APPENDIX H

PRELIMINARY REVIEW OF THE EFFECTS OF IONIC STRENGTH OF WATER ON FRESHWATER AQUATIC ORGANISMS,WITH EMPHASIS ON WHOLE EFFLUENT TOXICITY (WET) TEST ORGANISMS

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Preliminary Review of the Effects of Ionic Strength of Water on Freshwater Aquatic Organisms, with Emphasis on Whole Effluent Toxicity (WET) Test Organisms

Introduction

It has long been understood that the ionic strength of an aqueous solution can have a significant effect on the organisms inhabiting that solution. In general, ionic strength effects can act to structure or limit an aquatic community, in terms of species composition, abundance, etc. The most basic example of this is why freshwater fish cannot survive in salt water and, conversely, why marine fish cannot survive in fresh water. Ionic strength effects are also responsible for the lack of fish in the Great Salt Lake of Utah (Sigler and Sigler 1987). In fact, in an experiment often credited as the first toxicity test, Aristotle transferred freshwater fish into seawater to observe the effect (Cairns 1986). This first toxicity test was, in essence, a measure of the effects of ionic strength on the exposed fish.

Definition of Ionic Strength

Ionic strength is defined as a measure of the interionic effects resulting from electrical attraction and repulsion between ions in solution (Stumm and Morgan, 1981), which includes both positively charged ions (cations) and negatively charged ions (anions). Operationally, ionic strength is defined as μ in the following equation (Snoeyink and Jenkins, 1980):

$$\mu = 0.5\Sigma_i (C_i Z_i^2)$$

Where:

C_i = concentration of ionic species i

Z_i = charge of ionic species I

Unfortunately, a precise measurement of the ionic strength of a solution requires a complete chemical analysis of the solution (i.e., a complete cation-anion balance determination). However, Standard Methods (APHA *et al*. 1998) notes that a measure of conductivity or total dissolved solids (TDS) can be used to estimate the ionic strength of that solution in the absence of a complete analysis of the ions in solution. In addition to these two surrogate measures, salinity is also frequently used in the literature as a measure of the ionic strength of a solution. In fact, the literature regarding ionic strength effects typically do not include a

true measure of ionic strength, as strictly defined above, but rather uses one of these three surrogate measurements.

General Effects of Ionic Strength on Freshwater Organisms

There are a number of studies, detailed below, demonstrating that an excess of typically innocuous major ions in solution can create a toxic situation for aquatic organisms, especially those used in whole effluent toxicity testing. These excessive concentrations most likely overwhelm the osmotic capacities of the organisms, resulting in ionic strength toxicity. Notably, many of these studies are from sites or locales in the arid western United States, reflecting the regional influence of water quality in this part of the US.

Woodbury (1948) authored an early work reviewing the effects of salinity on organisms of the Great Basin, USA, that included, in general terms, the problems of elevated salinity for aquatic organisms in the arid American west. The primary conclusion of this work was that all organisms have a range of salinity in which they can survive and, within this broad range, a finer range which is optimum for that species. Although subsequent research has presented this concept in greater detail and with more empirical evidence, contemporary science has echoed this same theme.

The ideas suggested by Woodbury (1948) were confirmed for two United States Environmental Protection Agency (USEPA) standard zooplankton WET test organisms by Cowgill and Milazzo (1991). The authors studied the effects of both low and high concentrations of salinity, hardness, and alkalinity on the cladocerans *Daphnia magna* and *Ceriodaphnia dubia*. Relative to control organisms performance, *D. magna* exhibited enhanced brood sizes at salinity concentrations between 36 and 101 mg NaCl/L. However, at concentrations of 3,600 and 6,000 mg NaCl/L, the mean brood size and total number of broods were reduced. The no observed effects level (NOEL) was determined to be 1,296 mg NaCl/L for *D. magna*. *D. magna* was shown to have a salinity range over which reproduction was not restricted (0.08 - 1,296 mg NaCl/L) and an optimum range in which reproduction was enhanced (36 - 101 mg NaCl/L).

In the same study, Cowgill and Milazzo (1991) found that for *C. dubia*, NaCl concentrations above 280 mg NaCl/L were shown to reduce the size of the broods produced by exposed organisms. The highest total number of broods were produced at 168 and 280 mg NaCl/L. Thus, similar to *D. magna*, *C. dubia* was

shown to have a range over which reproduction was not impaired (0.08 - 280 mg NaCl/L), and a finer range over which reproduction was enhanced (168 - 280 mg NaCl/L), although the no-effect range was considerably lower than for *Daphnia*.

