BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:)
)
PROPOSED AMENDMENTS TO:) R07-009
35 Ill. Adm. Code 302.102(b)(6), 302.102(b)(8)) Rulemaking – Water
302.102(b)(10), 302.208(g), 309.103(c)(3),)
405.109(b)(2)(A), 405.109(b)(2)(B), 406.100((d))
REPEALED 35 Ill. Adm. Code 406.203 Part 407, and)
PROPOSED NEW 35 Ill. Adm. Code 302.208(h))

NOTICE OF FILING

TO: See Attached Service List

PLEASE TAKE NOTICE that the Environmental Law and Policy Center of the Midwest ("ELPC"), Prairie Rivers Network and the Sierra Club today have electronically filed POST HEARING COMMENTS OF PRAIRIE RIVERS NETWORK, SIERRA CLUB AND THE ENVIRONMENTAL LAW AND POLICY CENTER.

Respectfully submitted,

Albert F. Ettinger (Reg. No. 3125045)
Counsel for Environmental Law & Policy Center, Prairie Rivers Network and Sierra Club

DATED: June 7, 2007

BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

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POST HEARING COMMENTS OF PRAIRIE RIVERS NETWORK, SIERRA CLUB AND THE ENVIRONMENTAL LAW AND POLICY CENTER

Prairie Rivers Network ("PRN"), Sierra Club and the Environmental Law and Policy Center ("ELPC") continue to support the proposed changes to the Illinois water quality standards for sulfate and total dissolved solids. As was discussed during the hearing, these standards were developed following extensive new sulfate toxicity testing and numerous interest group discussions hosted by the United States Environmental Protection Agency (USEPA) in which members of the Illinois Coal Association and other organizations fully participated.

We remain concerned about a few elements of the proposal, particularly regarding the proposed changes to the mixing rules that were not addressed during the USEPA-hosted discussions.

Our specific comments are as follows:

Mixing

PRN, Sierra Club and ELPC, through the pre-filed testimony of Glynnis Collins, proposed language for 302.102(b)(8) that would codify IEPA's current practice with regards to allowed mixing in the situation in which 3:1 dilution is not available but in which the receiving water has a higher flow than a zero 7Q1.1. Somewhat to our surprise, IEPA has not accepted our proposal to write its current practice into the Board rules suggesting that it might in the future want to deviate from its current practice of assuring a zone of passage for aquatic life by insisting on at least 50% of the volume of flow.

IEPA also claims that the proposal made by PRN, Sierra Club and ELPC is arbitrary and has no scientific basis. IEPA, however, admits that the current Board rule covering the situation where 3:1 dilution is available was based on just the kind of consistent agency practice that is the basis for our proposed rule for the situation where less than 3:1 dilution is available. (Transcript of Proceedings, April 23, 2007 p. 56-61)

Clearly, there is something wrong here. Either it was wrong for the Board to adopt its current rule regarding the situations where more than 3:1 dilution is available or there is something wrong with IEPA's thinking regarding the PRN/Sierra Club/ELPC proposal as to how to treat the situation where less than 3:1 dilution is available.

In fact, drawing reasonable lines based on past practice and experience is both proper and a very common regulatory function. Administrative agency regulatory numerical standards are lawfully established if they are "within a zone of reasonableness." *Hercules Inc. v. EPA*, 598 F.2d 91, 107-08 (D.C. Cir. 1978). *See also, Reynolds Metal Co. v. United States EPA*, 760 F.2d 549, 558 (4th Cir. 1985) (upholding EPA numerical standard). Indeed, almost all water quality standards are ultimately based on rules of thumb and views of an appropriate safety factor. For example, there is no scientific proof that requires setting acute toxicity standards using 50% of the LC 50 instead of 10% of the LC 10. The decision to do so was based on drawing a reasonable line.

As to the problem now before the Board of setting limits on mixing necessary to provide the zone of passage necessary to protect aquatic life, PRN/Sierra Club/ELPC, writing on a blank slate, might prefer using the Board provision (25% of the volume of flow) for all cases including the many cases in which less than 3:1 dilution is available. However, IEPA's current use of 50% seems tolerable. While allowing a substantial number of areas in many streams to fail to meet water quality standards, the current IEPA practice reserves a substantial portion of the stream for passage of aquatic life.

However, it is not acceptable to allow IEPA to go on using an unpublished rule that is probably not enforceable because it has not gone through the Board approval process or the procedures set forth by the Administrative Procedure Act. *See Senn Park Nursing Center v. Miller*, 104 Ill.2d 169, 181, 470 N.E. 2d 1029 (1984). (agency could not use rule that had not been adopted pursuant to the Illinois Administrative Procedure Act) Nor is it acceptable for IEPA to have rules for required mixing from which it is free to deviate on an ad hoc basis because the discharger is providing a "vital function for society." (Transcript April 23 p. 56) As the Board is aware, there are other ways under the law, including site specific relief and variances, to reconcile the vital functions of society with the need to protect water quality that do not give IEPA permit writers unbridled discretion to compromise aquatic health based on their beliefs as to the vital needs of society.

Eliminating the requirement for allowing a zone of passage where there is little dilution certainly is not justifiable. The many small and medium sized streams that could be affected by loosening mixing protections can be extremely important to the overall health of the environment. *See* "Hydrological Connectivity of Headwaters Streams and Their Contributions to the Integrity of Downstream Waters," JAWRA, Vol. 43 pp1-280 (Feb. 2007), *available at* http://www.blackwell-synergy.com/toc/jawr/43/1.

PRN/Sierra Club/ELPC continue to believe that the language of Section 302.102(8) should be changed to state:

> (8) The area and volume in which mixing occurs, alone or in combination with other areas and volumes of mixing must not contain more than 25% of the cross-sectional area or volume of flow of a stream except for those streams where the dilution ratio is less than 3:1. In streams where the dilution ratio is less than 3:1, other than streams that have a zero flow for at least seven consecutive days recurring on average in nine years out of ten, the volume in which mixing occurs, alone or in combination with other volumes of mixing must not contain more that 50% of the volume of flow.

Interactions between sulfate toxicity and other dissolved solids

As explained in the pre-filed testimony of Ms. Collins, some data suggest that when calcium is the primary cation in a solution, it may serve to increase the toxicity of sulfate. Table 2 of the attached study, D.R., D.D. Gulley, J. R. Hockett, T. D. Garrison, and J.E.Evans. 1997. Statistical Models to Predict the Toxicity of Major Ions to *Ceriodaphnia dubia*, *Daphnia magna*, and *Pimephales promelas* (fathead minnows). *Environmental Toxicology and Chemistry* 16(10):2009-2019, shows that for the three species tested, mean

LC50 values for sulfate when calcium was the predominant cation in solution was lower than mean LC50 values for sulfate when sodium was the predominant cation (1910 mg $CaSO_4$/L of vs 3080 mg $NaSO_4$/L). When converted to the common term of toxicity per unit SO_4, the relationship still holds true, with LC50 values of 1348 mg SO_4/L vs. 2082 mg SO_4/L for calcium and sodium, respectively. The lower LC50 value for calcium indicates that the solution was more toxic to test organisms.

However, while noting this problem, we agree with the approach taken by IEPA at the April 23rd hearing to address these issues on a permit-by-permit basis by discouraging the use of $Ca(OH)_2$ in processing at mine sites, and requiring specific monitoring of water chemistry and toxicity in situations where $Ca(OH)_2$will be used.

Chloride above 500 mg/L

The draft standard does not clearly address the situation in which chloride concentrations in the receiving waters are greater than 500 mg/L and all of the participants at the hearing agreed that there may occasionally be situations in which this will be the case. IEPA has stated that it will address the situation on a case-by-case basis. PRN/Sierra Club and ELPC ask that the Board make clear in the rule that if chloride exceeds 500 mg/L, IEPA shall develop sulfate limits to prevent any danger that the sulfate discharge will increase the potential adverse effects on aquatic life caused by the violation of the chloride standard.

