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The acute toxicity of major ion salts to *Ceriodaphnia dubia*. III. Mathematical models for mixture toxicity

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Abstract

Based on previous research on the acute toxicity of major ions (Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Cl^- , SO_4^{2-} , and $\text{HCO}_3^-/\text{CO}_3^{2-}$) to *Ceriodaphnia dubia*, a mathematical model was developed for predicting the LC50 for any ion mixture, excepting those dominated by K-specific toxicity. One component of the model describes a mechanism of general ion toxicity to which all ions contribute, and predicts LC50s as a function of osmolarity and Ca activity. The other component describes Mg/Ca-specific toxicity to apply when such toxicity exceeds the general ion toxicity, and predicts LC50s as a function of Mg and Ca activities. This model not only tracks well the observed LC50s from past research used for model development, but also successfully predicts LC50s from new toxicity tests on synthetic mixtures of ions emulating chemistries of various ion-enriched effluents and receiving waters. It also performs better than a previously-published model for major ion toxicity. Because of the complexities of estimating chemical activities and osmolarity, a simplified model based directly on ion concentrations was also developed and found to provide useful predictions.

Keywords

Aquatic Toxicology; Mixture Toxicology; Toxicity Mechanism; Concentration Addition; Independent Joint Action

INTRODUCTION

As discussed in previous articles in this series [1,2] and references therein, anthropogenic increases in major geochemical ions (Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Cl^- , SO_4^{2-} , and $\text{HCO}_3^-/\text{CO}_3^{2-}$) pose significant and widespread risks to aquatic communities. Because mixtures of these

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Data availability – Data are available through the USEPA Environmental Dataset Gateway (<https://edg.epa.gov/metadata/catalog/main/home.page>) or by request from the corresponding author (erickson.russell@epa.gov).

ions in aquatic systems vary widely, adequately characterizing risk requires understanding how the toxicities of these ion mixtures depend on their composition.

Mount et al. [1] investigated the acute toxicity of individual major ion salts to *Ceriodaphnia dubia* in various dilution waters, and reported several outcomes important for describing major ion toxicity. First, toxicity relationships are best understood based on chemical activities rather than concentrations. Second, median lethal concentrations (LC50s) for Na and Mg salts are positively correlated with the concentration of Ca in the dilution water, expanding on previous work by other investigators [3–7]. Third, LC50s for K salts are positively correlated with the concentration of Na in the dilution water. Fourth, for K, Mg, and, possibly, Ca salts, toxicity is primarily dependent on the cation rather than the anion. Fifth, for Na salts, both the cation and the anions apparently contribute to toxicity, and using osmolarity as the exposure metric improves coherence of LC50s among the different salts and dilution waters. This association of the toxicities of Na salts with osmolarity is strengthened by mannitol having an LC50 similar to those of the Na salts on an osmolarity basis.

To better understand the effects of more complex ion mixtures, Erickson et al. [2] investigated the acute toxicity of binary mixtures of various major ion salts to *C. dubia*. These studies reinforced the findings of Mount et al. [1] and provided several new insights. First, the joint toxicities of salts with a shared cation are concentration additive, suggesting a shared mechanism of action. Second, mixtures of a Na salt with a Mg salt indicate independent toxic action for toxicity expressed as osmolarity versus Mg activity, with both salts contributing to the osmolarity. Third, the toxicity of KCl is independent of that of MgCl_2 and CaCl_2 when toxicity is expressed as cation activities, and mixtures of KCl with NaCl indicate independence for toxicity expressed as osmolarity versus K activity (with both salts again contributing to osmolarity). Fourth, the joint toxicities of Ca salts and Mg salts show concentration-additivity, superimposed on the positive correlation of Mg LC50s with Ca at non-toxic Ca concentrations. Fifth, when expressed as osmolarity versus Ca activity, the joint toxicities of Ca and Na salts show less-than-additive, but not completely independent, behavior, suggesting factors affecting toxicity that are not resolved. Sixth, the association of Na salt toxicity with osmolarity is further strengthened by the joint toxicity of mannitol and NaCl being approximately additive with similar LC50s on an osmolarity basis, and by mixtures of mannitol with MgCl_2 or CaCl_2 showing interactions very similar to those of NaCl with MgCl_2 or CaCl_2 .

In the present study, we use the data and insights from Mount et al. [1] and Erickson et al. [2] to develop a mathematical model for the acute LC50 of mixtures of major ions to *C. dubia*. This model is tested using additional toxicity tests with synthetic mixtures of ions based on chemistries reported from various ion-enriched effluents and receiving waters. These simulated field mixture tests also are used to compare this new model to an existing model for major ion toxicity [8].

MODEL FORMULATION

General considerations

As discussed in the Introduction, Mount et al. [1] and Erickson et al. [2] reported findings upon which a mathematical model for the acute toxicity of major ions to *C. dubia* can be developed. Based on potency differences and on the existence of both additive and independent joint toxic action among different salts, multiple toxic mechanisms are indicated: a general ion toxicity to which all ions contribute and that is well related to an exposure metric of osmolarity; and cation-specific toxicities for K, Mg, and Ca that are best related to an exposure metric of cation activity. The Mg-specific and Ca-specific toxicities appear to be concentration additive, so could therefore represent a shared mechanism. Overlying the joint toxicity relationships are the positive correlation of Na and Mg salt LC50s with Ca at nontoxic Ca concentrations, and the positive correlation of K salt LC50s with Na at nontoxic Na salt concentrations.

To capture these relationships, a model with two submodels was developed – one for Ca-dependent general ion toxicity (“general ion toxicity submodel”) and the other for joint Mg-specific and Ca-specific toxicity (“Mg/Ca toxicity submodel”). Because of the observed independent action between Mg-specific and general ion toxicities [2], these submodels are formulated and applied separately, whichever indicating greater toxicity being used for an exposure of interest.

Although K salts are more toxic to *C. dubia* than salts of other cations when Na concentrations are low, a submodel for K-specific toxicity was not developed in the present study because exposure scenarios in which K-specific toxicity is dominant would be relatively rare, especially given reduced K-specific toxicity when Na is even moderately elevated [1]. Modeling this toxicity would be a simple extension of the information in [1] and [2] and would involve a third submodel independent of the other two.

The general ion toxicity submodel uses exposure metrics of osmolarity ($\{Osm\}$, mOsm/L) and Ca activity ($\{Ca\}$, mM). The Mg/Ca toxicity submodel uses exposure metrics of Mg activity ($\{Mg\}$, mM) and Ca activity. Both submodels have the same two components: Ca-dependent toxicity in the absence of Ca toxicity and joint toxic action with Ca-specific toxicity at higher Ca levels.

