Ecology Laboratory.

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dict the toxicity of organic compounds. The aim of a QSAR is to relate fundamental or surrogate characteristics of a compound to observed toxicity, and account for variation in toxicity for a related group of chemicals. A secondary aim, if the primary is accomplished, is to predict the additional effects of molecular substitutions. The QSAR approach, developed first in pharmacology, was quickly adopted by environmental toxicologists.

Like QSARs for organic toxicants, predictive models for relative metal ion toxicity are based on both fundamental (primary) and surrogate (indirect) molecular characteristics. Fundamental characteristics are represented by electrical qualities (ionization potentials) or total molecular surface area. Surrogate molecular characteristics are represented by measures such as lipophilicity (K_{ow}) and electrical qualities (Hammett constants). QSARs based on fundamental characteristics provide a richer understanding of underlying processes or mechanisms than those based on surrogate molecular characteristics; consequently, they can be more useful for prediction of toxicity of compounds beyond those compounds used to develop the QSAR (Newman and McCloskey, 1996).

Although the QSAR is a widely used approach for organic compounds, its application to inorganic toxicants is poorly represented in the environmental toxicology literature. Characteristics of metal ions have been used to predict the relative toxicity or sublethal effects of metal ions (Biesinger and Christensen, 1972; Jones and Vaughn, 1978; Kaiser, 1980; Williams and Turner, 1981; Babich et al., 1986; Fisher, 1986; Magwood and George, 1996). Many of these characteristics reflect the binding tendencies of metals to ligands, and are conceptually linked to metal binding of biomolecules and consequent toxic effects (McCloskey et al., 1996). Fisher (1986) found that the log of the solubility product for the metal hydroxide (log $-K_{SO}MOH$) was correlated with metal inhibition of algal growth. The solubility of the metal sulfide, an indicator of a metal's tendency to bind with sulfhydryl groups of biomolecules, has been correlated with growth, reproduction, and survival of *Daphnia magna* exposed to different metals (Biesinger and Christensen, 1972). Based on hard and soft acid and base (HSAB) theory, the toxic effects of metals to mice were correlated with the softness parameter, σ_p (Jones and Vaughn, 1978; Williams and Turner, 1981). The softness parameter is a measure of the ability of a metal ion to give up its valence electrons. Babich et al. (1986), and Magwood and George (1996) also correlated the NR50 (concentration that resulted in the reduction of uptake of neutral red dye by 50%) for fish cell cultures with the softness parameter. Kaiser (1980) found correlations between sublethal and toxic effects to aquatic biota and log $AN/\Delta IP$ or ΔE_0, where AN reflects the size of the ion, and ΔIP and ΔE_0 reflect the effects of atomic ionization potential and the ability of the ion to change its electronic state, respectively.

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 several ion characteristics from the above studies. Predictive models were based on 15 min EC50s using the Microtox® bioassay, with the endpoint being a reduction in light output by a bioluminescent bacteria. Several useful models were generated using this simple microbial assay. Here we extend this approach to a

metazoan (*C. elegans*) exposed for longer durations. The models used for the microbial data were examined again with this more complicated model system.

Caenorhabditis elegans, a free living soil nematode, was chosen as the test organism for several reasons: methods for testing *C. elegans* in an aquatic medium had previously been developed (Williams and Dusenbery, 1990; Donkin and Williams, 1995); 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 lifespan. Use of *C. elegans* as a test organism allowed us to retain many elements of a simple model system. *Caenorhabditis 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. Also *C. elegans* can survive over 24 h without a food source (Williams and Dusenbery, 1988; Donkin and Williams, 1995). Elimination of a food source prevented the potential sorption of metals to food and consequent removal from the test media.

The same nine metals (Ca^{2+}, Cd^{2+}, Cu^{2+}, Hg^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+}) as well as ion characteristics chosen by Newman and McCloskey (1996) were used to test the hypothesis that development of QSAR-like models is possible for longer exposure durations using metazoans. The metals tested included two class A metal ions (Mg^{2+} and Ca^{2+}) with hard spheres and electronic configurations of noble gases. The covalent interactions of these cations with ligands were generally much weaker than those of the other seven metals, which ranged from borderline to class B metals with different tendencies for covalent interaction with soft ligands (Nieboer and Richardson, 1980; Newman and McCloskey, 1996).