Respectfully submitted,

Albert Ettinger

Albert Ettinger
Counsel for Prairie Rivers Network,
Sierra Club and the Environmental Law & Policy Center

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CERTIFICATE OF SERVICE

I, the undersigned, on oath state that I have served the attached POST HEARING COMMENTS OF PRAIRIE RIVERS NETWORK, SIERRA CLUB AND THE ENVIRONMENTAL LAW AND POLICY CENTER upon the persons listed in the attached service list via U.S. Mail.

Respectfully submitted,

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3125045)
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DATED: June 7, 2007

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STATISTICAL MODELS TO PREDICT THE TOXICITY OF MAJOR IONS TO *CERIODAPHNIA DUBIA, DAPHNIA MAGNA* AND *PIMEPHALES PROMELAS* (FATHEAD MINNOWS)

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Abstract—Toxicity of fresh waters with high total dissolved solids has been shown to be dependent on the specific ionic composition of the water. To provide a predictive tool to assess toxicity attributable to major ions, we tested the toxicity of over 2,900 ion solutions using the daphnids, *Ceriodaphnia dubia* and *Daphnia magna,* and fathead minnows (*Pimephales promelas*). Multiple logistic regression was used to relate ion composition to survival for each of the three test species. In general, relative ion toxicity was $K^+ > HCO_3^- \approx Mg^{2+} > Cl^- > SO_4^{2-}$; Na^+ and Ca^{2+} were not significant variables in the regressions, suggesting that the toxicity of Na^+ and Ca^{2+} salts was primarily attributable to the corresponding anion. For *C. dubia* and *D. magna,* toxicity of Cl^-, SO_4^{2-}, and K^+ was reduced in solutions enriched with more than one cation. Final regression models showed a good quality of fit to the data (R^2 = 0.767–0.861). Preliminary applications of these models to field-collected samples indicated a high degree of accuracy for the *C. dubia* model, while the *D. magna* and fathead minnow models tended to overpredict ion toxicity.

Keywords—Ions   Total dissolved solids   Salinity   Toxicity   *Ceriodaphnia dubia*

INTRODUCTION

Natural fresh waters contain several ionic constituents at greater than trace levels. Indeed, ions such as Na^+, Ca^{2+}, Cl^-, and others are required at a minimum level to support aquatic life, and these major ions are components of most formulas for "reconstituted" water used in aquatic toxicity testing [1,2]. However, many natural and anthropogenic sources can increase ion concentrations to levels toxic to aquatic life. Studies of oil and gas produced waters [3–5], irrigation drain waters [6,7], shale oil leachates [8], sediment pore waters [9,10], and industrial process waters [11,12] have shown toxicity caused by elevated concentrations of common ions.

Typically, integrative parameters such as conductivity, total dissolved solids (TDS), or salinity are used as a measure of the concentrations of common ions in fresh waters. While for a given ionic composition there is undoubtedly a correlation between increasing conductivity or TDS and increasing toxicity, these parameters are not robust predictors of toxicity for a range of water qualities. For example, Burnham and Peterka [13] noted that fathead minnows could tolerate TDS concentrations up to 15,000 mg/L in Saskatchewan lakes dominated by Na^+ and SO_4^{2-}, but populations did not persist above 2,000 mg/L in $Na^+/K^+/HCO_3^-$-dominated lakes of Nebraska. In studies of irrigation drain waters, Dickerson et al. [7] found *Ceriodaphnia dubia* 50% lethal concentration (LC50) values corresponding to approximate conductivities of 3,500 to 4,000 μS/cm (calculated), while Jop and Askew [11] showed major ion toxicity to *C. dubia* in an industrial process water with a conductivity of only 1,800 μS/cm (K.M. Jop, personal communication). Studies by Dwyer et al. [14] demonstrated that the toxicity of high TDS waters to *Daphnia magna* and striped bass *Morone saxatilis* was dependent on the specific ionic composition of those waters.

Given the substantial differences in toxicity among major ion salts [15], these differing responses in waters with different ionic compositions are to be expected. Still, they emphasize the inadequacy of generic measures for assessing the potential toxicity of major ions and the need for a broader understanding of major ion toxicity. This paper presents research to develop more comprehensive tools for assessing major ion toxicity. Acute toxicity tests using three freshwater organisms were conducted on solutions enriched with varying combinations of major ions. Results of these tests were incorporated into multivariate logistic regression models that predict survival of the three test species based on major ion concentrations.

MATERIALS AND METHODS

Test organisms

All organisms used in testing were obtained from in-house cultures (ENSR, Fort Collins, CO, USA); daphnids were less than 24 h old at test initiation, while fathead minnows were 1 to 7 d old. *Ceriodaphnia dubia* were cultured in either moderately hard reconstituted water (MHRW) or 20% mineral water [1] at 25°C, while *D. magna* were cultured in hard reconstituted water [1] at 20°C. Fathead minnow brood stock were cultured at 20 to 25°C in tap water that was pretreated with activated carbon. Eggs and larva were held in MHRW; larva were fed brine shrimp nauplii (*Artemia* sp.) twice daily until they were used in testing.

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† Deceased.

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Test procedures

Toxicity tests followed the general guidance of the U.S. Environmental Protection Agency (USEPA) [1,16] for conducting acute whole effluent toxicity tests. All tests were conducted in 30-ml plastic beakers containing 10 ml of test solution and five organisms per chamber. Tests were conducted under a 16-h:8-h light : dark photoperiod; *C. dubia* and fathead minnows were tested at 25°C, while *D. magna* were tested at 20°C. Dilution/control water for all tests was MHRW. Exposure periods were 48 h for *C. dubia* and *D. magna* and 96 h for fathead minnows, with daily observations of mortality. The criteria for death were no visible movement and no response to prodding.

Standard guidance for acute effluent toxicity testing [1] is to withhold food during testing of daphnids, presumably because of concerns that the addition of food might alter the toxicity of the sample. However, in water devoid of food (e.g., reconstituted laboratory water), withholding food likely places some stress on the test organisms. Moreover, effluents and ambient waters, to which the results of these experiments apply, can be expected to contain bacteria, algae, and other sources of food. Hence, addition of daphnid food (yeast/cerophyl/trout chow [YCT] and algae [2]) to clean laboratory water might better simulate the characteristics of field-collected samples. To assess the potential effect of feeding on major ion toxicity, initial tests using *C. dubia* were conducted both with and without feeding. Analysis of these initial experiments (see Results) showed that the addition of food represented only a small influence on *C. dubia* survival. Because the effect of feeding was small and its inclusion was believed to provide a more representative test matrix, remaining *C. dubia* tests included feeding, as did all *D. magna* and fathead minnow tests. For daphnid tests, 100 μl of a 1:1 mix of YCT and algal suspension was added to each test chamber at test initiation. For fathead minnow tests, 100 μl of concentrated brine shrimp nauplii was added after 48 h of exposure, though solutions were not subsequently renewed as recommended by the USEPA [1].

Because toxicity testing of salt solutions was to be completed over several months, we recognized the possibility that systematic drift in test organism sensitivity could bias the results of toxicity tests conducted at different times. In an attempt to account for this potential variability, each set of toxicity tests included a reference toxicant test using NaCl. LC50 values were computed for each of these tests and were included in the statistical modeling as another independent variable. Thus, if drifts in organism sensitivity did occur and were reflected in the response to NaCl, they could be accounted for in the regression modeling.

Chemical measurements

Concentrations of major ions were determined analytically in all stock solutions used in testing. Ca^{2+}, Na^+, Mg^{2+}, and K^+ were determined using inductively coupled plasma emission spectroscopy (ICP) according to USEPA method 200.7 [17]; Cl^- and SO_4^{2-} concentrations were determined by anion chromatography [18]; and HCO_3^- concentrations were determined indirectly by the measurement of phenolphthalein alkalinity [19]. As HCO_3^- is the predominate carbonate species present in the pH range of interest (pH 6.5–9.0), alkalinity equivalents were converted directly to HCO_3^- concentration.

Dissolved oxygen (DO) and pH were measured in selected test solutions during actual toxicity testing, primarily on solutions near the threshold for acute toxicity. DO was measured with a Yellow Springs Instrument model 54 DO meter (Yellow Springs, OH, USA) while pH was measured with a Orion pH meter model SA250 (Boston, MA, USA). Measured DO concentrations were always within an acceptable range (>40% saturation) [1]. Measured pH varied according to the components of the solution but was generally between pH 7.5 and 9.0.

