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# Effects of Anions and Anion Mixtures on the Reproduction of *Ceriodaphnia dubia*

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## ABSTRACT

Municipal and industrial effluents often fail chronic toxicity tests due to elevated levels of osmotic or ionic strength. However, there is significant variability in the toxicity of effluents ranging from 0.1 to 1 part per thousand total dissolved solids (TDS) attributable to the effects of the individual anions (chloride, sulfate and bicarbonate) and their interactions. Manipulation of the anion concentrations in effluents has the potential to reduce toxicity. Three-brood, 7-d toxicity tests with *C. dubia* were conducted on serial dilutions of chloride ($Cl^-$), sulfate ($SO_4^{-2}$) and bicarbonate ($HCO_3^-$) and solutions containing mixtures of $Cl^-$ (3, 193, 243, 340 mg/L), $SO_4^{-2}$ (38, 308, 367, 496 mg/L) and $HCO_3^-$ (115, 238, 336, 486 mg/L) prepared in deionized water reconstituted to 40 mg/L hardness and 100 mg/L alkalinity. Tests were conducted using Whole Effluent Toxicity testing procedures, repeated three times and accompanied by KCl reference toxicant tests. $IC_{50}$ values for $Cl^-$, $SO_4^{-2}$ and $HCO_3^-$ averaged 637 mg/L, 780 mg/L, and 767 mg/L (alkalinity of 629 mg/L as $CaCO_3$), respectively. These toxic concentrations are often exceeded in effluents. In the tested mixtures, the $IC_{50}$ value for TDS was determined to be 1184 mg/L (29.7 mM/L), with an $IC_{25}$ value of 787 mg/L (19.7 mM/L). Differences in TDS accounted for about 80% of the variability in average reproduction among the tested solutions. Across the range of TDS represented by the 64 mixture solutions and within the range of tolerable TDS (< $IC_{50}$ value), reproduction increased as the ratio of $SO_4^{-2}$ to $Cl^-$ increased. However, reproduction decreased as the ratio of $HCO_3^-$ to $Cl^-$ increased. Information from these tests was used to develop multivariate models using the solution-strength variables (TDS and ionic strength) with the $SO_4^{-2}$:$Cl^-$ ratio and the $HCO_3^-$:$Cl^-$ ratio to predict *C. dubia* reproduction in slightly-saline solutions. The models produced can be used to evaluate the potential toxicity of effluents and to suggest substitutions that may reduce their toxicity.

## INTRODUCTION

Municipal and industrial effluents that enter the nation's waterways are generally complex mixtures of common inorganic salts and organic chemicals that are components of the myriad of commercial products used for industrial and residential purposes. Due to this complexity, their toxicities to aquatic biota are difficult to predict. This research has been undertaken as a first step in understanding interactions among effluent components and the resulting influence on the toxicity of the whole effluent. These effluents are generally dominated by elevated levels of common salts (compounds containing the cations sodium, calcium, magnesium, or potassium and the anions chloride, sulfate or bicarbonate) that can be toxic to freshwater biota in their own right. Information concerning the toxicity of these salts is limited to their acute effects which are caused primarily by the anionic portion of the salt, with the exception of potassium. However, permitted effluents are usually assessed by their chronic toxicity under the National Pollution Discharge Elimination System within the Clean Water Act. Information about the chronic effects of these salts does not exist or is limited to a small amount of ancillary data generated from their use as reference toxicants. Salinity, or total dissolved solids (TDS), is often cited as the most probable source of toxicity in many effluents permitted for discharge to freshwater systems. Yet, the actual cause of toxicity is usually unknown. This study was designed to determine the chronic toxicity of the three common anions and the interaction effects among them in order to relate this information to the impacts of many effluents on freshwater biota and to provide a foundation for further work that will examine the effects of the anions on the toxicities of common organic chemicals also present in the effluents.

The freshwater cladoceran, *Ceriodaphnia dubia*, is a standard test organism for assessments of effluents permitted for discharge to freshwater systems. It is sensitive to elevated TDS (>1000 mg/L), but there are often significant differences in the toxicities of effluents with similar TDS concentrations that indicate differences in the individual toxicities of the predominant anions. Differences in toxicity and interaction effects should reflect physiological responses of freshwater invertebrates to osmotic stress and may provide additional information to the understanding of those processes. Results from this study can be used to better understand the impact of slightly-saline effluents on freshwater communities and the significance of the various components of the effluents. This information has the potential to be used to reduce the toxicity of some effluents through substitution with less toxic components.

