BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:
PROPOSED AMENDMENTS TO:)
35 Ill. Adm. Code 302.102 and 302.208(g)) R18-32
WATER QUALITY STANDARDS)
FOR CHLORIDES)

NOTICE OF FILING

TO: Don Brown
Clerk of the Board
Illinois Pollution Control Board
100 West Randolph Street, Suite 11-500
Chicago, Illinois 60601
(Via Electronic Mail)

(See Persons on Attached Service List)

PLEASE TAKE NOTICE that I have today filed with the Office of the Clerk of the Illinois Pollution Control Board the attached **AMENDED PETITION TO AMEND 35 ILL. ADM. CODE 302.102 and 302.208(g) WATER QUALITY STANDARDS FOR CHLORIDES, INCLUDING PROPOSED RULE LANGUAGE,** copies of which are herewith served upon you.

Respectfully submitted,

Huff & Huff, Inc.

By: James E Huff
Senior Consultant

Dated: March 14, 2019

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INTRODUCTION

Based on the hearing in this matter held January 23, 2019, this Amended Petition is intended to present a revised water quality standard for chlorides based upon stream temperature at the time of sampling. Recent literature regarding chloride toxicity has been incorporated herein. The proposed language has been modified with respect to winter temperatures, and the toxicity data used to derive the standards have been updated with additional recent literature. The toxicity data have been normalized based on hardness and sulfate. In addition, a temperature relationship was derived based upon a recent article by Jackson and Funk (2019)[1] coupled with the temperature data generated as part of this project. The result is a single year-round water quality standard based upon the stream temperature, hardness, and sulfate at the time of sampling for chlorides.

STATEMENT OF REASONS

The Illinois Pollution Control Board (Board), in R08-9 (Subdocket D) adopted water quality standards on the Chicago Area Waterways (CAWS) and the Lower DesPlaines River (LDPR), including for chlorides. With the exception of the Chicago Sanitary & Ship Canal (CSSC), the Board adopted a chloride water quality standard of 500 mg/L from May 1st through November 30th, and the same standard for the remainder of the year, effective three years after the effective date of those rules. The intent of the three-year delay was “to allow time for the work group to develop a proposal to address chloride and a water body wide variance.” (Final Notice Opinion and Order of the Board, R80-9, p 32.)

The focus of the various work groups has been on developing and implementing Best Management Practices (BMPs) to reduce the application of highway de-icing salts, the principal cause of elevated chlorides during the winter (and spring) months in the receiving streams. Elevated chlorides are not unique to the CAWS and LDPR during the winter months; elevated chloride concentrations occur on all urban streams in Illinois. The General Use Water Quality Standard for chlorides, as found in 302.208(g), is 500 mg/L, identical to what the Board has adopted for the CAWS and LDPR, excluding the CSSC.

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[1] Jackson, J.K. and Funk, D.H. Temperature affects acute mayfly responses to elevated salinity implication for toxicity of road de-icing salts. *Phil. Trans. R. Soc. B 374: 20180081.*

More efficient application of sodium chloride for highway de-icing is being implemented. However, as the 500 mg/L standard is a *not-to-exceed* standard, the question is: can BMPs achieve the necessary reduction under the worst storm events? For example, on the North Branch of the Chicago River, data from 2004 to 2014 reveal a maximum chloride concentration of 1,134 mg/L, necessitating a reduction of 55 percent in salt application during the worst events. (Huff, October 2015.[2]) It is appropriate to question whether simply implementing BMPs can consistently achieve this type of reduction. For smaller streams, the required reductions can be even greater. For example, Hickory Creek at Vine Street reached 1,476 mg/L in 2014, necessitating a reduction of 66 percent in salt application during the worst storm events to achieve 500 mg/L. (Huff, Feb 2015.[3]) Despite these elevated winter concentrations some of the impacted waterways still host aquatic communities who score at the upper end of the moderately impaired category of the state aquatic life scale. This observation supports the position that elevated winter concentrations are less destructive to aquatic communities than elevated warm weather concentrations.