Ca-dependence of general ion toxicity or Mg-specific toxicity in the absence of Ca toxicity

In the absence of Ca toxicity, the relationships of general ion toxicity and Mg-specific toxicity to Ca activity are designated as $LC50_{OsmOnly@ \{Ca\}}$ and $LC50_{MgOnly@ \{Ca\}}$, respectively. The results of Mount et al. [1] show that expressing these relationships on a log-log basis makes them more linear and better illustrates trends. However, even on a log-log basis, these relationships are nonlinear, with the log-log slopes becoming steeper at low Ca and flattening at high Ca (Figures 7 and 8 in [1]). A hyperbolic relationship is used to model such a shape

$$\left(\log \text{LC50}_{\text{XOnly@}\{\text{Ca}\}} - \log \text{LC50}_{\text{XMax}}\right) \times \left(\log \{\text{Ca}\} - \log \{\text{Ca}\}_{\text{Min}}\right) = -S \quad (1)$$

where logarithms are base 10; 'X' designates either Mg or Osm; $\log \text{LC50}_{\text{XMax}}$ is the horizontal asymptote at high Ca; $\log \{\text{Ca}\}_{\text{Min}}$ is the vertical asymptote at low Ca; and S determines the sharpness of the hyperbola. Therefore, $\text{LC50}_{\text{XOnly@}\{\text{Ca}\}}$ as a function of Ca will be

$$\log \text{LC50}_{\text{XOnly@}\{\text{Ca}\}} = \log \text{LC50}_{\text{XMax}} - \frac{S}{\log \{\text{Ca}\} - \log \{\text{Ca}\}_{\text{Min}}} \quad (2)$$

Although this equation is empirical, the use of such an asymptotic relationship does have some toxicological justification. Regarding $\{\text{Ca}\}_{\text{Min}}$, there should be a limit on how low Ca can be before survival is directly affected even without elevated osmolarity or Mg activity. Similarly, although $\text{LC50}_{\text{XOnly@}\{\text{Ca}\}}$ is not directly observable once Ca becomes high enough to contribute to toxicity, it should not, even conceptually, increase without limit.

Because $\text{LC50}_{\text{XMax}}$ is not directly observable, Equation 2 was modified to use the parameter $\text{LC50}_{\text{XOnly@}\{\text{Ca}\}=1}$, which is in the range of, and can be better related to, observed LC50s. This is accomplished by solving Equation 2 for $\log \text{LC50}_{\text{XMax}}$ being equal to $\log \text{LC50}_{\text{XOnly@}\{\text{Ca}\}=1} - S/\log \{\text{Ca}\}_{\text{Min}}$; substituting this back into Equation 2; and combining the terms with S and $\log \{\text{Ca}\}_{\text{Min}}$ to result in

$$\begin{aligned} \log \text{LC50}_{\text{XOnly@}\{\text{Ca}\}} &= \log \text{LC50}_{\text{XOnly@}\{\text{Ca}\}=1} \\ &\quad - \frac{S}{\log \{\text{Ca}\}_{\text{Min}}} \\ &\quad - \frac{S}{\log \{\text{Ca}\} - \log \{\text{Ca}\}_{\text{Min}}} \\ &= \log \text{LC50}_{\text{XOnly@}\{\text{Ca}\}=1} \\ &\quad - \frac{S \times \log \{\text{Ca}\}}{\log \{\text{Ca}\}_{\text{Min}} \times (\log \{\text{Ca}\} - \log \{\text{Ca}\}_{\text{Min}})} \quad (3) \end{aligned}$$

Joint toxic action Ca-specific toxicity with general ion toxicity or Mg-specific toxicity

Three model options for the joint action of Ca-specific toxicity with either general ion toxicity or Mg-specific toxicity were considered.

Concentration addition joint action—The results of Erickson et al. [2] suggest concentration additivity between Mg and Ca, so this option was included in model development. This is based on the standard equation for concentration addition in a binary mixture

$$1 = \text{TU}_X + \text{TU}_{\text{Ca}} = \frac{\{X\}_M}{\text{LC50}_{\text{XOnly@}\{Ca\}}} + \frac{\{Ca\}_M}{\text{LC50}_{\text{CaOnly}}} \quad (4)$$

where TU refers to toxic units; $\{X\}_M$ and $\{Ca\}_M$ are, respectively, the osmolarity or Mg activity and the Ca activity at the LC50 for a mixture “M”; and $\text{LC50}_{\text{CaOnly}}$ is the LC50 for Ca activity in the absence of significant toxicity from osmolarity or Mg activity. Equation 4 can be rearranged and combined with Equation 3 to express $\{X\}_M$ as a function of $\{Ca\}_M$

$$\log\{X\}_M = \left(\log\text{LC50}_{\text{XOnly@}\{Ca\}=1} - \frac{S \times \log\{Ca\}_M}{\log\{Ca\}_{\text{Min}} \times (\log\{Ca\}_M - \log\{Ca\}_{\text{Min}})} \right) + \log \left(1 - \frac{\{Ca\}_M}{\text{LC50}_{\text{CaOnly}}} \right) \quad (5)$$

Intermediate joint action—The results of Erickson et al. [2] indicate that the joint toxic action of osmolarity and Ca is neither concentration additive nor independent (Fig. 9 in [2]). Concentration additivity was not expected because osmolarity already includes an additive contribution from the Ca salt, and any Ca-specific toxicity should act at least somewhat independently from the general ion toxicity; the lack of complete independence indicates some uncharacterized interaction of general ion toxicity with Ca. This is further complicated by the fact that “Ca-only” toxicity cannot be assessed in the absence of considerable osmolarity from the Ca salt. Whatever interactions exist, a more empirical approach is required to model such joint action, which is accomplished by modifying the standard equation for concentration addition (Eq. 4) by raising each toxic unit to the power “p”

$$1 = \left(\frac{\{X\}_M}{\text{LC50}_{\text{XOnly@}\{Ca\}}} \right)^p + \left(\frac{\{Ca\}_M}{\text{LC50}_{\text{CaOnly}}} \right)^p \quad (6)$$

Figure 1 illustrates how this formulation produces a useful gradation between concentration addition and independent action isoboles, but this is strictly empirical and there is no specific mechanistic interpretation of “p”. Equation 6 can be rearranged and combined with Equation 3 to express $\{X\}_M$ as a function of $\{Ca\}_M$

$$\log\{X\}_M = \left(\log\text{LC50}_{\text{XOnly@}\{Ca\}=1} - \frac{S \times \log\{Ca\}_M}{\log\{Ca\}_{\text{Min}} \times (\log\{Ca\}_M - \log\{Ca\}_{\text{Min}})} \right) + \log \left(\left(1 - \left(\frac{\{Ca\}_M}{\text{LC50}_{\text{CaOnly}}} \right)^p \right)^{1/p} \right) \quad (7)$$

Independent joint action—Although the results of Erickson et al. [2] suggest that general ion toxicity and Ca-specific toxicity are not completely independent, modeling efforts still included this option so that evaluations could be made of the statistical significance of the deviation from independence and whether independent action could still serve as a useful approximation. Independent action of two mechanisms is modeled by assuming the probabilities of mortality via each mechanism (P_X , P_{Ca}) combine to produce 50% mixture mortality as follows

$$0.5 = (1 - P_X) \cdot (1 - P_{Ca}) \quad (8)$$

which can be rearranged to express P_X as a function of P_{Ca}

$$P_X = 1 - \frac{0.5}{(1 - P_{Ca})} \quad (9)$$

Consistent with the exposure-effects analysis of Mount et al. [1] and Erickson et al. [2], the tolerance distributions of the test organisms to $\log\{X\}$ and $\log\{Ca\}$ are assumed to be normally distributed with means $\log LC50_{XOnly@ \{Ca\}}$ and $\log LC50_{CaOnly}$ and with standard deviation σ_M . If the cumulative distribution for the standard normal variate z is designated as $F(z)$, $P_X = F((\log\{X\} - \log LC50_{XOnly@ \{Ca\}})/\sigma_M)$ and $P_{Ca} = F((\log\{Ca\} - \log LC50_{CaOnly})/\sigma_M)$. $\{X\}_M$ then can be expressed as a function of $\{Ca\}_M$ as follows

$$\begin{aligned} \log\{X\}_M &= \log LC50_{XOnly@ \{Ca\}} \\ &+ \sigma_M \cdot F^{-1}(P_X) \\ &= \log LC50_{XOnly@ \{Ca\}} \\ &+ \sigma_M \cdot F^{-1}\left(1 - \frac{0.5}{(1 - F((\log\{Ca\}_M - \log LC50_{CaOnly})/\sigma_M))}\right) \end{aligned} \quad (10)$$

which when combined with Equation 3 results in

$$\begin{aligned} \log\{X\}_M &= \left(\log LC50_{XOnly@ \{Ca\}=1} - \frac{S \times \log\{Ca\}_M}{\log\{Ca\}_{Min} \times (\log\{Ca\}_M - \log\{Ca\}_{Min})} \right) \\ &+ \sigma_M \cdot F^{-1}\left(1 - \frac{0.5}{1 - F((\log\{Ca\}_M - \log LC50_{CaOnly})/\sigma_M)}\right) \end{aligned} \quad (11)$$