Several fundamental ion characteristics were examined. Polarizing power, Z^2/r (where Z is ion charge and r is ionic radius), is a measure of electrostatic interaction strength between a metal ion and ligand (Turner et al., 1981). (Polarizing power is composed of two fundamental characteristics (Z and r), but the quotient Z^2/r can be interpreted as a surrogate characteristic that indicates ionic bond stability.) Also used was the quotient $AN/\Delta IP$, where AN is atomic number, and ΔIP is the difference in ionization potentials between ion oxidation number OX and OX-1. Atomic number (AN) reflects the size or inertia of the ion, while ΔIP reflects the effect of atomic ionization potential (Kaiser, 1980). The ΔE_0 (where ΔE_0 is the absolute difference in electrochemical potential between an ion and its first stable reduced state) reflects the ability of an ion to change its electronic state. The $X^2{}_m r$ (X_m is electronegativity and r is ionic radius) reflects the importance of covalent interactions in the metal-ligand complex relative to ionic interactions. Electronegativity (X_m) is correlated with the energy of an empty valence orbital and reflects the ability of a metal to accept electrons. Combining electronegativity with the ionic radius yields an index that quantifies the importance of covalent interactions relative to ionic interactions (Nieboer and Richardson, 1980).

Several surrogate ion characteristics were also examined. The softness parameter, σ_p ([coordinate bond energy of the metal fluoride-coordinate bond energy of the metal iodide]/coordinate bond energy of the metal fluoride), is a measure of the ability of a metal ion to give up its valence electrons (Jones and Vaughn, 1978;

Williams and Turner, 1981). A "hard" metal ion is one which retains its valence electrons very strongly and is not readily polarized. Ions of small size and high charge are "hard" (e.g. Mg^{2+}, Ca^{2+}). A "soft" metal ion is relatively large, does not retain its valence electrons firmly, and is easily polarized (Vouk, 1979).

Metal hydroxide solubility product (log $-K_{so}MOH$) and the log of the constant for the first hydrolysis (K_{OH} for $M^{n+}+H_2O \rightarrow MOH^{n-1}+H^+$) are surrogate indexes reflecting metal ion affinity to intermediate ligands like those with O donor atoms. The ΔB ([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.

2. Materials and methods

2.1. 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). 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 of 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.

2.2. Test media and solution preparation

All tests were conducted in K-medium. Metal solutions were prepared with the metal chloride salts using K-medium as the diluent. The temperature and pH of the

test solutions were recorded at the beginning 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.

2.3. Experimental design and test procedure

Nematodes were tested in Costar-3512 12-well tissue culture plates (Corning Costar Corp., 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 of 10) 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 dead was determined by visual inspection and probing the worms with a platinum wire under a dissecting microscope. This concentration response experiment was repeated three times for each metal.

2.4. 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 Corp., Norwalk CT). Concentrations of metal species including those of free (hydrated) ion and neutral chloro-complexes were predicted with PC MINTEQA2 Version 3.10 (Brown and Allison, 1987). The concentrations of Na^+ (51.3 mmol l^{-1}), Cl^- (83.0 mmol l^{-1}), K^+ (31.7 mmol l^{-1}), pH (varied with metal), and total alkalinity (0.001 mmol l^{-1} as $CaCO_3$) of the K-medium plus the dissolved metal and Cl^- 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.

Table 1
Metal ion characteristics used in regression models

Metal ion	ΔE_0 (V)	$\Delta\beta$	$\lvert\log K_{OH}\rvert$	$\log -K_{SO}MOH$	$X^2_m r$	Z^2/r	AN/ΔIP	σ_p
Mg^{2+}	2.38	5.76	11.61	10.50	1.236	5.56	1.62	0.167
Ca^{2+}	2.76	4.80	12.72	5.00	1.000	4.00	3.47	0.181
Mn^{2+}	1.03	0.66	10.59	12.70	1.994	5.97	3.04	0.125
Ni^{2+}	0.23	0.50	9.86	16.00	2.517	5.79	2.66	0.126
Cu^{2+}	0.16	1.12	8.00	19.80	2.635	5.48	2.31	0.104
Zn^{2+}	0.76	0.66	8.96	16.50	2.015	5.40	3.50	0.115
Cd^{2-}	0.40	−0.89	10.08	14.00	2.713	4.21	6.07	0.081
Hg^{2+}	0.91	−5.80	3.40	25.50	4.080	3.92	9.62	0.065
Pb^{2+}	0.13	0.48	7.71	18.70	6.406	3.39	10.78	0.131

See Newman and McCloskey (1996) for original sources of data in table.