While focusing on efficient utilization of de-icing salts is appropriate, there are concerns that implemented alone, BMPs will not achieve the target of 500 mg/L for these worst storm events. These concerns were the basis behind assembling a group of municipalities and sanitary districts, industries, The Salt Institute, a watershed group, and the Illinois Tollway to fund additional research on cold-temperature toxicity of chlorides. The results from these additional toxicity tests form the basis behind this Rulemaking request, as the findings show chlorides are less toxic at colder temperatures, thereby justifying a relaxed chloride standard during the colder months. Illinois already utilizes a similar approach for ammonia for which there are less stringent winter standards, so there is precedent for such an approach. In addition, the Board adopted higher winter chloride standards for the CSSC in R08-9 (Subdocket D).

Given the current water quality violations, chlorides are or should be identified as a cause of impairment for nearly all urban streams in Illinois. The ones not identified as impaired due to chlorides are likely due to the lack of sufficient monitoring. The impact of chlorides being identified as a cause of impairment is a serious impediment to future growth of any kind in the urban areas of Illinois. If increased pavement, housing, or parking lots are planned, then the required de-icing salts will need to be more than offset within the watershed. Finding these offsets is becoming more difficult. BMPs are being implemented as part of the watershed variances currently before the Board, and these same BMPs cannot be used as offsets for new growth. Alternatives to chloride de-icing are not technically feasible on a region-wide basis, when considering safety and mobility. This has been demonstrated by the Connecticut Department of

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[2] Huff, J.E., The Science Behind the Chloride Water Quality Standard, Presentation at the Chicago Area Waterways Chloride Workshop, MWRDGC Stickney Plant, October 29, 2015.

[3] Huff, J.E., Chloride Regulatory Update, Presentation at the Hickory Creek Watershed Planning Group, New Lenox, IL February, 19, 2015.

Transportation, that for a seven-year period used a mixture of sand-salt (7:2) and compared the accidents to a seven-year period with just salt and found a 19 percent increase in nonfatal injuries and a 33 percent increase in accidents with the sand-salt mixture compared to just salt.[4] So while a reduction in salt usage is an achievable goal, it is technically infeasible to reduce its use for de-icing practices sufficiently to consistently meet the 500 mg/L water quality standard.

Another consideration would simply shut down the highway system during snow events where there would be a potential to exceed the water quality standard. From the information generated in the Technical Support Document, this would vary from an average one storm per year to over five storm events, and the duration of each shut down would last for multiple days. Nationwide, a one-day shutdown of the snowbelt states would yield a loss of $2.6 billion per day, and a loss of retail of $870 million per day.[5] Not only would this be economically unreasonable, the social impacts would not be acceptable to the citizens of Illinois.

I. History and background to the present proceeding

The 500 mg/L Illinois general use water quality standard for chloride was adopted by the Board in 1972 in R71-14, based on the testimony of a "recognized expert in fish biology," that 500 mg/L would be a safe limit. (Opinion of the Board, March 7, 1972.) This Illinois General Use Water Quality Standard has remained in effect for the past 45 years.

U.S. EPA in 1988 published the *Ambient Water Quality Criteria for Chloride-1988* (EPA 440/5-88-001, February 1988) that recommended a four-day limit of 230 mg/L and a one-hour average limit of 860 mg/L that should not be exceeded more than once every three years on the average.

In January 2009, C. Stephan of the USEPA issued a draft update deriving chloride toxicity, factoring in hardness and sulfate concentrations, and utilizing a normalized hardness of 300 mg/L and normalized sulfate concentration of 65 mg/L. Temperature was not a factor in the Stephan document, with data generated at the USEPA guideline temperatures, 25°C for most species. Much of the more recent literature on chloride toxicity relates to the impact of sulfate and hardness concentration on the toxicity.

As nearly all of the toxicity data derived for chlorides have been generated at summer-type temperatures, the results do not accurately reflect the toxicity of chlorides at winter temperatures. This proposal sets forth the findings of both a literature search and toxicity testing with the four sensitive aquatic species (Fingernail clams, mayflies, Amphipod, and *C. dubia*) at 10°C and 25°C. Due to limited funding, hardness and sulfate were not varied as part of the testing, just temperature.

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[4] Mahoney, JU. D.S. Larsen, and E. Jackson. Reduction in nonfatal injury crashes after implementation of anti-icing technology. Transportation Research Record: *Journal of the Transportation Research Board,* No. 2613. Transportation Research Board of the National Academies, Washington, D.C., 2017, pp. 77-86. See Attachment 4

[5] Benefit-Cost of Various Winter Maintenance Strategies, Project 99006/CR13-03, Western Transportation Institute, September 2015. See Attachment 5.