Preparation of test solutions

Test solutions were prepared by dissolving individual ion salts in MHRW. Salts used in testing were NaCl, Na_2SO_4, $NaHCO_3$, KCl, K_2SO_4, $KHCO_3$, $CaCl_2$, $CaSO_4$, $MgCl_2$, $MgSO_4$, $CaCO_3$, and $MgCO_3$; all were of reagent grade or better (Sigma Chemical Company, St. Louis, MO, USA). Stock solutions were prepared from these salts by dissolving 10,000 mg/L of a salt in MHRW. $CaSO_4$ was not fully soluble at 10,000 mg/L; for this reason, $CaSO_4$ solutions were filtered through a 1-μm glass fiber filter prior to testing and ion concentrations were measured in filtered solutions. Test solutions using $CaCO_3$ and $MgCO_3$ had pH in excess of 10 and were acidified with HCl or H_2SO_4 until pH stabilized at approximately 8.5.

For tests evaluating only one salt (one cation and one anion), test solutions were prepared by serially diluting the 10,000-mg/L stock solutions with MHRW to develop a series of test concentrations spaced on a 0.5 × dilution factor (i.e., 10,000, 5,000, 2,500, 1,250 mg/L). For tests involving two salts, solutions were prepared by combining equal volumes of the two stock solutions, then diluting as necessary. As testing proceeded and effect thresholds were determined, test concentrations were often spaced much more closely (e.g., 2,500, 2,000, 1,500, 1,000, 500 mg/L) to better define responses near the effect threshold.

All ion concentrations measured in the stock solutions were compared to nominal values. If the measured concentrations differed from the nominal value by more than 20%, the actual measured concentrations were substituted for the nominal concentrations. Aside from $CaSO_4$, which did not completely dissolve, substantial discrepancies between nominal and measured concentrations occurred in two instances, once for a $MgCl_2$ stock solution and once for a $CaCl_2$ stock solution. In some analyses, the measured concentrations of cations and anions (expressed as milliequivalents or meq) in a salt solution were not similar. Because charge balance is a physical/chemical requirement, such solutions were further evaluated to determine which concentration (cation or anion) was closer to the nominal value. In all cases, the cation concentration was closer to the nominal value; based on this, the anion concentration in the stock solution was changed to the concentration (in meq) of the corresponding cation.

To calculate ion concentrations in actual test solutions, the concentrations in the applicable stock solutions were multiplied by the relative proportion of each solution in the test solution. Because the dilution water (MHRW) also contained small concentrations of each ion, these background concentrations were then added to the calculated contributions from the stock solutions.

In cases where an SO_4^{2-} salt (e.g., Na_2SO_4) was combined with a Ca^{2+} salt (e.g., $CaCl_2$), the potential existed for supersaturation of test solutions with respect to $CaSO_4$. This potential was confirmed by the appearance of white precipitates in some test solutions. Because precipitation would affect the dissolved ion concentrations in the test solutions, all ion combinations tested were checked for $CaSO_4$ supersaturation by comparing the nominal test concentrations of Ca^{2+} and SO_4^{2-} with the solubility product for $CaSO_4$ (226.5) calculated from measured concentrations of Ca^{2+} and SO_4^{2-} in a saturated $CaSO_4$ solution. If a particular solution was supersaturated with respect to $CaSO_4$, Ca^{2+} and SO_4^{2-} concentrations were reduced on an equimolar basis until the concentrations reached the calculated saturation point. These corrected concentrations were then used for data analyses.

Replication

To incorporate intertest variability into the data set, emphasis was placed on replication between batches of tests conducted through time rather than on having replicate chambers tested simultaneously. Accordingly, most ion combinations evaluated were tested on at least two and as many as five different occasions (see results). The exception was for two cation/one anion solutions tested with *D. magna* and fathead minnows, and two cation/two anion solutions tested with *C. dubia*; for these tests, duplicate chambers (10 animals total) were tested simultaneously. When calculating LC50 values, replicate tests conducted on different days were analyzed separately, but duplicate chambers tested simultaneously were combined into one analysis.

Data collection, management, and analysis

Data generated by all toxicity tests were entered into a database using Paradox® 3.1 software (Borland International, Scotts Valley, CA, USA). Regression modeling was based on individual ion concentrations rather than salt concentrations. By converting salts to ion concentrations, we were able to separate out the effects of individual cations and anions instead of the effects of cation–anion pairs. Statistical modeling of the toxicity data consisted of stepwise logistic multiple regression using the LR program within BMDP statistical software [20].

Logistic regression relates binary observations (e.g., alive or dead) to one or more independent variables (in this case, ion concentrations). The completed regression predicts a probability of survival based on concentrations of ions showing relationships to survival. The linear logistic regression model used is of the form

$$\begin{aligned}\text{logit}(P) &= \ln[P/(1 - P)] \\ &= \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_n X_n \qquad (1)\end{aligned}$$

where P = proportion surviving, β = regression coefficient, X = ion concentration, and n = total number of significant terms in the model.

During development of the final models, various data transformations (e.g., log) and independent variable interactions (e.g., $Cl \times SO_4$ interaction) were considered. Each potential model was evaluated using the following criteria: (1) each independent variable in the model must significantly improve the fit of the model to the data ($\alpha = 0.05$); (2) the model should maximize R^2 (maximize the amount of variance in the data that is explained by the model) and minimize the number of independent variables; and (3) the model should provide reasonable predictions even when extrapolating outside the limits of the data used to generate the model.

Data collection and model development were iterative processes in which a series of statistical models (regressions) were developed followed by supplemental data collection. To begin, data were generated for single ion pairs or salts (e.g., Na_2SO_4, $CaCl_2$). Based on these data, an initial regression equation was developed (F_1). Next, additional toxicity data were generated using combinations of two cations and one anion (e.g., Na^+, Ca^{2+}, and SO_4^{2-}) and one cation and two anions (e.g., Na^+, Cl^-, and SO_4^{2-}). The F_1 equation was then used to predict survival for these additional data. In addition, a second regression equation (F_2) was then developed using all data generated to date. The predictive abilities of both models were then compared by examining the relationship between predicted and observed survival for all of the ion combinations tested. If F_2 had notably better predictive ability than F_1, we concluded that important relationships in the data were not accounted for in the F_1 equation. The process was repeated by testing more complex ion solutions and developing additional regression equations, until the incorporation of additional data did not substantially alter the basic equation. This iterative process of data generation, model development, and additional data generation continued throughout model development.

As part of this iterative process, characteristics of specific points that had poor correlation between predicted and observed survival were considered. In some cases, it was found that such data points had poor agreement between replicate tests of the same ion combination, hence it was impossible for the regression equation to fit both responses. In these instances, additional toxicity tests were conducted using that particular combination of ions to better characterize the response. Of 2,904 total data points, 59 were discarded as spurious; of these, 46 were for *C. dubia,* 5 for *D. magna,* and 8 for fathead minnows. Thirty-eight of the 59 discarded points were cases where mortality (typically one or two dead out of five organisms) was observed two or more concentrations below the primary concentration response, suggesting that ion toxicity may not have been the cause of mortality. Though these points may represent innate variability in the survival of test organisms, our intent was to represent mortality due to ion stress; random mortalities at low ion concentrations tended to decrease the slope of the regression model and obscure the response threshold. Of the remaining discarded points, 10 were discarded because the $CaSO_4$ solution was not filtered prior to testing (*C. dubia*); 10 were from a K_2SO_4 dilution series in which there was erratic and substantial mortality without evidence of a concentration response (*C. dubia*); and one was from a test chamber that was spilled after the 24-h observation (*P. promelas*).