Because this model describes LC50 data, whereas the parameter σ_M relates to the steepness of the survival versus exposure curves, σ_M will need to be estimated separately from the other model parameters (see *Model Parameterization*).

MATERIALS AND METHODS

Model parameterization

Parameterization for the general ion toxicity submodel was based on osmolarities and Ca activities ($\{Osm\}_{Obs}$, $\{Ca\}_{Obs}$) at the observed median lethal exposure for all tests from [1] and [2] for individual inorganic Na and Ca salts; for Na×Na, Ca×Ca, and Na×Ca salt mixtures (“×” denoting a binary mixture experiment); and for those treatments in other mixtures in which toxicity could be solely attributed to the Na or Ca salt, but excluding tests using organisms reared in other than the standard culture waters for these studies (n=149, Supplemental Material Table S1). Parameterization was conducted not only for the intermediate joint action option (Equation 7), but also for the independent joint action option (Equation 11) to evaluate whether the deviation from independence was significant.

Parameterization for the Mg/Ca toxicity submodel was based on Mg and Ca activities ($\{Mg\}_{Obs}$, $\{Ca\}_{Obs}$) at the observed median lethal exposure for all tests from [1] and [2] for individual inorganic Mg and Ca salts; Mg×Mg, Ca×Ca, and Mg×Ca salt mixtures; and those treatments in other mixtures in which the toxicity could be solely attributed to Mg or Ca, again excluding tests using organisms reared in other than the standard culture waters for these studies (n=128, Supplemental Material Table S2). Parameterization was conducted not only for the concentration addition joint action option (Equation 5), but also for the intermediate joint action option (Equation 7) to evaluate whether there were significant deviations from concentration additivity.

Model parameterization used maximum likelihood analysis to optimize the overall fit between the model-predicted and the observed LC50s. An important aspect of this analysis is that neither the model nor the measured LC50s have a simple dependent versus independent variable framework, but rather consist of $\{Ca\}$ and $\{X\}$ pairs that together define the median lethal exposure and contribute in different amounts to its variability. Optimization procedures must address the difference () in two dimensions between an observed LC50 ($\log\{Ca\}_{Obs}$, $\log\{X\}_{Obs}$) and a suitable corresponding point on the model ($\log\{Ca\}_{Mod}$, $\log\{X\}_{Mod}$)

$$\Delta = \sqrt{\left(\log\{X\}_{Obs} - \log\{X\}_{Mod}\right)^2 + \left(\log\{Ca\}_{Obs} - \log\{Ca\}_{Mod}\right)^2} \quad 12$$

Logarithms are used for this because relative errors are more homogeneous than absolute errors over the large range for the LC50s.

A key aspect of using Equation 12 is the specification of the $\{Ca\}_{Mod}$ and $\{X\}_{Mod}$ suitable for comparison to a given $\{Ca\}_{Obs}$ and $\{X\}_{Obs}$, which will vary with the nature of the toxicity test. For tests with Na or Mg salts at a fixed initial Ca concentration, the comparison was at the same Ca activity, so $\{Ca\}_{Mod}$ was set to $\{Ca\}_{Obs}$ and $\{X\}_{Mod}$ was calculated with the applicable model equation (Equations 5, 7, or 11); Equation 12 therefore simplifies to $=\log\{X\}_{Obs} - \log\{X\}_{Mod}$. When parameterizing the Mg/Ca toxicity submodel or the general ion toxicity submodel with Equation 11, tests with just Ca salts have negligible

toxicity from other ions, so that $\{Ca\}_{Mod}$ will be $LC50_{CaOnly}$ and Equation 12 simplifies to $=\log\{Ca\}_{Obs}-\log LC50_{CaOnly}$. However, $\{Ca\}_{Mod}$ and $\{X\}_{Mod}$ cannot be assigned so simply for mixture tests in which both salts vary by a fixed ratio, or for tests with Ca salts when parameterizing the general ion toxicity model using Equation 7, for which the Ca salts contribute both to Ca-specific toxicity and to osmolarity. In these cases, $\{Ca\}_{Mod}$ and $\{X\}_{Mod}$ were calculated by iterative methods so that the ratio $\{X\}_{Mod}/\{Ca\}_{Mod}$ equaled $\{X\}_{Obs}/\{Ca\}_{Obs}$, thereby addressing both exposure variables.

The maximum likelihood analysis was conducted using custom software written with Intel Professional Fortran Composer XE 2015 and Winteracter Version 9.2. The Complex Method of Box [9] was used to iteratively search for the set of parameters maximizing the likelihood for the observed LC50s, assuming σ_R is normally distributed with standard deviation σ_R . Confidence limits for parameter estimates were computed by iterative searches using the likelihood-ratio method [10]. The Akaike Information Criterion (AIC) was used to compare the fits of alternative models.

As noted in *Model Formulation*, for Equation 11, σ_M could not be estimated in this parameterization procedure because it reflects the variability of the probability of death around the LC50s, thereby requiring information from the individual effects versus exposure relationships. Instead, σ_M was set separately to 0.060, the average value for this variable for 10 tests from [2] for Na×Ca salt mixtures and Mg×Ca salt mixtures near equitoxicity (where the value for this parameter in Equation 11 has a noticeable effect).

Because these models require extensive activity and osmolarity calculations, the possibilities for and the performance of simpler alternatives were of interest. Therefore, parameterizations also were conducted using the same equations, but with total Mg and Ca concentrations instead of Mg^{+2} and Ca^{+2} ion activities and with nominal osmolarities (=the sum of the total molar concentrations of each major ion) instead of calculated osmolarities.

Overview of field-based mixture toxicity tests

Natural waters often have ion ratios markedly different than the single-salt tests of Mount et al. [1] and the binary mixtures of Erickson et al. [2], upon which the submodels were parameterized. To test the models with more field-relevant ion compositions, additional toxicity tests were conducted on synthetic waters patterned after effluents and surface waters with anthropogenic elevations of major ions. Various published data [11–19] and some unpublished data of the authors were examined and used to formulate 10 test waters that encompassed the range of variability observed for cation and anion ratios from these sources and which were feasible to prepare in the laboratory. Table 1 provides the fractional cation and anion concentrations (on a molar basis) in these “field-based mixtures”. Mixtures F0 to F4 were Na dominated (>83% of total cations), but with a considerable range of Ca concentrations and with dominant anions including Cl (F0, F1), HCO_3^-/CO_3^{2-} (F2), SO_4^{2-} (F3), and mixed (F4). Mixtures F5 to F9 had much higher combined Ca and Mg concentrations (>40% of total cations), with SO_4^{2-} as the dominant anion, but also with cases with substantial Cl (F7) and HCO_3^-/CO_3^{2-} (F8). These test waters are not identified or characterized regarding the sources of the ions because the present study was not intended to assess specific risks;

rather, it was intended to evaluate model performance for more complex mixtures like those that would exist in the field, compared to the simpler mixtures of our earlier work [1,2].