The original Petition called for a winter chloride standard during the months of December through April. It was clear from the first hearing that a standard based upon actual stream temperature at the time of sampling was a preferred approach, and this Amended Petition has made this change. Factoring in sulfate and hardness in the derivation of chloride water quality standards was suggested during the hearing as well. The proposed language incorporates this request, assuming the same relationship for hardness and sulfate that was derived at temperature at 25°C applies at 10°C. This also seems like a reasonable approach until such time as further research is completed, so in a sense, the proposed standard herein can be viewed as interim water quality standard. Given that the hardness and sulfate relationship for aquatic species was based on one species, utilizing the temperature data from Jackson and Funk, combined with our data to establish a temperature relationship, is appropriate.

A. Purpose and Effect of Regulatory Proposal

The Board's existing 500 mg/L General Use Standard for chlorides is exceeded in all urban streams during snow melt periods in Illinois. For the CAWS and LDPR, the winter 500 mg/L water quality limit goes into effect on December 1, 2019. Most of the urban watersheds in Illinois are working on seeking watershed variances from the 500 mg/L standard. As noted in the introduction, the compliance plans are centered around BMPs for de-icing practices. This is a sound and necessary approach that will reduce chloride concentrations in our waterways. However, it is not likely to consistently achieve the not-to-exceed 500 mg/L standard in many of the urban streams.

This work was undertaken to improve the understanding of chloride toxicity under winter temperatures. The results show that chlorides are less toxic at colder temperatures, and therefore higher water quality standards can be derived for the winter months. The recent publication referenced earlier by Jackson and Funk (2019) demonstrated that the temperature effect on chloride toxicity is a linear relationship, which allowed us to use our data derived at two different temperatures to develop a mathematical relationship, combined with the Jackson and Funk data. If the proposed chloride water quality standards are adopted, it is expected that many smaller streams will be able to achieve the water quality standards through the implementation of BMPs. On larger streams, due to the duration of the chloride spikes, achieving the proposed chronic standard during snow melt periods will prove difficult.

Specifically, the proposed standard consists of the following amendments.

1. **Remove Chloride from Section 302.208(g), Numeric Standards for Chemical Constituents, Single-Value Standards**: Based on the difference in chloride toxicity at colder temperatures, it is appropriate to draft a new section to include chloride standards.

g) Single-value standards apply at the following concentrations for these substances:

Constituent	Unit	Standard
~~Chloride (total)~~	~~mg/L~~	~~500~~

2. Add Section 302.214 Chlorides

a. Acute chloride standard (Cl_{AC}):

$$Cl_{AC} = (1+(0.045)(25-T))* NFAV*(Hardness/300)^{0.205797} *(Sulfate/65)^{-0.07452}$$

Where NFAV is the Normalized Acute Value of 518 mg/L.
Hardness (as $CaCO_3$ in mg/L), Sulfate (as SO_4 in mg/L), and Temperature (^oC) are the stream results at the time the sample was collected.

b. Chronic chloride standard (Cl_{CV}):

$$Cl_{CV} = (1+(0.045)(25-T))* CCC*(Hardness/300)^{0.205797} *(Sulfate/65)^{-0.07452}$$

Where CCC is the Normalized Criteria Continuous Concentration of 300 mg/L.
Hardness (as $CaCO_3$ in mg/L), Sulfate (as SO_4 in mg/L), and Temperature (^oC) are the stream results at the time the sample was collected.

To calculate the attainment status of chronic standards, the concentration of the chloride result is divided by the calculated water quality standard for the sample to determine a quotient. The water quality standard is attained if the mean of the sample quotient is less than or equal to one for the duration of the averaging period.

The Cl_{CV} shall not be exceeded more than once every three years by the arithmetic average of at least four consecutive samples collected over any period of four days.