In other cases, it was found that outlier points tended to share certain characteristics. For example, it was noted that for *C. dubia,* early regressions showed poor predictive ability for ion combinations containing Cl^- opposed by two cations (e.g., Na^+ and Ca^{2+} with Cl^-); these solutions showed lower toxicity than those with just one Cl^- salt (e.g., NaCl). Further testing with these ion combinations showed that this response was reproducible. To account for this phenomenon, a new variable called NumCat was created. The value of NumCat is equal to the number of cations representing at least 10% of the total molar concentration of cations and present at greater than 100 mg/L. The development and implications of the NumCat variable are discussed in detail in the Results.

In addition to the more rigorous statistical modeling described above, LC50 concentrations were also calculated using a computer program following the trimmed Spearman–Karber method [21]. Independent LC50 values were calculated for each unique (i.e., nonsimultaneous) test of ion toxicity. For

Table 1. Number of ion solutions tested for toxicity[a]

Species	Number of cations/anions[b] 1/1	1/2	2/1	2/2	3/1	4/1	Subtotal	Reference toxicant and controls	Total
Ceriodaphnia dubia	464	449	438	401	108	20	1,887	232	2,119
Daphnia magna	354	147	65	0	0	0	566	122	688
Fathead minnows	242	142	59	0	0	0	451	56	499

[a] Replicate analyses counted separately.

[b] Number of ions enriched above background concentrations.

ion combinations that were tested repeatedly, average LC50s were calculated as the arithmetic mean of the values. In some cases, tests did not capture the effect threshold and an LC50 could only be expressed as a range (e.g., LC50 < 625 mg/L). Where this range did not conflict with the other calculated values, the indefinite value was dropped and the mean was calculated from the remaining values (e.g., 500, 700, and <625 would average to 600 with $n = 2$). If the indefinite value represented an extreme value, the mean was calculated as an inequality relative to the mean of the numerical values (e.g., 775, 700, and <625 would average to <700 with $n = 3$).

RESULTS AND DISCUSSION

In total, survival data were collected for 2,904 ion solutions, excluding reference toxicant tests and controls (Table 1). Data collection and modeling were conducted first for *C. dubia,* and the resulting data set encompasses both greater replication and a greater variety of ion combinations. The full data sets are too extensive to provide here but are provided in print in Mount and Gulley [22].

To present the data in a more condensed form, LC50 values were calculated for all ion solutions tested (Tables 2 and 3). Coefficients of variation for LC50 values for individual ion combinations were typical for acute toxicity tests [1], with means of 17% for *C. dubia* (SD = 14; range 0.0–61), 17% for *D. magna* (SD = 7.5; range 4.8–31), and 24% for fathead minnows (SD = 15; range 1.4–62).

The effect of feeding on the response of *C. dubia* was assessed during the first three sets of tests conducted. In each of these, toxicity of each single salt solution was tested both with and without the addition of food. Average LC50 values for tests with and without feeding were similar (Fig. 1), although there was a tendency for tests without feeding to have slightly lower LC50 values. Logistic regression modeling of these data confirmed this trend; feeding was judged a significant variable by the regression algorithm, with a positive coefficient indicating that feeding did increase overall survival. However, the influence of feeding in the model was quite small, explaining less than 1% of the overall variance. Because we believed that the addition of food might provide a more natural test matrix, all remaining tests were conducted with feeding.

To determine whether the results of reference toxicant tests related to the responses observed in the concurrent exposures to ion combinations, LC50 values were calculated for the reference toxicant tests from the first 11 test groups with *C. dubia* (total of 1,045 ion solutions tested). During this period, 48-h LC50 values for NaCl averaged 1,042 mg/L as Cl^- with a coefficient of variation equal to 24%. The LC50 value from the concurrent reference toxicant test was included as an independent variable for each ion solution and thus considered by the stepwise logistic regression. In this analysis, the reference toxicant variable was not selected as being statistically significant, explaining only 0.12% of the overall variance. From this, we surmised that there was no consistent relationship between the sensitivity of the test organisms (as measured by the reference toxicant test) and the responses of organisms in the concurrent ion exposures. For this reason, the reference toxicant test results were not considered further in subsequent regressions.

As described previously, the development of the final predictive models was an iterative process in which a series of regression models was developed. Initial regressions were developed based on more limited data sets (e.g., results from toxicity tests using single salts only); as data collection proceeded to more complicated solutions (enrichment with three and four ions), these equations were refined. Throughout the project, 74 distinct models were developed and considered. The majority of these models were discarded, either because they were superseded by later models that incorporated larger data sets, or were found to have undesirable characteristics (e.g., poor predictive ability). Several of these analyses involved experimentation with alternative variables or data transformations. To illustrate the model development process, we selected three intermediate models that demonstrate major advances in the model development, including the creation of a new variable, referred to as NumCat. The three example models are referred to as the single salt, double salt, and double salt with NumCat models and are based on 48-h survival data for *C. dubia.*

The single salt model was developed relatively early in the data collection process using 362 data points involving single salt solutions only (i.e., enriched with one cation and one anion; Fig. 2). This regression equation fit the observed survival values very well, with an R^2 value of 0.950. Significant variables in this equation were the concentrations of K^+, Mg^{2+}, HCO_3^-, Cl^-, and SO_4^{2-}; Na^+ and Ca^{2+} were not significant variables indicating that the toxicity of Na^+ and Ca^{2+} salts could be accounted for primarily by the toxicity of the co-occurring anion. No first-order interaction terms (e.g., $K \times Cl$) were selected as significant.

Data collection was then expanded to include solutions with one cation and two anions and two cations and one anion. When the single salt model was used to predict survival for this expanded data set (1,045 data points) it showed considerably less predictive ability than it had for the smaller initial data set. Accordingly, a new model was developed using data from all test solutions. This double salt model had the same significant variables as did the single salt model but did a better job of predicting survival for the entire data set than did the single salt model. Although it did have better predictive ability for the combined data set, the R^2 value of 0.837 indi-

Table 2. Mean 24-h (upper right) and 48-h (lower left) LC50 values for salt combinations tested with *Ceriodaphnia*[a]

	NaCl	Na_2SO_4	$NaHCO_3$	KCl	K_2SO_4	$KHCO_3$	$CaCl_2$	$CaSO_4$	$MgCl_2$	$MgSO_4$	24-h
	3,380 [3] (3,080–3,540)	3,320 [4] (3,110–3,540)	2,200 [4] (1,770–2,680)	1,650 [2] (1,540–1,770)	>1,800 [1]	1,360 [1]	3,340 [3] (2,960–3,540)	>2,430 [1]	3,230 [4] (3,080–3,460)	3,400 [1]	NaCl
		3,590 [4] (3,540–3,740)	2,800 [5] (2,220–3,540)	1,730 [1]	1,390 [2] (1,020–1,770)	1,300 [1]	4,120 [2] (3,800–4,150)	>4,940 [2] (4,170–>5,700)	3,100 [2] (2,750–3,460)	3,480 [3] (3,080–3,820)	Na_2SO_4
			1,420 [4] (1,240–1,770)	1,200 [1]	1,110 [1]	920 [3] (880–1,000)	2,680 [2] (2,320–3,080)	>1,040 [1]	1,800 [1]	2,210 [1]	$NaHCO_3$
NaCl	1,960 [3] (1,770–2,330)			630 [3] (580–630)	620 [3] (250–880)	550 [3] (290–770)	1,740 [3] (1,690–1,770)	1,580 [1]	1,400 [2] (1,030–1,770)	1,070 [2] (880–1,260)	KCl
Na_2SO_4	3,070 [4] (2,530–3,540)	3,080 [4] (1,770–3,540)			770 [3] (770–780)	390 [3] (290–440)	2,250 [1]	1,140 [3] 480–1,870	>1,550 [1]	1,510 [4] (1,340–1,770)	K_2SO4
$NaHCO_3$	1,890 [3] (1,770–2,030)	2,630 [4] (1,880–3,540)	1,020 [4] (880–1,170)			630 [2] (580–670)	1,910 [1]	1,560 [1]	860 [1]	940 [1]	$KHCO_3$
KCl	1,560 [3] (1,540–1,600)	1,730 [1]	1,140 [1]	630 [3] (580–670)			2,260 [3] (1,770–2,680)	3,880 [2] (3,660–4,100)	3,500 [3] (3,420–3,540)	>3,690 [2] (3,670–>3,700)	$CaCl_2$
K_2SO_4	1,660 [1]	1,590 [3] (1,020–2,000)	<1,000 [1]	480 [3] (250–670)	<680 [3] (<620–710)			>1,940 [4] (>1,940–>1,990)	>2,760 [1]	>5,610 [3] (>2,610–>5,610)	$CaSO_4$
$KHCO_3$	1,360 [1]	1,300 [1]	800 [3] (580–950)	480 [3] (290–580)	390 [3] (290–440)	630 [2] (580–670)			1,270 [3] (880–1,770)	1,560 [3] (1,360–1,770)	$MgCl_2$
$CaCl_2$	3,030 [4] (2,240–3,540)	>3,940 [2] (3,800–>4,080)	<2,640 [2] (<2,250–3,030)	1,730 [3] (1,640–1,770)	1,820 [1]	1,810 [1]	1,830 [4] (1,770–2,030)			1,770 [3] (1,770–1,770)	$MgSO_4$
$CaSO_4$	>2,430 [1]	>4,940 [2] (4,170–>5,700)	>1,040 [1]	1,580 [1]	1,130 [3] (480–1,830)	1,560 [1]	3,050 [2] (2,450–3,660)	>1,910 [4] (1,910–>1,970)			
$MgCl_2$	2,380 [4] (1,770–2,730)	<2,520 [2] (<2,320–2,720)	1,510 [1]	1,270 [3] (1,000–1,770)	1,040 [1]	860 [1]	2,600 [3] (2,430–2,680)	<2,370 [1]	880 [3] (880–880)		
$MgSO_4$	3,250 [1]	3,190 [3] (2,680–3,540)	1,670 [1]	1,060 [2] (880–1,220)	1,480 [4] (1,340–1,770)	940 [1]	>3,690 [2] (3,670–>3,700)	>5,610 [2] (>5,610–>5,610)	1,490 [3] (1,360–1,560)	1,770 [3] (1,770–1,770)	
48-h	NaCl	Na_2SO_4	$NaHCO_3$	KCl	K_2SO_4	$KHCO_3$	$CaCl_2$	$CaSO_4$	$MgCl_2$	$MgSO_4$	