Burnham and Peterka (1975) exposed *Pimephales promelas* (fathead minnows), another common USEPA WET test species, to waters from four saline North Dakota lakes. The authors observed that the lakes were of two distinct salinity types; in the first thee, the salinity was mainly comprised by Na_2SO_4, while the salinity of the fourth lake was comprised by NaCl. Fry hatched in the lake water from two of the Na_2SO_4 study lakes (the third lake was not tested at this conductivity) experienced 100% mortality at conductivity values above 12,000 µmho/cm. However, fry hatching in water from the NaCl type lake experienced only 6% mortality at that conductivity level, and only 32% mortality in waters with a conductivity of 18,000 µmho/cm. Thus, the toxic effect of waters from these lakes to fathead minnows was not only dependant upon the ionic strength of the solution (measured as conductivity), but was also dependent on the types of ions making up that solution.

Dickerson *et al*. (1996) used fathead minnows and *C. dubia* in an investigation of the source of toxicity in several lakes and wetlands in Colorado, Montana, Utah, and Wyoming that received irrigation drain water. The authors concluded that in 6 of the 22 locations evaluated, the toxicity to *C. dubia* was caused not by a particular toxicant, but by elevated concentrations of common ions. The conductivity observed at these sites varied from 8,220 to 14,400 µmho/cm. The major cation at all sites with major ion toxicity was sodium, with concentrations ranging from 1,800 to 3,400 mg/L. The major anion at all of these sites was sulfate, with concentrations ranging from 3,500 to 8,200 mg/L.

Dwyer *et al*. (1992) conducted a similar study of the toxicity of irrigation drain water in the Stillwater Wildlife Management Area (SWMA) in Nevada. To differentiate between salinity-based and trace element-based toxicity in these irrigation drain waters, the authors used both a standard freshwater test organism, *D. magna,* and a euryhaline (i.e., tolerant of a wide range of salinity) test organism, the striped bass (*Morone saxatilis*). *D. magna* did not survive in waters with conductivity values exceeding 12,000 µmho/cm, which the authors noted was near the limits of that organism's salinity tolerance (7,000 - 8,000 mg/L). It was determined that toxicity to *D. magna* was due to elevated salinity and ionic strength effects, which were related to elevated concentrations of sodium, magnesium, sulfate, and alkalinity. In the absence of trace

elements, the striped bass was considerably less sensitive to increased salinity, with no mortality in some treatments up to 34,000 µmho/cm conductivity. In a study of the same system, using similar methodology, Ingersoll *et al*. (1991) attributed observed toxicity to their test organisms to not only elevated concentrations of salinity and major ions, but also to atypical ion ratios in solution.

These authors also investigated the salinity tolerance of a number of standard test organisms. For the amphipod, *Hyallela azteca*, they reported that a salinity of 22,000 mg/L (as instant ocean salts) was acutely toxic to this species (Ingersoll *et al*. 1991). Using the same salts, salinity concentrations of 8,000 - 10,000 mg/L were acutely toxic to *D. magna* and fathead minnows. Interestingly, the authors reported that increasing the hardness of highly saline solutions decreased the toxicity of those solutions. This effect was attributed to the positive effect of calcium ions on the ability of aquatic organisms to osmoregulate.

Much like Dwyer *et al*. (1991) and Ingersoll *et al*. (1991), Jop and Askew (1994) determined that atypical ratios of common ions in solution can be responsible for observed toxicity to *C. dubia.* Jop and Askew (1994) also present "safe" concentrations, as well as preferable concentrations of several common ions to *C. dubia*. They determined that a range of safe concentrations of sodium was from 1 to 150 mg/L, with a preferred range of 5 to 100 mg/L. For potassium, these concentrations were 1 to 100 mg/L (safe) and 1 to 50 mg/L (preferable). For magnesium, the safe range was from 1 to 50 mg/L, with a preferred range of 1 to 25 mg/L. Finally, the safe concentration range of calcium was determined to be between 1 and 400 mg/L, with a preferred range of 50 to 300 mg/L.

Toxic conditions similar to those observed in the irrigation drain water discharges was observed in co-produced brines from a saline oil-field in Wyoming by Boelter *et al*. (1992). These waters became increasingly toxic to *C. dubia* as total flow decreased; however, the same waters were not toxic to the fathead minnow. The authors attributed the toxicity to elevated concentrations of the major ions in solution. These ions were sodium (≥1,200 mg/L), potassium (≥14 mg/L), and chloride (≥1,000 mg/L), as well as elevated alkalinity levels (500 - 900 mg/L as $CaCO_3$). In addition, when the discharge was toxic, the conductivity values exceeded 6,000 µmho/cm.