3. Amend Section 302.407 Chemical Constituents as follows:

g. Numeric Water Quality Standards for Other Constituents

2) ~~From July 1, 2015 until July 1, 2018, the following concentrations for Chloride and Total dissolved solids shall not be exceeded except in waters for which mixing is allowed pursuant to Section 302.102 of this Part.~~

~~Constituent~~	~~Unit~~	~~Standard~~
~~Chloride during the period of May 1 through November 30~~	~~mg/L~~	~~500~~
~~Total Dissolved Solids during the period of December 1 through April 30~~	~~mg/L~~	~~1,500~~

3) ~~Beginning July 1, 2018, the Chloride and Total Dissolved Solids standards in subsection (g)(2) of the Section are repealed and the following concentration for Chloride shall not be exceeded except in waters for which mixing is allowed pursuant to Section 302.102 of this Part:~~

~~Constituent~~	~~Unit~~	~~Standard~~
~~Chloride~~	~~mg/L~~	~~500~~

~~Where:~~

~~mg/L = milligram per liter~~

Add Section 407(g)(2) Chlorides

a. Acute chloride standard (Cl_{AC}):

$$Cl_{AC} = (1+(0.045)(25-T))* NFAV*(Hardness/300)^{0.205797} *(Sulfate/65)^{-0.07452}$$

Where NFAV is the Normalized Acute Value of 518 mg/L.
Hardness (as $CaCO_3$ in mg/L), Sulfate (as SO_4 in mg/L), and Temperature (^oC) are the stream results at the time the sample was collected.

b. a chronic chloride standard:

$$Cl_{CV} = (1+(0.045)(25-T))* CCC*(Hardness/300)^{0.205797} *(Sulfate/65)^{-0.07452}$$

Where CCC is the Normalized Criteria Continuous Concentration of 300 mg/L.
Hardness (as $CaCO_3$ in mg/L), Sulfate (as SO_4 in mg/L), and Temperature (^oC) are the stream results at the time the sample was collected.

To calculate the attainment status of chronic standards, the concentration of the chloride result is divided by the calculated water quality standard for the sample to determine a quotient. The water quality standard is attained if the mean of the sample quotient is less than or equal to one for the duration of the averaging period.

The Cl_{CV} shall not be exceeded more than once every three years by the arithmetic average of at least four consecutive samples collected over any period of four days.

II. Technical Feasibility and Economic Justification

A. The Board and the Agency have always supported science-based standards, and this proposed regulatory rule change is consistent with this approach. New research, financially supported by the consortium that was assembled to examine the chloride standards, forms the basis for the proposed changes. This work was undertaken because the current chloride water quality standard is neither technically feasible to achieve nor ecologically or economically justified. The Agency has promoted a pathway forward of watershed variances, relying on Best Management Practices to achieve a 500 mg/L, while at the same time USEPA is promoting even more restrictive water quality standards. However, there was no testimony in the CAWS proceedings that achieving the 500 mg/L winter chloride standard was technically feasible, economically reasonable, or ecologically justified. Proceeding with the Agency’s watershed approach will require the regulated community to continue striving to achieve an unachievable standard until someone comes up with an alternative approach, which is exactly what this proposal is intended to do.

B. The temperatures in the waters in Illinois are colder in the winter months. Yet, nearly all of the aquatic toxicity testing conducted prior to the testing contained in this Petition is at temperatures experienced during the summer months, with the majority of studies being conducted at what would be considered near maximum temperatures experienced in Illinois streams (25°C).

C. In summary, the expectation is that if the proposed winter water quality standards are adopted, the watershed approach based on implementing BMPs will become technically feasible and economically reasonable for many urban streams in Illinois, as supported by the stream data presented in the Technical Support Document (Attachment 2).

III. Facts in Support

The original Petition included several Attachments in support of the proposed changes and are not resubmitted herein. A supplement to the Technical Support Document has been prepared with additional supporting information regarding the proposed water quality standards proposed herein.

IV. Conclusion

The current general use chloride water quality standard is unattainable in many urban streams in Illinois during the winter months. Cold temperature toxicity testing of chlorides developed in support of this Petition has demonstrated that at colder temperatures chlorides are less toxic to aquatic organisms. The recent work by Jackson and Funk has established that a linear relationship exists between temperature and chloride toxicity, that has been used with the research we conducted to develop mathematically derived water quality standards based on temperature. Based on these findings, a new chloride standard is proposed for General Use waters, derived using the USEPA protocol for developing water quality standards.