2.5. Statistical analysis

Three LC50 values for each metal were calculated with the probit procedure of Toxstat® 3.4 (WEST Inc. and Gulley, 1994) using the measured metal concentrations (determined by atomic absorption spectroscopy) and a log transformation of concentration. The LC50 values obtained by the probit analysis were converted to percentage of free ion or percentage of free ion+neutral chloro-complexes with values obtained from MINTEQA2 Version 3.10. The three LC50 values for total metal, free ion, and free ion+neutral chloro-complexes were averaged and the log of the averages 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 SAS (SAS Institute, 1988). Ion characteristics were obtained from Table 1 of Newman and McCloskey (1996), and are summarized in Table 1 of this paper.

3. Results

Twenty-four hour LC50 values (±standard deviation) for total metal, free ion, and free ion plus neutral chloro-complexes are provided in Table 2. These three 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-complex plus free ion concentration was considered for mercury because its neutral chloro-complex is lipophilic (K_{ow} = 3.33, Mason et al., 1996) and consequently can be bioavailable (Gutknecht, 1981; Bienvenue et al., 1984;

Table 2
Total LC50s, free ion based LC50s, and free-ion+neutral chloro-complex based LC50s (mean ± SD) in mmol l^{-1} for nine metals (added as chloride salts) for *C. elegans*

Metal	*n*	Total LC50	Proportion free-ion[a]	Free-ion based LC50	Proportion free-ion +neutral chloro-complexes[a]	Free-ion+neutral chloro-complexes LC50
Ca	3	400.3 ± 9.06	1.000	400.3 ± 9.06	1.000	400.3 ± 9.06
Cd	3	8.39 ± 0.56	0.201	1.69 ± 0.11	0.201[b]	1.69 ± 0.11
Cu	3	1.42 ± 0.09	0.931	1.33 ± 0.08	0.931	1.33 ± 0.08
Hg	3	0.072 ± 0.003	3.85E-11	2.77E-12 ± 1.17E-13	0.522	0.038 ± 0.002
Mg	3	180.6 ± 10.14	1.000	180.6 ± 10.14	1.000	180.6 ± 10.14
Mn	3	97.9 ± 6.94	0.777	76.1 ± 5.40	0.777	76.1 ± 5.40
Ni	3	63.4 ± 8.96	0.841	53.4 ± 7.54	0.841[b]	53.4 ± 7.54
Pb	3	1.06 ± 0.23	0.444	0.47 ± 0.10	0.444[b]	0.47 ± 0.10
Zn	3	2.16 ± 0.24	0.925	2.00 ± 0.22	0.925	2.00 ± 0.22

[a] Estimated using MINTEQA2 Version 3.10.
[b] Neutral chloro-complexes existed for these metals, but were not included in LC50s used for modelling. Only the neutral chloro-complex for Hg was included because it is lipophilic.

Table 3
Results from the regression analysis of log LC50 based on total metal concentration and several ion characteristics

Log LC50 = $f(x)$	r^2	Model (log LC50 =)	MSE[b]
ΔE_0	0.38	$2.22+0.79(\Delta E_0)$	1.113
$X^2_{\mathrm{m}}r$[a]	0.49	$2.44-0.53(X^2_{\mathrm{m}}r)$	0.916
$\Delta\beta$[a]	0.65	$0.75+0.31(\Delta\beta)$	0.620
log $-K_{\mathrm{so}}MOH$[a]	0.86	$4.03-0.20(\log -K_{\mathrm{so}}MOH)$	0.257
$\lvert\log K_{\mathrm{OH}}\rvert$[a]	0.89	$-3.02+0.44(\lvert\log K_{\mathrm{OH}}\rvert)$	0.192
Softness (σ_{p})[a]	0.63	$-2.28+26.91(\sigma_{\mathrm{p}})$	0.654
$AN/\Delta IP$[a]; ΔE_0	0.63	$1.43-0.20(AN/\Delta IP)+0.54(\Delta E_0)$	0.767
$X^2_{\mathrm{m}}r$; Z^2/r	0.49	$2.57-0.54(X^2_{\mathrm{m}}r)-0.02(Z^2/r)$	1.068
$\Delta\beta$[a]; Z^2/r	0.69	$-0.43+0.28(\Delta\beta)+0.24(Z^2/r)$	0.655

[a]Variable had a significant effect on log LC50 ($\alpha = 0.05$).
[b]Mean square error from model.