Preliminary modeling efforts in the present study were used to estimate total ion concentrations for mixtures with the relative compositions in Table 1 expected to cause 50% mortality (“mixture LC50”). The highest test concentrations (100% mixture strength) for each composition were set to 2.01-fold of this mixture LC50 estimate, except a lower value of 1.41-fold was used for F5 due to solubility limitations for CaSO₄. Formulas using various major ion salts were developed to produce these test concentrations. Tests were conducted in two sets, the first including the Na-dominated solutions (F0 to F4) and the second including the higher Ca and Mg solutions (F5 to F9). To compare organism sensitivities in these field-based mixture tests with previous efforts, simultaneous reference toxicity tests were also conducted with NaCl in the first set and with MgSO₄ in the second. Because the LC50 was not reached for test solution F6, this test was repeated with the highest test concentration increased by 40%, along with an additional MgSO₄ reference test.

Toxicity testing procedures

Toxicity testing procedures followed that of Mount et al. [1], which are just summarized in this section, noting any differences specific to these field-based mixture tests. The dilution water was amended Lake Superior water (ALSW), which was developed by Mount et al. [1] to address concerns about ion ratios in synthetic dilution waters being atypical of natural waters. At the mixture LC50s, the major ions contributed by the ALSW were generally negligible compared to the the added, field-based ion mixtures. However, for waters with extremely low concentrations for some ions (e.g., Table 1, no alkalinity for F3) the ALSW was a substantial source and thus caused the ion ratios to differ from Table 1.

Major ion salts used to prepare ALSW and the test solutions were obtained from Sigma-Aldrich Chemical Company or Fisher Scientific. All chemicals were ACS reagent grade or better. Salt mixtures to achieve the highest test mixture were dissolved directly in ALSW, reducing pH with carbon dioxide to dissolve calcium carbonate when this salt was used (F5, F6, F8) and then restoring the ALSW pH by aeration. This solution was then diluted with ALSW to produce 9 test concentrations ranging from 16% to 100% mixture strength with a 0.8× dilution factor.

Test organisms were <24 h old *C. dubia*, cultured in-house in ALSW, with general culture procedures as described by [20]. Static 48-h toxicity tests were conducted in 30-ml plastic cups containing 10 ml of test solution and held in polystyrene boards floated in a temperature-controlled water bath with a glass sheet covering all test cups. Each cup received food in the form of 100 µl of a 50:50 mixture of YCT (a mixture of yeast, cereal leaves, and trout chow [20]) and algae (*Pseudokirchneriella subcapitata* at 3.5×10^7 cells/ml). Test temperature was $25 \pm 1^\circ \text{C}$ with fluorescent lighting on a 16:8 h (light:dark) photoperiod. Five *C. dubia* neonates were added to each of 2 duplicate cups for each treatment, and survival was determined after 24 and 48 h of exposure; death was defined as no visible movement after gentle prodding and at least 10 s of observation. For the second set of tests, a third cup was also prepared to provide additional volume for alkalinity measurements. Temperature, pH, dissolved oxygen, hardness, alkalinity, and conductivity were monitored

as described in [1], with additional alkalinity measurements as needed to document CaCO_3 precipitation.

For analysis of the major ions other than $\text{HCO}_3^-/\text{CO}_3^{2-}$, analytical samples were collected at the start of exposure from the dilution water and from the highest concentration of each exposure series. In addition, analytical samples were collected from each exposure series at 24 h in the lowest concentration with 100% mortality, and at 48 h from 1 (for the first set) or 2 (for the second set) concentrations nearest the LC50. The samples from the start of the exposure were analyzed for all 4 cations plus Cl and SO_4 . The other samples were analyzed only for the 4 cations. Sample processing and analysis were as described in [1].

Toxicity test data analysis and model comparisons

Median lethal concentrations and their confidence limits were estimated as described in [1], except that these were calculated as a percentage of the highest test mixture for the test, and then converted to nominal added concentrations for each ion. Ion concentrations at the mixture LC50 were calculated either as the sum of these added concentrations and those in the dilution water if ion measurements were within 10% of nominal, or by interpolation of measurements if CaCO_3 or CaSO_4 precipitation was indicated. This interpolation was to the second day of the test to better relate to toxicity increasing with time if Ca concentrations declined. These estimated ion concentrations at the LC50 were subjected to chemical speciation/ion activity and osmolarity calculations (as described in [1]) to provide $\{\text{Ca}\}_{\text{Obs}}$, $\{\text{Mg}\}_{\text{Obs}}$ and $\{\text{Osm}\}_{\text{Obs}}$ for comparison to model predictions. Confidence limits for LC50s expressed as chemical activities or osmolarities were assigned the same relative values as those for the percent test mixture, and do not reflect any additional uncertainty associated with estimating these metrics.

The final general ion toxicity and Mg/Ca toxicity submodels were used to predict $\{\text{Mg}\}_{\text{Mod}}$ and $\{\text{Osm}\}_{\text{Mod}}$ at the LC50 for each field-based mixture and reference salt based on $\{\text{Ca}\}_{\text{Obs}}$. The ratios of observed to predicted $\{\text{Mg}\}$ and $\{\text{Osm}\}$ at the mixture LC50s were used to assess model performance. Similar comparisons were made for models parameterized using Mg and Ca concentrations and nominal osmolarities.

Unlike the model of the present study, the model of Mount et al. [8] does not directly predict LC50s, but rather the expected level of mortality for a specified set of major ion concentrations. To estimate LC50s, the observed concentrations of ions at the mixture LC50s were iteratively and proportionately adjusted up or down to estimate a mixture at which the Mount et al. [8] model predicted 50% mortality. These LC50s were then used to calculate observed to predicted LC50 ratios to judge the performance of this model and compare it to the model developed in the present study.

RESULTS AND DISCUSSION

Mg/Ca toxicity submodel

The final Mg/Ca toxicity submodel uses Equation 5 (concentration addition joint action option):

$$\begin{aligned}
 \log\{\text{Mg}\}_{\text{Mod}} &= \left(8.99 + \frac{2.95 \times \log\{\text{Ca}\}_{\text{Obs}}}{3.52 \times (\log\{\text{Ca}\}_{\text{Obs}} + 3.52)} \right) \\
 &+ \log \left(1 - \left(\frac{\{\text{Ca}\}_{\text{Obs}}}{7.74} \right) \right) \Rightarrow \{\text{Mg}\}_{\text{Mod}} \\
 &= 8.99 \times 10^{\frac{0.84 \times \log\{\text{Ca}\}_{\text{Obs}}}{\log\{\text{Ca}\}_{\text{Obs}} + 3.52}} \\
 &\times \left(1 - \frac{\{\text{Ca}\}_{\text{Obs}}}{7.74} \right)
 \end{aligned} \tag{13}$$

Parameter estimates are provided in Table 2. The model relationship and the parameterization data are plotted on Figure 2, using both logarithmic scaling (Fig. 2A, which better shows the Ca-effects on Mg toxicity in the absence of Ca toxicity) and arithmetic scaling (Fig. 2B, which shows the approximate linearity of {Mg} vs. {Ca} at higher Ca, indicative of concentration addition). Figure 2 also shows the inferred relationship of Mg toxicity vs. Ca in the absence of Ca toxicity (dotted curves) against which concentration addition is calculated, including the hypothetical part of this relationship at high Ca levels.