This Amended Petition, along with the original Petition, satisfies the requirements of Section 102.202 of the Board's rules because the Petition:

- Details the language of the proposed rule change;
- Presents the facts that support the proposal including the environmental, technical, and economic justification;
- Includes a statement of the purpose and effect of the proposal;
- Includes a synopsis of the expected testimony;
- Describes the results of the current knowledge on cold temperature toxicity of chlorides and the findings of the research associated with the request;
- Demonstrated the proposed rule change is consistent with federal law.

WHEREFORE, Petitioner, Huff & Huff, Inc. respectfully requests that the Illinois Pollution Control Board adopt revised chloride water quality standards for the winter months, as proposed herein.

HUFF & HUFF, INC.

Dated: March 14, 2019

By: James E Huff
James E. Huff, P.E.

James E. Huff, P.E.
Huff & Huff, Inc., a Subsidiary of GZA, Inc.
915 Harger Road
Oak Brook, IL 60523
James.Huff@gza.com

CERTIFICATE OF SERVICE

I, James E. Huff, the undersigned, on oath state the following:

That I have served the attached **PETITION TO AMEND 35 ILL. ADM. CODE 302.102 and 302.208(g) WATER QUALITY STANDARDS FOR CHLORIDES,** via electronic mail upon:

Don Brown Clerk of the Board Illinois Pollution Control Board 100 W. Randolph Street, Suite 11-500 Chicago, Illinois 60601 Don.Brown@illinois.gov	Division Chief of Environmental Enforcement Office of the Attorney General 100 West Randolph Street, Suite 1200 Chicago, Illinois 60601 enviro@atg.state.il.us
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That my email address is James.Huff@gza.com.

That the number of pages in the email transmission is__327 pages__.

That the email transmission took place before 5:00 p.m. on the date of March 14, 2019

HUFF & HUFF, INC.

Dated: March 14, 2019 By: James E Huff

James E. Huff, P.E.

AFFIDAVIT OF E-MAIL SERVICE

I, the undersigned, on oath [or affirmation] state the following:

That I have served the attached **Petition to Amend 35 Ill. Adm. Code 302.102 and 302.208(g) Water Quality Standards for Chlorides, including Proposed Rule Language, and 200 Signatures in Support of this Petition** by email upon Mr. Don Brown, Clerk of the Board, at Don.Brown@illinois.gov.

That my e-mail address is James.Huff@gza.com

That the number of pages in the e-mail transmission is 327 pages .

That the e-mail transmission took place before 5:00 p.m. on the date of March 14, 2019.

James E Huff

SUBSCRIBED AND SWORN TO BEFORE ME this 14th day of March, 2019.

Notary Public

[Notary Seal]

MAUREEN T WUNDERLICH
Official Seal
Notary Public - State of Illinois
My Commission Expires Oct 2, 2020

TECHNICAL SUPPORT DOCUMENT SUPPLEMENT FOR A WINTER WATER QUALITY STANDARD

March 2019

By

James E. Huff, P.E.

1. INTRODUCTION

The Illinois Pollution Control Board's General Use Water Quality Standard for chlorides has been 500 mg/L since the early 1970s. Since the adoption of this water quality standard, sales of de-icing salts in the United States have doubled, with a similar increase in chloride concentrations in the receiving streams (Kelly et al., 2012). In Docket R8-09 (Subdocket D), the Board expanded the 500 mg/L chloride water quality standard to include the Chicago Area Waterways (CAWS) and Lower DesPlaines River, excluding the Chicago Sanitary & Ship Canal (CSSC), where site-specific standards were adopted. However, the Board provided three years before the chloride water quality standard would apply during the winter months to the CAWS and Lower DesPlaines River, in recognition of the current water quality exceedances of this 500 mg/L level. Chloride concentrations above the 500 mg/L level are not unique to these two waterways but occur during snow melt periods in nearly all urban streams within Illinois.

The current focus in Illinois to address these chloride exceedances is in pursuing variances from the Board, with a commitment to developing and implementing Best Management Practices (BMPs) to reduce the application of highway de-icing salt. While implementing BMPs is a worthwhile activity, the potential to achieve a *not-to-exceed* limit of 500 mg/L in urban streams under the worst storm conditions is not realistic. As USEPA has also proposed a more restrictive chloride water quality criterion, the 500 mg/L standard has a potential to be significantly reduced at some future date.