## METHODS

- Test animal – *Ceriodaphnia dubia* (< 8 h old)
- Test type – 7-day, 3-brood static renewal reproduction test (USEPA 1994)
  - Repeated three times for each test solution
- Test procedures
  - 25-mL plastic cup used as test chambers
  - 15 mL of test solution / chamber
  - 10 replicates / test solution
  - 1 *C. dubia* / replicate
  - Fed daily – 0.1 mL *Selenastrum capricornutum* and 0.1 mL YCT
  - Maintained at 25°C with 16-h light / 8-h dark photoperiod
  - Animals transferred daily into fresh test solutions
  - Nominal concentrations used
    - Activities and ionic strength calculated by MINTEQA2
  - Average reproduction as endpoint (percent of control reproduction)
- Base water
  - 40 mg/L hardness, 100 mg/L alkalinity - similar to many receiving streams and effluents in the Southeast
  - Reconstituted deionized water
  - $MgSO_4$ (28 mg/L)
  - $NaHCO_3$ (158 mg/L)
  - KCl (6 mg/L)
  - $CaSO_4 \cdot 2H_2O$ (28 mg/L)
  - Fortified with commercial vitamin and mineral premixes
- Test solutions
  - Sodium salts (NaCl, $Na_2SO_4$ and $NaHCO_3$)
  - Individual anion solutions
    - Serial dilutions of chloride, sulfate or bicarbonate
  - Mixture solutions
  - Based on inhibition concentrations ($IC_6$, $IC_{12}$, $IC_{25}$) determined for individual anions
- Mixture effects evaluated using multivariate selection techniques to identify models with the greatest $R^2$ values
  - Units of terms (mass, molar, activity) kept consistent within a model
  - All selected terms added significantly ($p \leq 0.05$) to the model
  - Terms selected could not be significantly ($p \leq 0.05$) correlated
- Response models developed using full data set (n=64) and reduced data set (only solutions with concentrations of osmotic and ionic strength less than their $IC_{50}$ values, n=24)

**Toxicities of Individual Anions and Anion Mixtures**

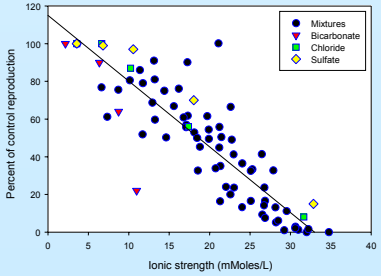


**Figure 1.** Response curves depicting *C. dubia* reproduction versus ionic strength generated from toxicity tests of solutions predominated by individual anions and solutions of anion mixtures. Regression line represents equation calculated from data for mixture solutions.

## RESULTS

- Anions were toxic at concentrations often encountered in municipal and industrial effluents

Average inhibition concentrations (mg/L) that reduce reproduction of *C. dubia* by 50 and 25 percent

| | $IC_{50}$ | $IC_{25}$ |
|---|---|---|
| Chloride | 563 | 340 |
| Sulfate | 715 | 496 |
| Bicarbonate | | |
| calculated | 763 | 568 |
| Alkalinity (as $CaCO_3$) | 626 | 466 |

- Ranking of anions according to their chronic toxicity was dependent on the metric of measurement

(Anions ranked from most to least toxic)

**Concentration**

mg/L $Cl^- > SO_4^{-2} = HCO_3^-$

mM/L $SO_4^{-2} > HCO_3^- > Cl^-$

**Normality** (for neutralization purposes)

meq/L $HCO_3^- > SO_4^{-2} = Cl^-$

**Anion activity**

mM/L $HCO_3^- > SO_4^{-2} > Cl^-$

**Solution strength**

mg/L $HCO_3^- > Cl^- = SO_4^{-2}$

mM/L $HCO_3^- = SO_4^{-2} > Cl^-$

**Ionic strength** (of solution)

mM/L $HCO_3^- > Cl^- = SO_4^{-2}$

- Osmotic (TDS) and ionic strength (IS) were the most influential variables controlling the toxicity of solutions with anion mixtures (Table 1, Figure 1)

Inhibition concentrations based on solution strength (with 95% CI, n=64)

| | $IC_{50}$ | $IC_{25}$ |
|---|---|---|
| TDS (mg/L) | 1184 (1137 – 1231) | 787 (693 – 854) |
| IS (mM/L) | 18.8 (17.7 – 19.9) | 11.7 (9.4 – 13.2) |

- In addition to TDS and IS, the best terms for predictive models (Table 2) were $SO_4^{-2}$ and $HCO_3^-$ normalized to $Cl^-$ ($SO_4^{-2}/Cl^-$, $HCO_3^-/Cl^-$)
  - Consistent for the full and reduced data sets
  - Consistent for mass, molar and activity measurements
- $SO_4^{-2}/Cl^-$ and $HCO_3^-/Cl^-$ terms provided an additional 3 to 12% to the amount of variability explained by the models using the full data set and an additional 33 to 45% using the reduced data sets (Table 1, Table 2)

## DISCUSSION

The toxicity of many effluents is due to elevated concentrations of chloride, sulfate and bicarbonate. Test results indicated that bicarbonate was more toxic than chloride and sulfate, but solution strength (osmotic or ionic) was the primary factor affecting *C. dubia* reproduction. There was, however, substantial variability in this response, particularly in mixtures with solution strength less than the $IC_{50}$ value (1184 mg/L TDS). Models that explained the most variability in reproduction contained variables that represented the ratio of sulfate to chloride and the ratio of bicarbonate to chloride, in addition to the solution-strength term. Coefficients for these terms indicated that increasing the $SO_4^{-2}$ to $Cl^-$ ratio increased reproduction, and increasing the $HCO_3^-$ to $Cl^-$ ratio reduced reproduction. Therefore, $SO_4^{-2}$ and $HCO_3^-$ were antagonistic; increasing concentrations of $Cl^-$ modified the effects of both $SO_4^{-2}$ and $HCO_3^-$; and all three contributed to the overall toxicity associated with osmotic or ionic strength.