The model relationship in Figure 2 tracks the data well. Based on the estimate of 0.087 for σ_R (Table 2), a 2 standard deviation variation of LC50s from the model is about 1.51-fold. The degree of scatter of observed LC50s from the model is greater at low {Ca} than at higher {Ca}, but such variation is not addressed by the model. The data scatter could reflect a variety of sources – uncertainties in LC50 estimation, Ca concentrations, and speciation calculations, as well as factors affecting toxicity not addressed by the model. An important aspect of the scatter is some correlation of Mg salt LC50s to the test salt anion even when Mg is expressed as activity (i.e., MgSO₄-dominated exposures tend to be above the model curve and the other Mg salts tend to be below). The model thus would represent the average mix of anions in the parameterization data, which can have some impact on predictions for mixtures dominated by a different anion mix (see *Model performance*).

To test for non-additivity, Equation 7 (intermediate joint action option) was also applied. This resulted in an increase rather than decrease in the Akaike Information Criterion (–251.3 for Equation 5 vs –249.8 for Equation 7) and an estimate for the power parameter “p” that was not significantly different from unity (1.12, 95% confidence limits=0.93–1.43), so this model was rejected. This strengthens the case for concentration additivity of Mg and Ca, suggesting a shared mechanism for these two ions.

General ion toxicity submodel

The final general ion toxicity submodel uses Equation 7 (intermediate joint action option), consistent with the observation of Erickson et al. [2] that interactions of Na and Ca salts are not additive but also not completely independent:

$$\begin{aligned}
 \log\{\text{Osm}\}_{\text{Mod}} &= \left(85.4 + \frac{0.51 \times \log\{\text{Ca}\}_{\text{Obs}}}{2.43 \times (\log\{\text{Ca}\}_{\text{Obs}} + 2.43)} \right) \\
 &+ \log \left(\left(1 - \left(\frac{\{\text{Ca}\}_{\text{Obs}}}{7.81} \right)^{3.6} \right)^{1/3.6} \right) \Rightarrow \{\text{Osm}\}_{\text{Mod}} \\
 &= 85.4 \times 10^{\frac{0.210 \times \log\{\text{Ca}\}_{\text{Obs}}}{\log\{\text{Ca}\}_{\text{Obs}} + 2.43}} \\
 &\times \left(1 - \left(\frac{\{\text{Ca}\}_{\text{Obs}}}{7.81} \right)^{3.6} \right)^{1/3.6}
 \end{aligned} \tag{14}$$

Parameter estimates are provided in Table 2, and the model relationship and the parameterization data are plotted on Figure 3, again using both logarithmic and arithmetic scaling.

This model also tracks the data well. The variability of the data around the model ($\sigma_R=0.046$, Table 2) is even less than for the Mg/Ca toxicity submodel, with 2 standard deviations representing a factor of only 1.25 for the data scatter from the model line, primarily due to less scatter at low {Ca} than for Figure 2. Unlike for {Mg} vs. {Ca} in Figure 2B, there is no indication of a linear relationship between {Osm} and {Ca} at higher Ca in Figure 3B, and the model's power parameter "p" is significantly greater than 1.0 (3.6, 95% confidence limits=2.5–6.6, Table 2). Based on Figure 1, this value for the power indicates joint action much closer to independent action than concentration addition.

The alternative of Equation 11 (independent joint action option) resulted in an increase in the Akaike Information Criterion (−478.9 for Equation 7 vs −474.0 for Equation 11), substantiating that there is some deviation of the data from independent action. This independent action model is also plotted on Figure 3 (dashed curve), with the deviation of this model from the data and primary model (solid curve) being most evident in Figure 3B for the {Ca}=5–7 mM range. However, the differences between the lethal osmolarities of the two models are relatively modest except at Ca activities near $\text{LC50}_{\text{CaOnly}}$. The differences are less than 20% for {Ca}<6.5 mM and exceed 40% only when {Ca}>95% of $\text{LC50}_{\text{CaOnly}}$. These large differences are not likely to be of practical significance because they would require nearly lethal Ca levels with substantial levels of other ions (to contribute to osmolarity), but with low levels of Mg (because otherwise the Mg/Ca submodel would apply). As such, the discussion in *Alternative models* will consider the suitability of simpler models based on independent action.

Model performance – field-based mixture tests

For the field-based mixture tests, Supplemental Information Table S3 documents values at the mixture LC50s for pH, total concentrations and activities of all major ions, and osmolarities. To assess model performance, Table 3 provides {Ca}_{Obs}, {Mg}_{Obs} and {Osm}_{Obs}; {Mg}_{Mod} and {Osm}_{Mod} at {Ca}_{Obs}; and ratios for these observed to predicted values. The ratios are provided only for whichever model predicts the greater toxicity and

thus is applicable to that test solution. Table 3 also indicates the percentage loss of [Ca] from nominal concentrations due to CaCO_3 or CaSO_4 precipitation. These Ca losses (and the associated anion losses) cause the ion ratios in the final test solutions to differ substantially from the field data upon which these test solutions were based (Table 1). These losses can be attributed both to our highest test concentration being greater than the field data used to establish the ion proportions (to reach toxic levels) and to factors in field waters that might reduce CaCO_3 oversaturation and precipitation (e.g., higher CO_2 levels). Nevertheless, these solutions still provide more diverse and realistic ion combinations useful for assessing model performance. Figure 4 compares the observed LC50s for the field-based mixtures to the model lines and parameterization data.

The Mg/Ca toxicity submodel predicts more toxicity than the general ion toxicity submodel for only 2 field-based mixtures (F5, F6) and the MgSO_4 reference tests (Table 3). For the MgSO_4 reference tests, the observed:predicted {Mg} ratios are moderately greater than 1.0 (1.36 for the initial test and 1.29 for the retest); this is consistent with the aforementioned anion-related deviations of data from the model (Figure 2), especially at low {Ca}. For mixture F5, {Mg}_{Mod} is within the confidence limits of {Mg}_{Obs} and the observed:predicted ratio (0.94) is not significantly different than 1.0. For mixture F6, no toxicity was observed at the highest mixture concentration in the initial test, with an observed:predicted ratio >1.05. The retest indicated the LC50 is only slightly higher, with an observed:predicted ratio of 1.11.

For the other 8 field-based mixtures and for the NaCl reference test, the general ion toxicity submodel predicts greater toxicity (Table 3). For all 9 tests, the observed:predicted {Osm} ratios are close to 1.0, ranging from 0.94 to 1.20, with the two highest ratios (F2 and F4) being for tests with very low Ca activities and higher uncertainties. For 6 of these tests {Osm}_{Mod} at the LC50 falls within the confidence limits of {Osm}_{Obs}.

For all 9 tests for which the general ion toxicity submodel was identified as predicting more toxicity, Figure 4B illustrates how the observed field-based LC50s track this model well, within the data scatter. The 3 test solutions predicted to be more toxic by the Mg/Ca toxicity submodel fall below the general ion toxicity submodel, albeit slightly so for mixture F6. In contrast, Figure 4A illustrates how most of the tests fall far below the curve for the Mg/Ca toxicity submodel, except for the 3 test solutions identified as being more toxic by this model. Mixture F8 is also fairly near the Mg/Ca toxicity submodel line, its observed:predicted ratio being 0.74.