Delnomdedieu et al., 1992; Girault et al., 1995; Mason et al., 1996). Neutral chloro-complexes existed for cadmium, lead and nickel, but were not accounted for in the LC50s used in modelling. The neutral chloro-complex of cadmium was not lipophilic ($K_{\mathrm{ow}} = 0.21$, Mason et al., 1996). The lipophilicity of lead and nickel neutral chloro-complexes was unknown. The results of the regression analyses based on total metal concentration, percentage of free ion, and percentage of free ion+neutral chloro-complexes for several ion characteristics are presented in Tables 3–5 respectively.

When the total metal LC50 and free ion+neutral chloro-complex LC50 concentration metameters were used, six ion characteristics ($\Delta\beta$, $\lvert\log K_{\mathrm{OH}}\rvert$, σ_{p}, log $-K_{\mathrm{SO}}MOII$, $X^2_{\mathrm{m}}r$, and $AN/\Delta IP$) were statistically significant ($\alpha = 0.05$) in the regression models (Tables 3 and 5). However, when the free ion LC50 concentration metameters were used only four ion characteristics ($\Delta\beta$, $\lvert\log K_{\mathrm{OH}}\rvert$, σ_{p}, and log

Table 4
Results from the regression analysis of the log LC50 based on percentage of free-ion and several ion characteristics

Log LC50 = $f(x)$	r^2	Model (log LC50 =)	MSE[b]
ΔE_0	0.05	$-1.27+0.98(\Delta E_0)$	20.727
$X^2_{\mathrm{m}}r$	0.22	$3.06-1.23(X^2_{\mathrm{m}}r)$	17.058
$\Delta\beta$[a]	0.71	$-1.21+1.11(\Delta\beta)$	6.335
log $-K_{\mathrm{so}}MOH$[a]	0.61	$8.64-0.58(\log -K_{\mathrm{so}}MOH)$	8.507
$\lvert\log K_{\mathrm{OH}}\rvert$[a]	0.83	$-13.83+1.47(\lvert\log K_{\mathrm{OH}}\rvert)$	3.702
softness (σ_{p})[a]	0.50	$-10.47+83.49(\sigma_{\mathrm{p}})$	10.890
$AN/\Delta IP$; ΔE_0	0.43	$3.93-0.87(AN/\Delta IP)-0.08(\Delta E_0)$	14.458
$\chi^2_{\mathrm{m}}r$; Z^2/r	0.25	$-3.13-0.86(X^2_{\mathrm{m}}r)+1.06(Z^2/r)$	19.008
$\Delta\beta$[a]; Z^2/r	0.75	$-5.54+1.03(\Delta\beta)+0.90(Z^2/r)$	6.459

[a]Variable had a significant effect on log LC50 ($\alpha = 0.05$).
[b]Mean square error from model.

Table 5
Results from the regression analysis of the log LC50 based on percentage of free-ion+percentage of neutral chloro complexes and several ion characteristics

Log LC50 = $f(x)$	r^2	Model (log LC50 =)	MSE[b]
ΔE_0	0.41	$-0.06+0.90(\Delta E_0)$	1.228
$X^2_m r$[a]	0.53	$2.46-0.60(X^2_m r)$	0.976
$\Delta\beta$[a]	0.70	$0.54+0.34(\Delta\beta)$	0.634
log $-K_{SO}MOH$[a]	0.81	$4.02-0.21(\log -K_{SO}MOH)$	0.397
$\lvert\log K_{OH}\rvert$[a]	0.85	$-3.41+0.46(\lvert\log K_{OH}\rvert)$	0.322
Softness (σ_p)[a]	0.70	$-2.91+30.64(\sigma_p)$	0.626
$AN/\Delta IP$[a]; ΔE_0	0.72	$1.39-0.24(AN/\Delta IP)+0.60(\Delta E_0)$	0.687
$X^2_m r$[a]; Z^2/r	0.53	$2.02-0.57(X^2_m r)+0.07(Z^2/r)$	1.134
$\Delta\beta$[a]; Z^2/r	0.75	$-1.14+0.31(\Delta\beta)+0.35(Z^2/r)$	0.599

[a]Variable had a significant effect on log LC50 ($\alpha = 0.05$).
[b]Mean square error from model.