The effects of these terms can be explained by their roles in osmoregulation. Bicarbonate is excreted via a mechanism that exchanges it for $Cl^-$ taken from the external solution. Increasing external concentrations of $Cl^-$ may facilitate this exchange, while increasing external concentrations of $HCO_3^-$ may make the exchange more difficult. The role of $SO_4^{-2}$ is more obscure, but the beneficial effect may be derived by the ability of $SO_4^{-2}$ to stabilize macromolecular structures produced internally to offset external osmotic or ionic pressure. Industrial processes that provide a substitution of $SO_4^{-2}$ and/or $Cl^-$ for $HCO_3^-$ should be evaluated as potential means to reduce effluent toxicity.

**Table 1.** Response variability within the anion-mixtures data set explained ($R^2$) by individual continuous variables representing concentration or ionic strength of dissolved solutes in solution.

| | Variable | $R^2$ |
|---|---|---|
| Full data set (n=64) | | |
| | Total Dissolved Solids (mg/L) | 0.82 |
| | Total Dissolved Solids (mM/L) | 0.86 |
| | Ionic Strength (mM/L) | 0.74 |
| Reduced data set (n=24) | | |
| | Total Dissolved Solids (mg/L) | 0.45 |
| | Total Dissolved Solids (mM/L) | 0.34 |
| | Ionic Strength (mM/L) | 0.31 |

**Table 2.** Models predicting the toxicity of anion mixtures to *C. dubia*.

Model: % control reproduction = intercept + terms (term coefficient)

| | Terms in model | Coefficient | p > \| t \| | $R^2$ |
|---|---|---|---|---|
| Full data set (n=64) | | | | |
| | Intercept | 116.99 | <0.0001 | 0.90 |
| | Total Dissolved Solids (mg/L) | -0.06 | <0.0001 | |
| | $SO_4^{-2}/Cl^-$ | 0.21 | <0.0001 | |
| | $HCO_3^-/Cl^-$ | -0.06 | 0.0313 | |
| | Intercept | 121.18 | <0.0001 | 0.89 |
| | Total Dissolved Solids (mM/L) | -2.43 | <0.0001 | |
| | $SO_4^{-2}/Cl^-$ | 0.41 | <0.0001 | |
| | $HCO_3^-/Cl^-$ | -0.16 | 0.0033 | |
| | Intercept | 108.33 | <0.0001 | 0.86 |
| | Ionic Strength (mM/L) | -3.33 | <0.0001 | |
| | $SO_4^{-2}/Cl^-$ | 1.09 | <0.0001 | |
| | $HCO_3^-/Cl^-$ | -0.20 | 0.0071 | |
| Reduced data set (n=24) | | | | |
| | Intercept | 104.88 | <0.0001 | 0.78 |
| | Total Dissolved Solids (mg/L) | -0.04 | <0.0001 | |
| | $SO_4^{-2}/Cl^-$ | 0.17 | <0.0001 | |
| | $HCO_3^-/Cl^-$ | -0.07 | 0.0180 | |
| | Intercept | 99.77 | <0.0001 | 0.67 |
| | Total Dissolved Solids (mM/L) | -1.46 | 0.0002 | |
| | $SO_4^{-2}/Cl^-$ | 0.45 | 0.0003 | |
| | $HCO_3^-/Cl^-$ | -0.14 | 0.0184 | |
| | Intercept | 108.37 | <0.0001 | 0.79 |
| | Ionic Strength (mM/L) | -3.20 | <0.0001 | |
| | $SO_4^{-2}/Cl^-$ | 0.94 | <0.0001 | |
| | $HCO_3^-/Cl^-$ | -0.28 | 0.0013 | |

## CONCLUSIONS

- The chronic toxicity of anion solutions to *C. dubia* was primarily dependent on the concentration or activity of dissolved solutes.
- Effluents (or effluent dilutions) that exceed 800 mg/L TDS will have problems consistently meeting $IC_{25}$ requirements
- Substituting $SO_4^{-2}$ and/or $Cl^-$ for $HCO_3^-$ can reduce the toxicity of solutions despite similar TDS concentrations
- Producers of industrial and municipal effluents with TDS concentrations in this marginal range may potentially reduce the toxicity of the effluents by selecting materials or processes that minimize $HCO_3^-$ inputs.

## LITERATURE CITED

US Environmental Protection Agency. 1994. Short-term methods for estimating the chronic toxicity of effluents and receiving water to freshwater organisms, 3rd edition. EPA 600-4-91-002. USEPA, Cincinnati, OH.