Mixtures F7–F9 were in the second set of field-based solutions selected to have substantial combined Ca and Mg concentrations (Table 1), but were still estimated to be more subject to general ion toxicity than Ca/Mg-specific toxicity. This can be attributed to Na still being 49–57% of the initial total cation concentration (Table 1), and its relative concentration being even greater (63–68%) after Ca precipitation (Table 3) is accounted for. Combined with the contributions of the Ca and Mg and their associated anions to osmolality, total ion concentrations were enough to cause the general ion toxicity to be greater than Mg/Ca toxicity. The combined Ca and Mg concentrations need to substantially exceed that of Na for the Mg/Ca toxicity submodel to apply.

Model performance – binary salt mixture tests

Because model assumptions and parameterizations were based largely on the binary test results from Erickson et al. [2], model predictions would naturally be good for these tests, especially the Mg×Ca and Na×Ca tests so important to defining model shape at {Ca}>1.0 (Figures 2 and 3). However, it is useful to examine how well the models describe the NaHCO₃×MgCO₃ mixture experiment that was highlighted by Erickson et al. [2] as having an atypical, complicated shape (Fig. 4 in [2]) affected by the independent mechanisms of Mg-specific and general ion toxicity, the effect of Ca on these mechanisms, and CaCO₃ precipitation in these tests.

The observed LC50s from this experiment (Figure 5) deviate greatly from the simple rectangular (independent) shape for {Mg}_{Obs} vs {Osm}_{Obs} shown by other Na×Mg salt mixture experiments (Fig. 3 in [2]) that is indicative of independent joint action. Rather than having a flat upper portion at constant {Mg}_{Obs} where Mg-specific toxicity determines effects, this portion of the isobole has a substantial trend of decreasing {Mg}_{Obs} as {Osm}_{Obs} increases. And rather than having a vertical right portion at constant {Osm}_{Obs} when general ion toxicity dictates effects, this portion of the isobole shows considerable increase in {Osm}_{Obs} as {Mg}_{Obs} increases. Erickson et al. [2] noted that the observed data trends in Figure 5 are correlated with {Ca} and qualitatively consistent with the effect of {Ca} on both Mg-specific and general ion toxicity in the absence of Ca-specific toxicity.

The model was applied to each test in Figure 5 by first applying both submodels to {Ca}_{Obs}, which determined {Mg}_{Mod} if the Mg/Ca toxicity model predicted more toxicity (pink stars) and {Osm}_{Mod} if the general ion toxicity submodel predicted more toxicity (green stars). The plotted value for the less toxic exposure metric was calculated based on keeping constant the ratio of {Mg} to {Osm} for the test. For example, if {Mg}_{Mod} was predicted to determine toxicity, but at a value 20% higher than {Mg}_{Obs}, {Osm}_{Mod} was also increased 20% relative to {Osm}_{Obs}, keeping the ratio constant. A more exact calculation of the {Osm}_{Mod} to associate with {Mg}_{Mod} would be very complicated, so this simple calculation was used to provide a reasonable approximation in Figure 5 for illustrative purposes.

The model predictions in Figure 5 reproduce the observed trends, showing both a similar downward slant for the upper portion of the isobole and a similar rightward tilt for the right portion of the isobole. For the points in the upper portion of the isobole driven by Mg-specific toxicity, the deviation between the predicted and observed points can be attributed to the aforementioned anion effects for the Mg/Ca toxicity submodel that result in underestimation of MgSO₄ LC50s and overestimation of MgCO₃ LC50s, which in this case is about 10–20%.

Alternative models – Concentration-based models

The results of Mount et al. [1] and Erickson et al. [2] demonstrate that the effects of major ions are better related to chemical activities than concentrations, which is not surprising since this is a basic principle of chemical reactivity. However, activity-based models are not easily related to environmental measurements of major ions, because they require extensive calculations. Although this can be addressed with software that provides the necessary

calculations and relationships, this does raise the issue of whether alternative models based directly on measured concentrations could provide acceptable performance.

Equation 5 was parameterized using Mg and Ca concentrations from Supplemental Information Table S2 rather than activities, resulting in:

$$[\text{Mg}]_{\text{Mod}} = 20.1 \times 10^{\frac{1.16 \times \log\{\text{Ca}\}_{\text{Obs}}}{\log\{\text{Ca}\}_{\text{Obs}} + 2.90}} \times \left(1 - \frac{[\text{Ca}]_{\text{Obs}}}{19.7}\right) \quad (15)$$

This concentration-based Mg/Ca toxicity submodel is compared to the parameterization data and to the field-based mixture LC50s in Table 4 and Figure 6A. There is considerably more data scatter for this than the activity-based analysis (Figure 2, Figure 4A), with the estimated σ_R (0.13) being 50% greater than for the activity-based model, and 2 standard deviations corresponding to a nearly 2-fold deviation from the model (rather than the 1.5-fold deviation for activities). This scatter is mainly due to LC50s for the different Mg salts having more pronounced differences on a concentration basis than an activity basis, due to ignoring differences in the degree of cation complexation by anions, with MgSO_4 being substantially less toxic than the other Mg salts on a concentration basis (Figure 6A). As a result, the toxicities of the MgSO_4 reference tests and of mixtures F5 and F6 are now markedly overestimated, with observed:predicted LC50 ratios of 1.4–2.6 (Table 4, Figure 6A). For the concentration-based Mg/Ca toxicity submodel, field-based mixtures F7–F9 now have toxicities that appear to be more in accord with the model (Figure 6A), but this is misleading given the anion effect and the fact that general ion toxicity is more important in these mixtures than Mg/Ca toxicity. However, even though toxicity is overestimated for sulphate-dominated waters, this concentration-based Mg/Ca toxicity submodel might still serve as a useful screening tool for Mg and Ca concentrations. To improve applicability, the model could be reparameterized based on tests with anion mixtures closer to the field samples to which the model will be applied.

For general ion toxicity, because the activity-based model indicated limited differences between the intermediate and independent joint action options, our concentration-based model development not only used nominal osmolarity and calcium concentration data (Supplemental Information Table S1), but also treated Ca-specific toxicity as independent of general ion toxicity (Equation 11 rather than Equation 7). Furthermore, because this assumption of independence allows Ca toxicity to be addressed separately with the Ca/Mg toxicity submodel, the final concentration-based general ion toxicity submodel can be expressed just as the Ca-dependent osmolarity LC50 in the absence of Ca toxicity:

$$[\text{Osm}]_{\text{Mod}} = 83.5 \times 10^{\frac{0.38 \times \log[\text{Ca}]_{\text{Obs}}}{\log[\text{Ca}]_{\text{Obs}} + 2.39}} \quad (16)$$

where [Osm] denotes nominal osmolarity. This model is compared to the parameterization data and to the field-based mixture LC50s in Table 4 and Figure 6B. Unlike the Mg/Ca toxicity submodel, changing from an activity-based model to a concentration-based model

still provided good performance, σ_R increasing only from 0.048 to 0.053. The 8 field-based mixtures to which this general ion toxicity submodel applies again show close agreement between the observed and predicted LC50s (Table 4, Figure 6B), the ratios varying from 0.98–1.17, similar to those for the activity-based model (Table 3). Additionally, the observed and predicted LC50s for F5 and F6 are now very close (Figure 6B), suggesting that this general ion toxicity submodel might be useful for mixtures with rather high proportions of Mg and/or Ca. Therefore, this concentration-based general ion toxicity submodel deserves consideration as a simpler alternative to the activity-based one, provided there are also appropriate limits on combined Mg and Ca concentrations.