[a] Values are arithmetic means [*n*] (range) expressed as total ion concentrations added in mg/L. Tests with two salts involved 1:1 combinations of stock solutions containing 10,000 mg/L, except $CaSO_4$ (1,970 mg/L).

Table 3. Mean LC50 values for salt combinations tested with *Daphnia magna* and fathead minnows[a]

	Daphnia magna		Fathead minnow		
Salt	24-h	48-h	24-h	48-h	96-h
$NaCl$	6,380 [2] (6,160–6,600)	4,770 [2] (3,790–5,740)	8,280 [3] (7,240–10,000)	6,510 [3] (6,090–7,070)	6,390 [3] (6,020–7,070)
Na_2SO_4	6,290 [4] (5,790–7,070)	4,580 [4] (4,060–5,360)	>8,080 [3] (7,070–>10,000)	>7,960 [3] (6,800–>10,000)	7,960 [3] (6,800–10,000)
$NaHCO_3$	2,380 [4] (1,900–2,870)	1,640 [4] (1,170–2,030)	4,850 [2] (3,540–6,160)	2,500 [2] (950–4,060)	<850 [3] (<310–1,220)
KCl	740 [5] (580–880)	660 [5] (440–880)	950 [3] (750–1,090)	910 [3] (750–1,090)	880 [3] (750–1,020)
K_2SO_4	850 [4] (670–1,170)	720 [4] (580–880)	990 [4] (770–1,170)	860 [4] (580–1,170)	680 [4] (510–880)
$KHCO_3$	670 [4] (440–880)	650 [4] (380–820)	940 [4] (750–1,340)	820 [4] (750–880)	<510 [4] (<310–750)
$CaCl_2$	3,250 [4] (2,680–4,010)	2,770 [4] (2,330–3,230)	>6,660 [3] (4,700–>10,000)	>6,560 [3] (4,390–>10,000)	4,630 [3] (3,930–5,360)
$CaSO_4$	>1,970 [3] (>1,970–>1,970)	>1,970 [3] (>1,970–>1,970)	>1,970 [2] (>1,970–>1,970)	>1,970 [2] (>1,970–>1,970)	>1,970 [2] (>1,970–>1,970)
$MgCl_2$	1,560 [4] (1,250–1,810)	1,330 [4] (1,170–1,580)	3,520 [3] (2,520–4,490)	2,840 [3] (1,970–3,880)	2,120 [3] (1,580–2,740)
$MgSO_4$	2,360 [4] (2,180–2,500)	1,820 [4] (1,540–2,330)	4,630 [3] (3,180–7,070)	3,510 [3] (3,000–4,350)	2,820 [3] (2,610–3,080)
$NaCl/Na_2SO_4$	6,140 [2] (5,360–6,930)	5,700 [2] (5,360–6,030)	>9,040 [2] (8,080–>10,000)	>8,460 [2] (6,930–>10,000)	6,090 [2] (6,030–6,160)
$NaCl/NaHCO_3$	4,440 [2] (3,520–5,360)	2,950 [2] (2,830–3,080)	4,580 [2] (3,540–5,630)	3,790 [2] (2,330–5,250)	2,540 [2] (2,330–2,750)
$Na_2SO_4/NaHCO_3$	4,480 [2] (4,060–4,900)	3,180 [2] (2,830–3,540)	5,350 [2] (4,660–6,030)	5,050 [2] (4,060–6,030)	4,060 [2] (3,080–5,040)
KCl/K_2SO_4	740 [2] (600-880)	740 [2] (600–880)	900 [2] (790–1,020)	760 [2] (630–880)	760 [2] (630–880)
$KCl/KHCO5_3$	740 [2] (640–830)	740 [2] (640–830)	800 [2] (770–830)	770 [2] (700–830)	770 [2] (700–830)
$K_2SO_4/KHCO_3$	630 [2] (540–720)	630 [2] (540–720)	1,060 [2] (1,030–1,090)	720 [2] (610–830)	720 [2] (610–830)
$CaCl_2/CaSO_4$	3,250 [2] (3,140–3,360)	2,950 [2] (2,760–3,150)	>5,510 [1]	>5,510 [1]	>5,510 [1]
$MgCl_2/MgSO_4$	2,110 [2] (1,940–2,280)	1,510 [2] (1,340–1,680)	3,830 [2] (3,790–3,870)	3,330 [2] (3,300–3,370)	2,800 [2] (2,240–3,370)
$NaCl/KCl$	3,930 [1]	3,930 [1]	1,410 [1]	1,410 [1]	1,410 [1]
$NaCl/CaCl_2$	5,250 [1]	5,250 [1]	8,410 [1]	8,080 [1]	6,460 [1]
$NaCl/MgCl_2$	3,820 [1]	3,070 [1]	5,250 [1]	3,520 [1]	3,160 [1]
$KCl/CaCl_2$	2,620 [1]	2,450 [1]	2,810 [1]	2,810 [1]	2,810 [1]
$KCl/MgCl_2$	2,280 [1]	2,020 [1]	1,580 [1]	1,410 [1]	1,410 [1]
$CaCl_2/MgCl_2$	4,850 [1]	4,390 [1]	5,630 [1]	5,250 [1]	5,250 [1]
Na_2SO_4/K_2SO_4	4,800 [1]	4,610 [1]	1,580 [1]	1,580 [1]	1,580 [1]
$Na_2SO_4/MgSO_4$	8,400 [1]	7,980 [1]	8,840 [1]	5,740 [1]	4,800 [1]
$K_2SO_4/CaSO_4$	1,160 [1]	1,200 [1]	1,980 [1]	1,720 [1]	1,720 [1]
$K_2SO_4/MgSO_4$	2,760 [1]	2,210 [1]	1,380 [1]	1,290 [1]	1,290 [1]
$CaSO_4/MgSO_4$	>6,470 [1]	>6,470 [1]	NT[b]	NT	NT
$NaHCO_3/KHCO_3$	1,220 [1]	1,040 [1]	1,140 [1]	820 [1]	740 [1]

[a] Values are arithmetic means [*n*] (range) expressed as total ion concentrations added in mg/L. Tests with two salts involved 1:1 combinations of stock solutions containing 10,000 mg/L, except for $CaSO_4$ (1,970 mg/L), $MgCl_2$ (5,480 mg/L), and $CaCl_2$ (7,480 mg/L).