Toxicity testing for chlorides has consistently demonstrated the need for restrictive water quality standards. However, this laboratory testing has been conducted at water temperatures between 23 and 25°C, basically near peak summer temperatures. Winter temperature toxicity studies on chlorides are limited. We know that growth and reproduction for most aquatic organisms are limited at colder water temperatures, which raises the question of the appropriateness of the many chronic concerns during the winter months.

In addition, some species are absent from the water column during the winter months. As part of the justification for the site-specific water quality standards in R8-09, Citgo presented data on its collection of Cladocera (water fleas, including *Ceriodaphnia*) from the CSSC. Cladocera population peaked in the summer and steadily declined as the water temperatures cooled. By October 29th, no Cladocera were collected within the CSSC. This finding is not surprising when one considers the life cycle of zooplankton.

Based on the work funded by Citgo as part of R8-09, questions were raised about the impact temperature has on the toxicity of chlorides. Huff & Huff, Inc. solicited funding from a cross section of salt users to fund additional research on cold temperature toxicity of chlorides. The actual toxicity testing for three species (the amphipod *Hyalella azteca,* the fingernail clam *Sphaerium simile,* and mayfly *Neocloeon triangulifer)* was conducted by Dr. David Soucek and Amy Dickinson at the Illinois Natural History Survey (INHS), and the daphnia test (*Ceriodaphnia dubia*) was conducted by the New England Bioassay (NEB) Laboratory in Manchester, Connecticut. The INHS is recognized as the leading research laboratory on aquatic toxicity of chlorides and sulfates.

Presented herein are the findings from this research, which include more recent literature and cold temperature toxicity testing on four of the most sensitive species to chlorides. Also included herein is an analysis of the duration of exposure to elevated chlorides and derivation of suggested winter water quality standards for chlorides. The proposed water quality standards are then derived, incorporating temperature into the equation.

2. SUMMARY OF MORE RECENT LITERATURE

The literature survey on the toxicity of chlorides was completed in late 2017, with a focus on temperature effects on this toxicity. There has been minimal research on the effect of temperature on the toxicity of chlorides. Chloride toxicity in general has focused on some of the most sensitive aquatic species, including *C. dubia, N. triangulifer, H. azteca and S. simile,* the same organisms that were studied under the current work. Since completion, there have been additional studies published that are relevant to these proceedings.

Jackson and Funk (2019) of the Stroud Water Research Center presented salinity toxicity data on four species of mayflies representing three different families tested at winter and summer temperatures, using sodium chloride during 96-hour static tests at temperatures ranging from 5 to 25ºC. The four mayflies were *Neocloeon triangulifer, Leptophlebia cupida, Maccafertium modesum*, and *Procloeon fragile*. The mayflies were tolerant of high chloride values at low temperature and were less tolerant as temperature increased. For the four species of mayflies, the LC_{50} for chlorides increased with decreasing temperatures, at rates ranging from 201 to 305 mg/L chlorides for every degree C drop in temperature. Taking the least temperature sensitive mayfly species, when the stream temperature declines from 20ºC to 10ºC, the LC_{50} increases by over 2,000 mg/L as chlorides. The correlation the authors found extended from 5 to 25ºC.

Hardness and sulfates have both been recognized as having an impact on the toxicity of chlorides, with the following equation generally recognized today as reflecting this relationship:

$$\textit{Acute Water Quality Std} = \textit{Normalized Acute Value}*(\textit{Hardness}/300)^{0.205797}\ (\textit{Sulfates}/65)^{-07452}$$

This relationship was based on the two studies of one species (*C. dubia*), as explained by Stephan (2009). Stephan further explained that any covariance analysis would yield similar exponents. This equation was utilized by Stephan (2009) in his derivation of chloride water quality standards, as well as Pennsylvania and Iowa.

Jackson and Funk (2018) found a linear inverse relationship between temperature and chloride acute toxicity. They utilized actual stream water from White Clay Creek, with a mean hardness of 97 mg/L and sulfate of 17.3 mg/L. Without any adjustments to a *normalized* hardness and sulfate, they evaluated four species of Mayflies the LC_{50} for chlorides increased with decreasing temperatures, at rates ranging from 201 to 305 mg/L chlorides for every degree C drop in temperature. The relationship held for temperatures ranging from 5 to 25ºC. An additional key finding from Jackson and Funk is the linear relationship between temperature and toxicity.