As evidence of the recognition the toxicological community has given to ionic strength toxicity to aquatic organisms in general and USEPA test organisms specifically, McCulloch *et al*. (1993) set forth additional toxicity identification evaluation (TIE) procedures for identifying TDS toxicity in an effluent. In

addition to setting forth these new methods, the authors present toxicity data for several different salts to several USEPA test organisms. Both *C. dubia* and the less commonly used Cladoceran test species, *Daphnia pulex*, are reported to have the same sensitivity to NaCl (48-hr LC_{50} = 2.4 g/L). *Daphnia magna* is reported to be roughly twice as tolerant of NaCl with a 48-hour LC_{50} of 4.5 g/L, and the fathead minnow is reported to be greater than twice as tolerant as *D. magna* with a 96-hour LC_{50} of 10.8 g/L. In addition to NaCl, the authors provided *D. magna* toxicity values for KCl (48-hour LC_{50} = 128.5 mg/L), $CaCl_2$ (48-hour LC_{50} = 833.6 mg/L), and $MgCl_2$ (48-hour LC_{50} = 1.591.5 mg/L).

Toxicity Associated Specifically with Alkalinity and Hardness

While it is well known that the hardness of a solution may affect the toxicity of metals in that solution, it is less well known that elevated hardness levels themselves may be linked to toxicity. While hardness is reported in mg/L as $CaCO_3$, it is important to remember that hardness is primarily a measure of the divalent cations (chiefly Ca^{++} and Mg^{++}) in solution (APHA *et al*. 1998). Alkalinity is defined as the acid-neutralizing capacity of a solution, and is typically expressed in the same units as hardness (mg/L as $CaCO_3$). However, where hardness is a measure of the divalent cations in solution, alkalinity measures the titratable bases (chiefly HCO_3^- and $CO_3^=$) in solution (APHA *et al*. 1998). Thus, toxicity associated with hardness or alkalinity may be related to the amount of common divalent ions and/or common bases (carbonates) in solution. In essence, hardness and alkalinity are additional rough measures of the ionic strength of a solution.

Cowgill and Milazzo (1991) performed a series of bioassays using *D. magna* and *C. dubia* and varying levels of hardness. The authors observed that the reproductive success of both *D. magna* and *C. dubia* was negatively affected by hardness concentrations less than 72 mg/L as $CaCO_3$. At the other end of the scale, the no observed effect level (NOEL) for *D. magna* was reported at a hardness of 668 mg/L as $CaCO_3$. The NOEL for *C. dubia* was 638 mg/L as $CaCO_3$, a value slightly lower than that calculated for *D. magna*. Also, *C. dubia* showed signs of stress at hardness levels below 9 mg/L as $CaCO_3$. Based upon this study, both *D. magna* and C. dubia have ranges of hardness values over which survival and reproduction are not impaired. These hardness ranges are from 72 - 668 mg/L as $CaCO_3$ for *D. magna* and from 72 - 638 mg/L as $CaCO_3$ for *C. dubia*.

Cowgill and Milazzo (1991) also exposed *D. magna* and *C. dubia* to varying concentrations of alkalinity (mg/L as $CaCO_3$). For *D. magna*, the number of broods produced was reduced at all alkalinity concentrations above and below the control of 45 (or 55, as both values are mentioned as the control concentration) mg/L as $CaCO_3$. The NOEL for alkalinity to *D. magna* was reported to be 129 mg/L as $CaCO_3$. Thus, *D. magna* populations have an optimal alkalinity concentration of approximately 50 mg/L as $CaCO_3$ and can be sustained at levels below 129 mg/L as $CaCO_3$. Cowgill and Milazzo (1991) demonstrated that the total number of broods produced by *C. dubia* was reduced at alkalinity concentrations above that of the control (72 mg/L as $CaCO_3$) and below 13 mg/L as $CaCO_3$. Based upon this study, *C. dubia* populations appear to have a range of alkalinity levels from 13 to 72 mg/L as $CaCO_3$ over which their reproduction is not impaired.