A variety of factors could explain the poorer performance of the concentration-based Mg/Ca-specific toxicity model relative to its activity-based counterpart, in contrast to the general ion toxicity submodel which performed well on either basis. These include large differences between activities and concentrations for Mg and Ca (and thus large estimation uncertainties), compared to lesser differences between nominal and actual osmolarities; the apparent anion effect on Mg-specific toxicity, and to some extent Ca-specific toxicity, that is much greater on a concentration-basis but less of an issue for general ion toxicity; and the dependence of the general ion toxicity submodel on all ions, making it less sensitive to uncertainties for individual ions.

Alternative models – Mount et al. [8]

Mount et al. [8] developed a regression model for the percent survival of *C. dubia* as a function of major ion concentrations, based on extensive testing of single salts and 1:1 salt mixtures in moderately hard reconstituted water. Although the basic structure of this model involves additivity among the ions, their mixture data indicated less-than-additive toxicity for salts with different cations, which was addressed with an empirical correction based on whether single or multiple cations were present at substantial concentrations. This less-than-additive toxicity could reflect the amelioration of Na salt and Mg salt toxicity by elevated Ca, the amelioration of K salt toxicity by elevated Na, and the independent action of various exposure metrics noted in the present study.

Table 5 provides predictions for the field-based mixture experiments using the Mount et al. [8] model. The model was first used to predict percent survival at the observed ion concentrations at the LC50 for each field-based mixture (Supplemental Information Table S3). The model was then used to iteratively determine the factor by which this chemistry must be reduced or increased (proportionately for all ions) so that the survival was 50%. The multiplicative inverse of this factor would be the ratio of the observed to the predicted LC50 for the ion mixture, and is reported in Table 5.

For the NaCl and MgSO₄ reference tests and for the tests with the NaCl-dominated mixtures F0 and F1, the predicted survival is reasonably near 50% (considering the steepness of the toxicity curves for major ions) and observed:predicted LC50 ratios are within 14% of 1.0 (Table 5). However, for the other mixtures, predicted survival is close to zero and the observed LC50s are 33% to >100% higher than predicted. These mixtures are variously affected by different elements of joint toxicity and the effect of non-toxic Ca concentrations

on ion toxicity (Figures 4 and 6), which are not explicitly considered by this model and lead to this overestimation of toxicity.

Nonetheless, except for the Mg/Ca-dominated mixtures (F5 and F6), the deviations of the Mount et al. [8] model from the field-based mixture studies in the present study are rather modest. Also, in the face of uncertainty, the model errs on the side of overestimating toxicity. As such, this model has been a useful assessment tool. However, with the more extensive data and mechanistic insights of Mount et al. [1] and Erickson et al. [2], the models of the present study provide better performance.

SUMMARY AND IMPLICATIONS

Previous work on the acute toxicity of major ions to *C. dubia* [1,2] provided data and established relationships that support the development of models for predicting this toxicity, including: the existence of general ion and cation-specific toxicity mechanisms; whether interactions between different ions or metrics are additive or independent; and effects of nontoxic concentrations of some ions on the toxicity of others. In the present study, a mathematical model was developed consisting of a general ion toxicity submodel describing osmolarity-based LC50s as a function of Ca activity and a Mg/Ca toxicity submodel describing LC50s in terms of joint Mg and Ca activities, with the submodel indicating greater toxicity being that which is applied to an ion mixture of interest.

This model tracks the available data very well (Figures 2 and 3), although there are possible secondary effects not addressed by the model (e.g., some anion role in the toxicity of Mg salts). This model also predicts well the toxicity observed in synthetic mixtures of ions based on field studies (Figure 4), although few of these field-based mixtures were pertinent to assessing the Mg/Ca toxicity submodel.

Although chemical activities are the more accurate exposure metrics for this model, a simpler alternative model based on ion concentration (rather than activity) was also evaluated. A general toxicity submodel based on nominal osmolarities performed well, and could provide a viable alternative to the activity-based approach. A concentration-based Mg/Ca submodel showed poorer performance, but could still provide limits for combined Mg and Ca concentrations to supplement the general ion toxicity submodel.

The model from the present study provides a good basis for assessing the risk of major ions, but only for the acute toxicity to *C. dubia* and was based on a very large amount of data. This raises the issue of needing relationships for other species and other endpoints (growth, reproduction), and the practical need to develop these with fewer data and less effort. For chronic *C. dubia* toxicity, the acute model has been used to design a much smaller set of toxicity tests to evaluate its applicability to chronic toxicity and the utility of acute:chronic ratios to extrapolate from acute to chronic effects; the next paper in this series will address these efforts.

Extension of this model to other species is more uncertain because of the diverse ionoregulatory adaptations of different freshwater species. However, insights from the present study can also guide more limited and targeted testing with other species to evaluate where

the relationships in the *C. dubia* model can be applied, and where different relationships need to be addressed. This has been and is a focus of ongoing work in our laboratory and others, and forthcoming papers will show both similarities and differences among species, and discuss models for these species.

Finally, because natural aquatic communities are adapted to a wide range of ambient ion concentrations, regulatory applications of this evolving work need to consider the mix of species relevant to any site. Although species relevance is an issue for any aquatic risk assessment, this could be more of an issue for major ions than for xenobiotic contaminants, warranting consideration of mesocosm and field data to better understand how to apply single-species laboratory tests.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Degree of non-additivity produced by power term (p) in joint toxicity equation. TU_A refers to toxic units of chemical A and TU_B refers to toxic units of chemical B.

Figure 2. Relationship of magnesium activity to calcium activity at median lethal conditions for *C. dubia*, plotted on logarithmic (A) and arithmetic (B) scales. The solid curve denotes the fitted model based on a hyperbolic relationship (dotted curve) of Mg toxicity to Ca in the absence of Ca toxicity and concentration addition of Mg and Ca toxicities. Arrows between different symbols in the legend refer to graded colors representing varying mixture ratios.

Figure 3.

Relationship of osmolarity to calcium activity at median lethal conditions for *C. dubia*, plotted on logarithmic (A) and arithmetic (B) scales. The solid curve denotes the fitted model based on a hyperbolic relationship of osmolarity toxicity to Ca in the absence of Ca toxicity and joint toxicity of osmolarity and Ca intermediate between concentration addition and independent action. The dashed curve denotes a model based on independent joint action. Arrows between different symbols in the legend refer to graded colors representing varying mixture ratios.

Figure 4.

Comparison of model LC50 predictions (solid curve) to observed toxicity in experiments with field-based mixtures for model based on Ca activity, Mg activity, and osmolarity. Large circles denote the first set of tests with Na-dominated mixtures and large squares denote the second set of tests for mixtures with higher Ca/Mg concentrations. Mixture designations F0 to F9 are provided within the symbols, with Na denoting the NaCl reference test and Mg denoting the MgSO₄ reference test (see Supplemental Table S3 for test water compositions). Green denotes tests predicted to be more toxic by the general ion toxicity (osmolarity) model while pink denotes those predicted to be more toxic by the Mg/Ca toxicity model. Smaller symbols denote the data used in model parameterization (see Figures 2 and 3 for meaning of symbol shapes and colors).

Figure 5.