$-K_{SO}MOH$) were statistically significant ($\alpha = 0.05$) in the regression models (Table 4). The ion characteristic that provided the best fit using total metal LC50s as the dependent variable was $\lvert \log K_{OH} \rvert$ ($r^2 = 0.89$, Fig. 1), although log $-K_{SO}MOH$ also provided a very good model. Overall, models based on free ion concentration were generally less effective than those based on total metal concentration and free

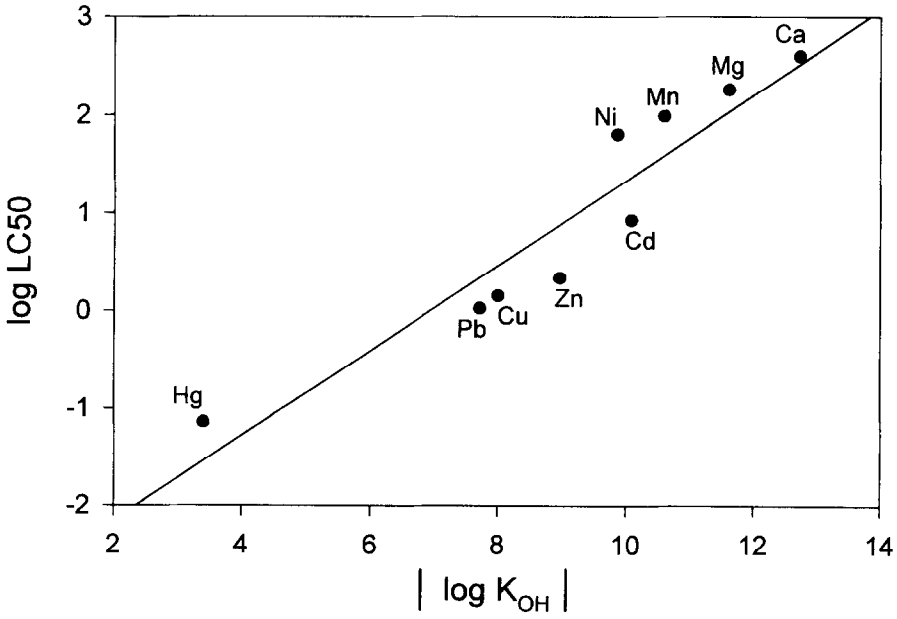

Fig. 1. The model for log LC50 (total metal concentration in mmol l^{-1}) and $\lvert \log K_{OH} \rvert$ for nine metals. For the equation of the regression line, see Table 5.

ion+neutral chloro-complex concentration. The best fitting two-independent variable model for all concentration metameters was log LC50 = $f(\Delta\beta, Z^2/r)$. For all three concentration metameters modelled, $\Delta\beta$ accounted for the majority of the variation in LC50 values, indicating that Z^2/r contributed little to the model fit.

4. Discussion

Predictive models of relative divalent metal ion toxicity to *C. elegans* were produced using ion characteristics. The model with the best fit used | log K_{OH} | as the independent variable, and the concentration metameter based on total metal concentration as the dependent variable ($r^2 = 0.89$, Fig. 1). The results of this study were consistent with earlier predictive modelling efforts based on the Microtox® microbial bioassay by Newman and McCloskey (1996), where the relative decrease in bioluminescence (EC50) was best predicted by the first hydrolysis constant ($r^2 = 0.93$). When McCloskey et al. (1996) modeled Microtox® EC50s for 20 metals with ion characteristics, the softness parameter (σ_p) produced the best model with all metals, although the | log K_{OH} | also produced a good model. Modelling the metals by valence produced better models, especially if | log K_{OH} | was the independent variable (McCloskey et al., 1996).

The use of the free ion and free ion+neutral chloro-complex LC50s did not produce the best fitting models, although they were expected to do so because they accounted for the bioactive species of metals in the test solutions. There appears to be some ambiguity as to which LC50 metameter is optimal for relative toxicity modelling. Upon reanalysis of the Newman and McCloskey (1996) data, free ion+neutral chloro-complex LC50s produced the best fitting models for relative metal toxicity. However, McCloskey et al. (1996) found that LC50s based on percentage free ion did not significantly improve model fits in comparison to LC50s based on total metal concentration.

Although the best model in this study ($r^2 = 0.89$) was produced using LC50s based on total metal concentration and | log K_{OH} | as the independent variable, a very good model ($r^2 = 0.85$) was also produced using free ion+neutral chloro-complex LC50s and | log K_{OH} | as the independent variable. The same is true for models produced using the metal hydroxide solubility product (log $-K_{SO}MOH$). The r^2 values for the total metal LC50s and free ion+neutral chloro-complex LC50s were 0.86 and 0.81, respectively. With such close r^2 values, it is difficult to say which LC50 metameter performs optimally. Of the nine models investigated, six achieved optimal fits when the free ion+neutral chloro-complex LC50 metameter was the dependent variable, two models achieved optimal fits when total metal LC50 metameter was the dependent variable, and only one model achieved optimal fit using the free ion LC50 metameter as the dependent variable.