[b] Not tested.

cated a lower quality of fit than was observed for the single salt model fit to the initial, less complex data set.

There were two basic explanations for the decreased quality of fit observed with the double salt model: (1) the larger data set contained greater inherent variability (measurement error) and hence it was not possible to achieve as high an R^2 value; or (2) there were important toxic interactions represented in the three ion solutions that were not represented in the solutions containing only a single salt (although the regression algorithm had not selected any interaction terms as being significant). When the ion combinations for which the model made poor predictions were analyzed, some patterns were apparent. In particular, it appeared that the model was overpredicting toxicity for solutions containing two Cl^- salts.

This phenomenon is perhaps best illustrated by data collected for solutions of $NaCl$ and $CaCl_2$ tested both alone and in combination. As explained above, the single salt model indicated that the toxicity of Na^+ and Ca^{2+} salts could be

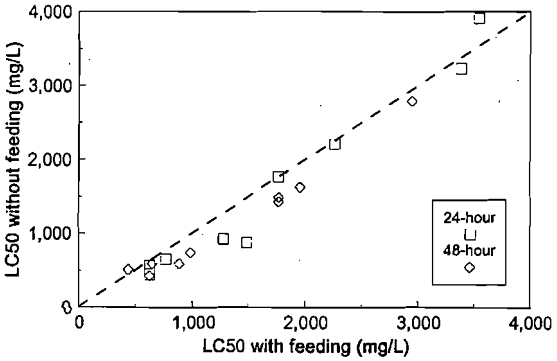

Fig. 1. Average LC50 values for *Ceriodaphnia dubia* exposed to single salts with and without feeding.

adequately explained on the basis of the anion concentration alone; in other words, NaCl and $CaCl_2$ had approximately the same toxicity when expressed on the basis of Cl^-. A plot of these data (Fig. 2) supports this conclusion and also shows a good fit of the single salt model to these data. However, when NaCl and $CaCl_2$ were tested in combination, the resulting solution was less toxic (on the basis of Cl^- concentration) than either of the solutions tested singly. The single salt model was unable to account for this decreased toxicity and, consequently, made poor predictions for the combined NaCl/$CaCl_2$ solutions (Fig. 2). The same trend toward lower toxicity of Cl^- in the presence of two cations was also evident for solutions containing K^+ or Mg^{2+}.

The double salt model compensated for the lower toxicity of two cation solutions but only partially. The double salt model simply fit a shallow response curve between the single cation and two cation data, predicting a "mean" probability of survival somewhere between the observed single salt and two salt survival values. While this compromise provided a better overall fit to the data than did the single salt model, it was clearly not a good representation of the response. Given that the regression algorithm did not find any interaction terms to be significant, it appeared that a new variable was required to provide a better fit to the data.

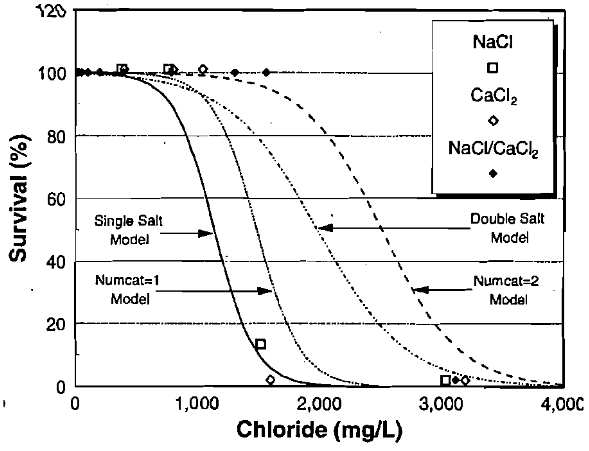

Fig. 2. The 48-h survival of *Ceriodaphnia dubia* exposed to solutions enriched with NaCl, $CaCl_2$, or a 1:1 combination of NaCl and $CaCl_2$, normalized to Cl^- concentration. Curves represent regression model predictions for the single salt, double salt, and double salt with NumCat models. Values at 0% and 100% offset slightly for clarity.

We attempted without success to derive a continuous variable that would respond appropriately to the relative concentration of cations in solution and thus identify the two cation solutions as different than solutions with a single cation. After our lack of success with continuous variables, we created a categorical variable called NumCat. The NumCat variable was intended to simply represent the number of major cations in the solution. For the initial modeling trials, the NumCat variable was arbitrarily defined as the number of cations in the solution that represented at least 10% of the total molar cation concentration and that were also present at a concentration greater than 100 mg/L. Our expectation was that the NumCat variable would show a significant interaction with Cl^- and any other ion whose toxicity was influenced by the number of cations present. The resulting model, called the "double salt with NumCat" model, showed a markedly improved fit ($R^2 = 0.899$); significant terms were the original five ions in the single and double salt models, plus NumCat and the NumCat × Cl, NumCat × SO_4, and NumCat × K interaction terms. The NumCat × Cl term allowed the model to better represent the toxicity of NaCl, $CaCl_2$, and NaCl + $CaCl_2$ solutions shown in Figure 2. NumCat also showed significant (positive) interactions with SO_4^{2-} and K^+, suggesting that the presence of two cations (or one additional cation in the case of K^+) ameliorated the toxicity of these ions as well.

After subsequent data collection and analysis, two additional steps were taken to optimize the NumCat variable. First, we conducted supplemental testing of *C. dubia* exposed to mixtures of three and four Cl^- salts (data not shown). Modeling of these data (NumCat = 3 or 4) yielded a substantial underprediction of toxicity. Direct inspection of these data confirmed that the protective effect observed with two cations did not seem to increase with the addition of three or four cations. Accordingly, we chose to limit the NumCat variable to values of 0, 1, or 2; for solutions where the >10% and >100-mg/L criterion yielded values of 3 or 4, these values were reset to 2.

The second step involved rigorously evaluating the definition criteria for the NumCat variable. Although the NumCat variable was clearly effective at increasing the predictive capability of the model, its original definition had been arbitrary. To provide a stronger technical basis for defining NumCat, we conducted a sensitivity analysis by varying the two components of the NumCat definition, the relative molar concentration (originally >10%), and the absolute concentration (originally 100 mg/L). A complete matrix of relative concentration (0, 5, 10, 15, 20, and 25%) and absolute concentration (0, 100, 200, and 300 mg/L) was modeled using 48-h *C. dubia* data. The resultant models were evaluated based on their R^2 values (Fig. 3). The NumCat criteria that produced the model with the highest R^2 (best fit of the model to the observed data) were the 15% with >100 mg/L ($R^2 = 0.8559$) and the 10% with >100-mg/L ($R^2 = 0.8553$) criteria. Given that the difference in R^2 was only 0.0006 (0.06% of the variance) and that we had already worked extensively with the 10% and >100-mg/L criteria, we elected to continue using these criteria in finalizing the model equations.

After completion of data collection, final regression equations were developed to predict *C. dubia* survival after 24 and 48 h of exposure. Through the course of these analyses, several additional variables and data transformations were evaluated and discarded. Aside from the feeding and reference toxicant variables discussed previously, we evaluated the sum of all ions, the sum of all cations, the sum of all anions, and NumAn

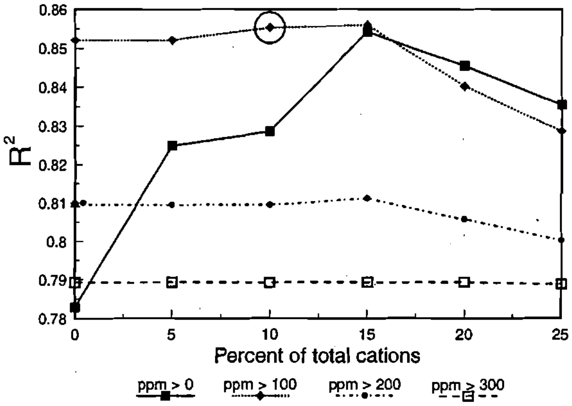

Fig. 3. Effect of varying criteria for the definition of the NumCat variable. Circled point represents the criteria selected initially and maintained for final derivation of the regression equations.