Comparison of model LC50 predictions (solid curves and stars) to observed osmolarity and magnesium activities at the LC50 (blue circles) for a binary mixture experiment with NaHCO_3 and MgCO_3 . Calcium activities (mM) for each test are provided adjacent to each point. For the model predictions, green denotes tests in which general ion toxicity was estimated to be greater than Mg/Ca-specific toxicity and pink denotes the reverse.

Figure 6.

Comparison of model LC50 predictions (solid curve) to observed toxicity in experiments with field-based mixtures for model based on Ca total concentration, Mg total concentration, and nominal osmolarity. Large circles denote the first set of tests with Na-dominated mixtures and large squares denote the second set of tests for mixtures with higher Ca/Mg concentrations. Mixture designations F0 to F9 are provided within the symbols, with Na denoting the NaCl reference test and Mg denoting the MgSO₄ reference test (see Supplemental Table S3 for test water compositions). Green denotes tests predicted to be more toxic by the general ion toxicity (osmolarity) model while pink denotes those predicted to be more toxic by the Mg/Ca toxicity model. Smaller symbols denote the data used in model parameterization (see Figures 2 and 3 for meaning of symbol shapes and colors).

Table 1Relative concentrations^a of major ions for formulation of field based mixtures

Mixture name	Percentage of total cations				Percentage of total anions			
	Na	K	Ca	Mg	Cl	SO ₄	Alkalinity	
F0	93.2	0.5	2.7	3.6	98.7	0.1	1.2	
F1	83.1	0.3	14.7	1.9	99.9	0.1	0.1	
F2	98.4	0.4	0.9	0.3	9.4	0.5	90.1	
F3	90.6	0.7	6.3	2.4	36.9	63.1	0.0	
F4	97.1	1.3	1.2	0.4	24.9	21.9	53.3	
F5	2.0	2.3	35.3	60.4	0.8	87.2	11.9	
F6	7.8	1.5	36.6	54.1	0.5	57.3	42.1	
F7	57.2	3.6	27.7	11.5	59.2	39.3	1.5	
F8	48.8	1.7	26.0	23.6	4.4	35.3	60.3	
F9	54.5	1.0	25.6	18.9	22.8	43.0	34.1	

^aOn molar basis, except equivalents basis for alkalinity

Table 2

Parameter estimates for activity-based model.

Mg/Ca toxicity submodel (Equation 5)		General ion toxicity submodel (Equation 7)	
Parameter name	Parameter value	Parameter name	Parameter value
LC50 _{CaOnly} (mM)	7.74 (7.16–8.36) ^a	LC50 _{CaOnly} (mM)	7.81 (7.50–8.15) ^a
LC50 _{MgOnly@{Ca}=1} (mM)	8.99 (8.38–9.64) ^a	LC50 _{OsmOnly@{Ca}=1} (mOsm/L)	85.4 (82.9–88.0) ^a
{Ca} _{Min} (mM)	0.00030 (0.00017–0.00051) ^a	{Ca} _{Min} (mM)	0.0037 (0.003–0.0044) ^a
S (Hyperbola Sharpness)	2.95 (2.45–3.48) ^a	S (Hyperbola Sharpness)	0.51 (0.41–0.63) ^a
		p (Power)	3.6 (2.5–6.6) ^a
σ _R (for log LC50)	0.087 (0.078–0.099) ^a	σ _R (for log LC50)	0.046 (0.041–0.052) ^a

^a95% confidence limits

Table 3

Performance of activity-based model for predicting toxicity of field-based ion mixtures.

Test solution	Ca activity @LC50 (mM)	Osmolarity @LC50 (mOsm/L)			Mg activity @LC50 (mM)		
		Observed	Predicted	Ratio ^a	Observed	Predicted	Ratio
NaCl	0.17	67 (62–71) ^b	68	0.97	0.077	5.1	
F0	0.55	79 (71–88)	81	0.98	0.65	7.2	
F1	2.85	94 (87–103)	91	1.03	0.37	7.1	
F2	0.031 (69%) ^c	44 (40–49)	38	1.15	0.041	2.1	
F3	0.90	85 (80–91)	85	1.01	0.36	7.7	
F4	0.055 (63%)	62 (58–67)	51	1.20	0.062	3.0	
MgSO ₄	0.10	27	61		5.7 (5.2–6.3)	4.2	1.36
F5	3.0 (14%)	61	92		6.5 (6.1–7.0)	6.9	0.94
F6	1.8 (66%)	>75	89		>8.4	7.9	>1.05
F7	3.7 (20%)	104 (95–113)	92	1.13	2.0	6.2	
F8	0.13 (96%)	61 (55–67)	65	0.94	3.4	4.6	
F9	1.8 (58%)	101 (90–117)	89	1.13	3.4	7.9	
MgSO ₄ repeat	0.11	25	62		5.5 (5.0–5.9)	4.2	1.29
F6 repeat	1.7 (68%)	79	89		8.8 (8.3–9.4)	7.9	1.11

^aRatios are observed:predicted osmolarities or Mg activities at the LC50 for the submodel predicting the greater toxicity.

^bParentheses under osmolarities or Mg activities at the LC50 denote 95% confidence limits for the submodel predicting the greater toxicity.

Ca activities denote estimated percent loss of Ca at the LC50.
c

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Table 4

Performance of concentration based model for toxicity of field based ion mixtures.

Test solution	Ca conc. @LC50 (mM)	Nominal osmolarity @LC50 (mOsm/L)		Mg concentration @LC50 (mM)	
		Observed	Predicted	Observed	Predicted
NaCl	0.36	71 (66–76) ^b	69	1.03	1.03
F0	1.24	84 (78–94)	86	0.98	1.5
F1	7.1	103 (94–112)	105	0.98	0.9
F2	0.13 (69%) ^c	51 (46–57)	50	1.03	0.13
F3	4.0	101 (94–107)	99	1.01	1.5
F4	0.24 (63%)	71 (67–77)	61	1.17	0.22
MgSO ₄	0.36	39	69	18.6 (17.0–20.3)	12.2
F5	14.6 (14%)	92	111	29.0 (27.1–31.0)	11.2
F6	9.4 (66%)	>112	108	>40	20.6
F7	14.7 (20%)	125 (115–137)	111	1.12	7.6
F8	0.57 (96%)	77 (70–85)	76	1.02	13.2
F9	8.7 (58%)	127 (113–146)	107	1.18	15.2
MgSO ₄ repeat	0.36	36	69	17.3 (15.9–18.9)	12.2
F6 repeat	9.3 (68%)	118	107	43.3 (40.7–46.1)	20.7

^aRatios are observed; predicted osmolarities or Mg activities at the LC50 for the submodel predicting the greater toxicity.

^bParentheses under osmolarities or Mg activities at the LC50 denote 95% confidence limits for the submodel predicting the greater toxicity.

^cParentheses under Ca activities denote estimated percent loss of Ca at the LC50.

Table 5

Performance of Mount et al. [8] model for predicting toxicity of field-based ion mixtures.

Test solution	Mount et al. [8] model predictions	
	% survival at observed LC50	Observed:predicted LC50
NaCl	72	0.89
F0	26	1.12
F1	76	0.85
F2	4	1.39
F3	6	1.33
F4	2	1.46
MgSO4	39	1.05
F5	0	1.71
F6	0	>2.1
F7	5	1.36
F8	3	1.43
F9	1	1.62
MgSO4 repeat	53	0.99
F6 repeat	0	2.22