It is possible that these "bioactive" LC50 metameters did not yield superior performance due to the use of calculated free ion and neutral chloro-complex concentrations. The calculated concentrations of these bioactive species may not be accurate, as assumptions are required for the models used to obtain these estimates.

Perhaps the free ion LC50s and the free ion+neutral chloro-complex LC50s would produce better fitting models if the concentrations of the free ion and the neutral chloro-complexes were measured instead of estimated. Nevertheless, there is sufficient reason and theory to continue investigation of these "bioactive" LC50 metameters.

Free ion LC50 metameters were investigated because it is accepted that the free ion is the most bioactive species. Campbell (1995) has proposed the free-ion activity model (FIAM) to explain how the free ion achieves its toxic effect. Like all models, FIAM is based on a number of assumptions. Violation of model assumptions can create exceptions to the model. Speciation estimations with PC MINTEQA2 in this and previous studies showed that neutral chloro-complexes existed for Hg, Cd, Pb, and Ni. It is known that the neutral chloro-complex of mercury is lipophilic and may be bioactive (Gutknecht, 1981; Bienvenue et al., 1984; Delnomdedieu et al., 1992; Girault et al., 1995; and Mason et al., 1996). Therefore the neutral chloro-complex should be considered in modelling efforts. This led us to consider LC50 metameters based on percentage free ion+neutral chloro-complexes.

The presence of lipophilic metal compounds (neutral chloro-complexes) violates assumption two of FIAM (Campbell, 1995) which states that the interaction of metal ions with the plasma membrane can be described as a surface complexation reaction, forming an M-X-cell (where X is a ligand on the cell surface). Metals exist in the exposure solution as hydrophilic species; lipophilic metal species present in solution that might traverse the plasma membrane without first forming a surface complex are not considered. Lipophilic metal complexes can be formed with organic ligands, and under certain test conditions (chloro-dominated systems like K-medium used in the concentration response experiments) with inorganic anions (Cl^-). K-medium contains 83.0 mmol l^{-1} of Cl^-. These bioactive metal species are probably important in addition to the free-ion in determining bioactivity.

The ion characteristics investigated in this experiment were chosen because each characteristic represented possible ways in which divalent metal ions and biologically significant ligands could interact. The best models were produced using $|\log K_{OH}|$, where K_{OH} is the first hydrolysis constant. A general mechanism to explain acute toxicity of divalent metal ions can be derived through an interpretation of K_{OH}. The first hydrolysis constant is a measure of the ability of a metal ion to form a metal hydroxide ($M^{n+}+H_2O \rightarrow MOH^{n-1}+H^+$). The first hydrolysis constant is, therefore, a measure of metal ion affinity to intermediate ligands like those with O or N donor atoms. Intermediate ligands such as carboxyls, hydroxyls, aldehydes, ketones, and amino compounds are ubiquitous throughout an organism, and are vital to proper protein structure and function. The first hydrolysis constant also reflects a metal's tendency to bind to soft ligands, like those with S centers. The first hydrolysis constant is correlated ($r^2 = 0.61$, for the nine metals used in this study) with the softness parameter (σ_p, a measure of metal ion affinity to soft donor atoms). Binding of metals to these ligands can alter the functional and/or structural nature of proteins resulting in cellular damage, possible inactivation of membrane transport proteins, and uncoupling of metabolic pathways leading to death.

In light of the metals investigated in this study, it makes sense that the first

hydrolysis constant is the best predictor of relative metal toxicity. The metals chosen included those that bind to hard, soft and intermediate ligands. Of the characteristics explored in this study, the first hydrolysis constant accounts best for the hard (Ca and Mg) and soft (Hg and Cd) metals because it contains more information about the affinities of metals for both hard and soft ligands than an ion characteristic that quantifies affinity only on the basis of "hardness" or "softness".

5. Conclusion

This study supports the hypothesis that general prediction of relative metal toxicity from ion characteristics is possible. It also suggests that the log of the first hydrolysis constant is a particularly good prediction variable in such models. This study also shows that identification and quantification of all bioactive metal species does not necessarily improve prediction of relative metal toxicity with ion characteristics. The best overall model was produced using LC50 metameters based on total metal concentration.

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