Lasier *et al*. (1997) noted that under certain environmental conditions (high ionic strength waters, elevated dissolved organic carbon, sulfate reduction, etc.), the alkalinity levels of sediment pore waters may become elevated. Sediment pore water alkalinities of up to 4,500 mg/L as $CaCO_3$ have been reported from arid regions of Utah. These elevated levels of alkalinity and its corresponding toxic threshold for *H. azteca* are important as these elevated alkalinity levels may impact the results of sediment toxicity tests, which often use *H. azteca* as a test organism (USEPA 1994). Lasier *et al*. (1997) reported mean 96-hour alkalinity LC_{50} values for both 14-day old and 7-day old *H. azteca*. The 96-hour LC50 value for 14-day old *H. azteca* was substantially higher than that determined for 7-day old *H. azteca* (alkalinity of 1,212 vs. 662 mg/L as $CaCO_3$). Again, these toxicity levels may be seen as the result of elevated concentrations of common ions (chiefly HCO_3^- and $CO_3^=$) in solution.

Community Effects of Salinity/Ionic Strength

In addition to the these toxicity tests using solutions with elevated ionic strength, the literature contains several references to the effects naturally occurring high ionic strength waters have on structuring the aquatic community. These studies demonstrate that high saline systems appear to limit both the abundance and diversity of benthic macroinvertebrate taxa, while moderate salinity levels may only serve to "order" the community.

In a study of a naturally saline lake in Central Washington State, Wiederholm (1980) determined that benthic species composition and abundance was correlated with the ion composition and salinity of the lake.

The naturally saline lake was slowly diluted with influx from irrigation waters over a 25-year period (1950 - 1975). As the salinity of the lake decreased, the abundance and diversity of benthic organisms increased. A particularly rapid increase in taxa was noted as the salinity of the lake decreased from 3 to 2 g/L.

Wollheim and Lovvorn (1995) studied three oligosaline (conductivities of 800 - 8,000 µmho/cm) and three mesosaline (8,000 - 30,000 µmho/cm) lakes in the state of Wyoming. The authors found that the observed variation in salinity values between the lakes failed to explain the variation in benthic macroinvertebrate biomass. However, there were noticeable differences in species composition between the lakes. Amphipods and gastropods were the dominant taxa in the oligosaline lakes, while these same taxa were absent or rare in the mesosaline lakes. Conversely, chironomid abundance increased with increasing salinity. Another interesting observation was that the biomass of zooplankton (including cladocerans) increased in the mesosaline lakes. These data demonstrate that in relatively stable environments, the level of salinity did affect the variety or abundance of aquatic invertebrates present.

Finally, Metzeling (1993) studied several Australian streams with historic, naturally occurring elevated TDS values (51 - 1,100 mg/L). Metzeling found that there was no correlation between the abundance and diversity of benthic macroinvertebrates in these streams. However, there were distinct aquatic communities associated with either high or low TDS waters. He concluded that these findings probably hold true only for similar streams (i.e., those with naturally occurring, historically elevated salinities), and that newly introduced elevated salinity or wildly fluctuating salinity levels would have a negative effect, rather than a simple structuring effect, on the benthic community.

Ion Toxicity Models

In an attempt to model the effects of major ion toxicity/ionic strength effects, Mount *et al*. (1997) developed an extensive model to predict toxicity based on the ionic composition of a solution. This model was developed based on data generated in toxicity tests of approximately 2,900 ion solutions using *C. dubia*, *D. magna*, and fathead minnows. The authors concluded that the relative toxicity of the common major ions to the test species was $K^+ > HCO_3- = Mg^{++} > Cl^- > SO_4^=$. Further, they concluded that toxicity associated with Na^+ and Ca^{++} was most likely due to the corresponding anion. In addition, this study presents ion toxicity data for a large number of salts and salt combinations to all three aforementioned species.

This model was tested by Tietge *et al*. (1997) on six samples of produced waters, and by Dickerson *et al*. (1996) on samples from several irrigation drain water catchments. Tietge *et al*. (1997) found that the major ion toxicity model developed by Mount *et al*. (1997), when used in conjunction with traditional phase I TIE procedures, was successful in identifying major ion toxicity. Dickerson *et al*. (1996) determined that the model was accurate enough to be used in prediction of acute toxicity associated with major ion concentration to *C. dubia*.

Conclusions

Ionic strength effects are generally reported by a number of water quality parameters, most commonly conductivity, TDS, salinity, and sometimes as alkalinity or hardness. Based upon this preliminary review, it is apparent that atypical ratios of major ions or elevated concentrations of major ions in solution may be toxic to aquatic organisms. The effects of these solutions may be straight toxicity to sensitive species or a structuring effect on the aquatic community. Recently, effective models have been produced to predict toxicity due to major ion effects, which are, in essence, ionic strength effects.

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