(the anion equivalent of NumCat). First-order interactions of these variables and ion concentrations were also evaluated. None of these variables was selected as significant by the regression algorithm. Models based on log-transformed ion concentrations consistently showed lower R^2 values than those based on untransformed data.

The final 24- and 48-h equations for *C. dubia* had K^+, HCO_3^-, Mg^{2+}, Cl^-, and SO_4^{2-} as significant variables (Table 4). Additionally, NumCat and the interaction terms NumCat × Cl, NumCat × SO_4, and NumCat × K were found to be significant. As had been the case since early in the modeling process, Na^+ and Ca^{2+} concentrations were not significant variables except as they affected the calculation of NumCat. R^2 for the final regressions were 0.861 and 0.842 for the 24-h and 48-h equations.

Model development for *D. magna* proceeded along the same lines as those described for *C. dubia.* The initial model developed using only single salt data fit those data very well (R^2 = 0.97) but was not as good at predicting survival for more complex ion mixtures. As was observed for *C. dubia,* solutions with multiple cations tended to be less toxic than comparable solutions with only one cation. As a result, when all *D. magna* data were analyzed, NumCat was again selected as a significant variable, both by itself and through its interactions with Cl^-, SO_4^{2-}, and K^+ (Table 4). In fact, all significant terms in the *C. dubia* double salt model with NumCat were also significant for *D. magna.* Quality of fit for the *D. magna* models was slightly lower than for the *C. dubia* models, though still quite good (0.812 and 0.799).

As for the daphnids, modeling of the fathead minnow data indicated that toxicity was a function of K^+, Mg^{2+}, HCO_3^-, Cl^-, and SO_4^{2-} concentrations, as neither Na^+ nor Ca^{2+} were selected as significant variables (Table 4). The primary difference in the fathead minnow equations was that NumCat was not a significant variable either by itself or in interaction with other terms. R^2 values for the three regression equations were generally comparable to those for the other models, ranging from 0.767 to 0.832.

Because of the large number of independent variables, the actual response surface of the regression models cannot be easily visualized. Nonetheless, marginal plots of the regression equations can be used to illustrate the relative sensitivity of each species to the various ions (Fig. 4). These plots show that *C. dubia* are, in general, the most sensitive of the three species to major ion toxicity, while fathead minnows are the least sensitive. K^+ was the most toxic ion to all species and SO_4^{2-} the least. The only inconsistency between species was that Mg^{2+} was more toxic than HCO_3^- for *D. magna* and fathead minnows, but the reverse was true for *C. dubia.*

As a means to visually evaluate the fit of the data sets to the regression equations, each regression equation was used to predict the ion concentrations producing 50% survival for each of the ion combinations tested during data collection. These values were then plotted against the average observed LC50 values from Tables 2 and 3 (Fig. 5). These plots indicate good overall agreement between the calculated and predicted LC50 values for all three species. Note, however, that this analysis is not a direct evaluation of quality of fit for the models because it actually compares a point estimate derived from individual logistic regression equations with the arithmetic mean of multiple point estimates for specific ion combinations derived by a different method (trimmed Spearman–Karber LC50 estimation [21]); it is not a plot of raw data versus model predictions. There are other biases in this comparison as well, such as different weighting of observations. Nevertheless, the concordance between the two methods does provide some assurance that the single multiple regression models provide a reasonable representation of the responses to a broad range of ion combinations.

The absence of interaction terms in the final regression equations, aside from those involving NumCat, suggests that

Table 4. Regression coefficients for final regression equations[a]

	Ceriodaphnia dubia		*Daphnia magna*		Fathead minnow		
	24-h	48-h	24-h	48-h	24-h	48-h	96-h
Constant	9.11	8.83	5.91	5.83	5.69	5.51	4.70
K^+	−0.0320	−0.0299	−0.0200	−0.0185	−0.0108	−0.0113	−0.00987
Mg^{2+}	−0.00594	−0.00668	−0.00450	−0.00510	−0.00225	−0.00316	−0.00327
Cl^-	−0.00706	−0.00813	−0.00330	−0.00395	−0.00117	−0.00125	−0.00120
SO_4^{2-}	−0.00424	−0.00439	−0.00204	−0.00255	−0.000728	−0.000750	−0.000750
HCO_3^-	−0.00745	−0.00775	−0.00276	−0.00397	−0.00200	−0.00274	−0.00443
NumCat	0.0332	−0.446	−0.410	−0.511	NS[b]	NS	NS
NumCat*K^+	0.00888	0.00870	0.00778	0.00677	NS	NS	NS
NumCat*Cl^-	0.00196	0.00248	0.00110	0.00146	NS	NS	NS
NumCat*SO_4^{2-}	0.00121	0.00140	0.000998	0.00132	NS	NS	NS
Model R^2	0.861	0.842	0.812	0.799	0.832	0.828	0.767

[a] Units for ion variables are mg/L.

[b] NS indicates that this particular variable was not significant and was excluded from the model.

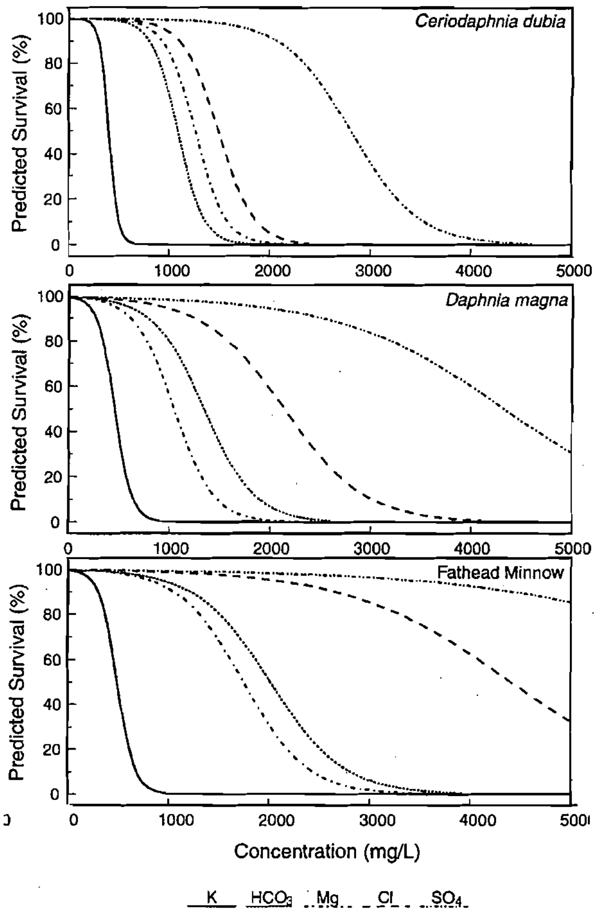

Fig. 4. Marginal plots of regression equations for each of the ions selected as significant. For *Ceriodaphnia dubia* and *Daphnia magna* models, NumCat = 1.