3. DERIVATION OF A TEMPERATURE RELATIONSHIP

Based on the Jackson and Funk study, a linear relationship exists between temperature and chloride toxicity. This is a key finding, as our study evaluated two different temperatures (10°C and 25°C) from which a linear relationship can be derived. Our research found the following changes:

Species	Change in Acute Toxicity
C. dubia	-68.8 mg/L/degree C
S. simile	-83.1 mg/L/degree C
N. triangulifer	-31.2 mg/L/degree C
H. azteca	-30.1 mg/L/degree C

Combining our data with the Jackson and Funk (2018) data yields the following:

Species (Common Name)	Change in Acute Toxicity, mg/L Cl/°C
C. dubia (Cladoceran)	-68.6
S. Simile (Fingernail clam)	-83.1
N. triangulifer (Mayfly)	-31.2
H. azteca (Amphipod)	-30.1
N. triangulifer (Mayfly)	-305.2
P. fragile (Mayfly)	-237.4
L. cupida (Mayfly)	-243.7
M. Modestum (Mayfly)	-201.1

Normalizing the change in toxicity based on the LC_{50} results yields a change of 4.5 percent per degree C decrease in temperature. Note that Jackson and Funk found a five-fold greater impact from temperature, which may be attributable at least to the use of actual stream water, as compared to the use of laboratory synthetic dilution water. Also, Jackson and Funk cultured its mayflies at 20°C, versus Soucek's culturing temperature of 25°C, and this may have impacted the sensitivity to temperatures.

This leads to the following equation for the acute water quality standard:

Acute Water Quality Std =

$$[NFAV+1.045NFAV(25-T)]*(Hardness/300)^{0.205797}\ (Sulfates/65)^{-07452}$$

Chronic Water Quality Std=

$$[1+(0.045)(25-T)]*[CCC*(Hardness/300)^{0.205797}\ (Sulfates/65)^{-07452}]$$

Where NFAV is the Normalized Final Acute Value and CCC is the Criterion Continuous Concentration at 300 mg/L hardness, 65 mg/L sulfate and 25°C.

These equations would be valid for all seasons, based upon the temperature, hardness, and sulfate in the stream at the time of sampling for chlorides.

4. DURATION OF ELEVATED CHLORIDES IN RECEIVING STREAMS

Elevated chlorides in Illinois streams are episodic events. The levels reached and the duration are functions of the amount of salt applied in response to a storm event, the subsequent temperatures, the base flow in the receiving streams, and antecedent period from the previous snow event (which determines the background chloride concentration before the storm runoff). The smaller the drainage area, the flashier the chloride response will be, typically with spikes of chlorides above 500 mg/L lasting one-to-two days. As the stream size increases, the duration where the chlorides will remain above 500 mg/L can last a week or more. However, these longer durations typically have a return interval longer than three years on most urban streams.

Three data sources for spikes in chlorides or surrogate measurements (conductivity or Total Dissolved Solids or TDS) were used to look at the duration of snow melt events. First, the Citgo Lemont Refinery has measured the chlorides concentration on the CSSC for over a decade. Second, the Metropolitan Water Reclamation District of Greater Chicago (MWRD) has monitored conductivity on many Chicago Area Waterways, each with an individually-derived chloride concentration. The third data source is from the DuPage River/Salt Creek Workgroup (DRSCW), which also has monitored conductivity, with correlation to chlorides. Each of these three datasets provides information on the duration to chlorides spikes on a variety of stream sizes. Each of the three is discussed below.

4.1 Lemont Refinery Chloride Monitoring on the Chicago Sanitary & Ship Canal

Presented in the original Technical Support Document, Appendix C, is the Lemont Refinery's chloride data table for the winter months. The average concentration each winter ranged from a low of 128 mg/L (based on limited data in 2012) to 393 mg/L in 2014. More relevant to our analysis is the duration of elevated chlorides when such events occur. The CSSC represents the largest stream segment, especially associated with urban runoff, so the durations of elevated chlorides would be expected to be longer on this waterbody than all others in Illinois. The chronic water quality standard on the CSSC is 620 mg/L, so using this value, the following table was constructed:

CSSC at Romeoville

Event	# of days 4-day running average above 620 mg/L Chlorides, days	Maximum Chloride Concentration Recorded, mg/L
2017	0	568
2016	0	660
2015	0	904
2014	5	720
2013	2	711
2012	0	145
2011	4	1,099
2010	1	870
2009	1	881

CSSC at Romeoville

Event	# of days 4-day running average above 620 mg/L Chlorides, days	Maximum Chloride Concentration Recorded, mg/L
2008	7	896
2007	4	998
2006	0	454
2005	1	835

*Note the duration in days is an extrapolation; typically, samples were collected two days per week.