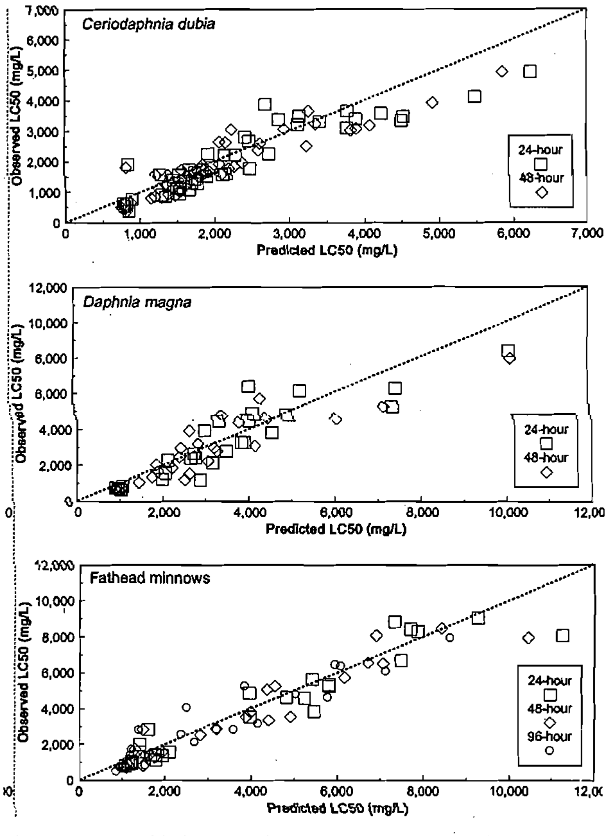

Fig. 5. Relationship between the ion concentrations predicted to cause 50% mortality and the average of LC50 values for individual salts and salt combinations (Tables 2 and 3). Line of unity (slope = 1) added for reference.

assuming additivity among individual ion toxicities is sufficient to describe the toxicities of the ion mixtures, at least from an empirical standpoint. The apparent amelioration of Cl^-, SO_4^{2-}, and K^+ toxicity by multiple cations could be construed as less than additivity. Alternatively, given that Na^+ and Ca^{2+} were not clearly identified as toxic by themselves, it might be more appropriate to consider those ions as water quality variables influencing toxicity, rather than as components of a toxic mixture.

We had little precognition of the important role that the NumCat variable would play in representing the combined toxicity of major ions. In a study of high-TDS irrigation return waters, Dwyer et al. [14] demonstrated that increasing the hardness (Mg^{2+} and Ca^{2+}) of an NaCl-dominated water decreased toxicity to *D. magna* and striped bass. For *D. magna,* this decreased toxicity would be predicted based on the current research, as the addition of hardness to these waters would have increased the value of the NumCat variable, thereby increasing predicted survival. However, results of our study also show that the effect of multiple cations is not an effect of hardness per se. For example, the *C. dubia* 48-h LC50 values for NaCl and $CaCl_2$ were almost identical when expressed on the basis of Cl^- concentration (1,187 and 1,172 mg/L, respectively; Table 2), even though the solutions had greatly different hardness. Moreover, the addition of NaCl to KCl increased the K^+ concentration at the *C. dubia* 48-h LC50 from 329 mg K/L for KCl to 458 mg K/L for an NaCl + KCl mix (Table 2), even though hardness was the same in both solutions.

Despite its importance in modeling the response of *C. dubia* and *D. magna,* the NumCat variable was not selected as significant for fathead minnows. Given that the addition of Mg^{2+}/Ca^{2+} improved survival of striped bass in high TDS solutions tested by Dwyer et al. [14], it seems that the protective effect in multiple cations is not restricted to cladocerans. It is worth noting that combinations of two cations and one anion were only tested once (in duplicate) for fathead minnows. If by chance those test results had a systematic bias, it might mask the presence of a cation-related effect for fathead minnows; coefficients of variation for fathead minnow LC50s were higher than for the other two species. More testing would be required to confirm or deny this possibility.

Though the effect of multiple cations is quite consistent both within the *C. dubia* and *D. magna* data sets and with other research [14], it must be emphasized that its identification and quantification through our modeling is empirical. Notwithstanding the effectiveness of the categorical NumCat variable in modeling our data set, it seems reasonable from a physiological standpoint to assume that the effect is in reality some type of continuous function, rather than the step function represented by our >10% and >100-mg/L criteria. In our modeling efforts, we were unable to devise a continuous variable that corresponded to the observed influence of multiple cations. Nonetheless, with continued research it seems likely that such a relationship could be uncovered and, if so, might provide a more rigorous representation of the actual relationship than that provided by NumCat as currently defined. A better understanding of the mechanisms of major ion toxicity would likely enhance this effort.

As a related matter, even though we conducted a sensitivity analysis to determine the optimum criteria for the NumCat variable, this analysis was subject to bias from the structure of our data set. Specifically, we tested binary combinations of salt solutions in 1:1 ratios only. As such, only certain areas within the total sampling space (all possible ion combinations) were represented in the data set. Thus, there is no assurance that the ion combinations tested were near critical points in the response surface that might alter the apparent thresholds for response. While we believe the NumCat variable is a significant advance in understanding the response of cladocerans to high TDS solutions, it is probably a somewhat crude representation of the actual physiological response.

Because most chemical reactions are related to molar concentrations, an argument could be made for modeling survival on the basis of molar concentrations rather than mass-based concentration. In retrospect, it seems this would have made little difference in the outcome of the modeling. As the equations are based on first-order concentrations of single ions only, transformation between mass-based and molar concentrations is a simple algebraic manipulation and does not affect the nature of the response surface. In fact, the equations in Table 4 can be converted to a molar basis by simply dividing each coefficient by the molecular weight of each ion. Conversion to chemical activity, however, would be much more involved.

Ultimately, the test of the toxicity models we have generated lies in their ability to make accurate predictions for samples outside those used to generate the original data set. Thus far, the equations have performed well in predicting major ion toxicity in field-collected samples, particularly so for the *C. dubia* equations. For example, Mount et al. [4] showed a strong correlation ($R^2 = 0.95$) between predicted and observed survival of *C. dubia* exposed to ambient samples from a watershed receiving oil field-produced waters. The *C. dubia* regression model was a better predictor of survival than any individual ion concentration, illustrating the ability of the model to predict the combined toxic effects of multiple ions. In a separate analysis, Mount et al. [15] showed a strong relationship between predicted and observed survival of *C. dubia* exposed to six produced waters collected from coalbed methane operations in Alabama. Obviously, these comparisons assume that major ions were the primary cause of toxicity in the field-collected samples.

Another application of the ion toxicity models that may prove equally or even more valuable lies in using model predictions to determine whether the presence of toxicants other than major ions is indicated. Research by Tietge et al. [5] both demonstrates this application and provides a rigorous evaluation of the predictive capability of the regression models. Six produced waters from various fossil fuel production sites were tested for toxicity and analyzed for major ion concentrations. The ion toxicity models presented here were used to predict survival of *C. dubia, D. magna,* and fathead minnows based on major ion concentrations. Differences between observed and predicted toxicity were used to make inferences as to whether the observed toxicity could be wholly explained by the major ion concentrations alone, or if the presence of other toxicants was indicated. The accuracy of these inferences was then evaluated by conducting Phase I TIE manipulations [16] and by testing the toxicity of laboratory waters reconstituted to the same major ion concentrations. This study indicated that the *C. dubia* model provided highly accurate predictions, while the fathead minnow and *D. magna* models tended to overpredict ion toxicity. The tendency of the *D. magna* and fathead minnow models to overpredict toxicity in field-collected samples was also noted in comparisons made by Mount et al. [4].

Dickerson et al. [7] used the *C. dubia* and fathead minnow models to evaluate toxicity in surface waters influenced by irrigation drain water. Although independent tests were not performed to confirm model predictions, it appeared that predictions by the *C. dubia* model correlated well with observed toxicity. As in the study by Tietge et al. [5], however, the fathead minnow model seemed to overpredict toxicity; several sites had higher observed survival than was predicted by the fathead minnow model.

In summary, applications of the *C. dubia* models and, to a lesser extent the *D. magna* and fathead minnow models, have proven them to be highly effective and comprehensive tools for evaluating major ion toxicity. To date, they have been successfully applied to studies of ambient waters [15], produced waters [4,5], irrigation drain waters [7], water purification byproducts [23], municipal effluents, and effluents from pulp and paper, refining, and manufacturing industries (J.R. Hockett, unpublished data). Because the models represent the combined toxicity of all seven ions, they have much broader application than ion toxicity studies based on generic measures like conductivity or TDS, or studies focusing on certain waters or ion combinations. Application of these models can reduce the need for extensive characterization and fractionation manipulations during TIE studies of high TDS waters [11]. They can also be used to project changes in toxicity resulting from modifications in industrial processes, effluent treatment, or other remedial measures.

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