There were 25 events over the 12-year period, with exceedances of the four-day 620 mg/L chronic standard, or an average of two events per year. The average duration of these exceedances was approximately 5 days. Three events had durations greater than 7 days, or a frequency of occurring once per 3.6 winter seasons. The acute standard on the CSSC is 990 mg/L, and documented exceedances occurred twice over the 11-year period, a recurrence frequency of once every 5.5 years.

4.2 MWRD Conductivity Monitoring on the Chicago Area Waterways

The MWRD has a series of conductivity probes throughout the CAWS, and data from 2007 through April 2017 were available for analysis. Using the MWRD conversion from conductivity to chlorides allows for more accurate estimates of duration. The results for the CSSC at Cicero, just above the discharge from the Stickney treatment plant, are summarized below:

CSSC at Cicero

Event	# of days 4-day running average above 620 mg/L Chlorides, days	Maximum Chloride Concentration Recorded, mg/L
Thru April 30, 2017	0	500
2016	0	465
2/15/2015	8	812
1/14/2014	9	916
3/6/2013	7	918
2/12/2013	3	822
2/19/2011	2	719
2010	0	612
12/26/2009	0	634
3/3/2008	0	690
2/12/2008	9	1,241
12/17/2007	11	860
3/1/2007	6	1,008

Over the eleven years on the CSSC at Cicero Avenue, the number of four-day periods when the existing chronic standard of 620 mg/L was exceeded was 55 days, or an average of five days per year. The acute standard (990 mg/L) was exceeded twice over this period, or a recurrence interval of approximately every five years. Durations above the chronic standard longer than seven days occurred four times over the period of record, or a recurrence interval of once every 2.7 years. These results are similar to the Lemont Refinery's data presented above.

On the North Branch of the Chicago River, Kinzie Street is just above the confluence with the Main Stem Chicago River. This station has been monitored by the MWRD from 2007 to 2013. Using 640 mg/L for the chronic winter threshold from the original proposal, and 1,010 mg/L for the acute threshold, also from the original proposal, yields the following on the North Branch of the Chicago River.

North Branch at Kinzie Street

Event	# of days 4-day running average above 640 mg/L Chlorides, days	Maximum Chloride Concentration Recorded, mg/L
3/8/2013	1	876
3/1/2013	4	1,075
2012	0	644
2011	0	718
2010	0	699
2009	0	690
2/9/2008	11	1,160
12/13/2007	3	914
2/27/2007	8	1,092

Over the seven years, the number of days when the chronic winter level of 640 mg/L was exceeded was 27 days, or an average of four days per year. An acute value of 1,010 mg/L was exceeded in three events over this seven-year period, or a recurrence interval of approximately every 2.3 years. Durations above the chronic standard longer than seven days occurred twice over the period of record, or a recurrence interval of once every 3.5 years.

For the Cal-Sag Channel, the MWRD has robust records at Cicero Avenue, from 2007 to 2017 for conductivity. Like the North Branch, this station is downstream of one of the major water reclamation facilities.

Cal-Sag at Cicero

Event	# of days 4-day running average above 640 mg/L Chlorides, days	Maximum Chloride Concentration Recorded, mg/L
2017	0	341
2016	0	454
2015	0	646
2014	0	387
2011	0	551
2/25/2010	2	723
2009	0	568
2008	0	668
2007	0	622

Over the nine years, the number of days when the chronic winter level of 640 mg/L was exceeded was 2 days, or an average of once every 4.5 years. An acute value of 1,010 mg/L has not been exceeded on the Cal-Sag at Cicero over the nine-year period of record, with a maximum estimated chloride of 723 mg/L that occurred on February 25, 2010.

A summary of the MWRD data on the Chicago Area Waterways indicates that most elevated chloride periods last less than seven days, with the peak duration above some chronic level of 11 days.

4.3 DuPage River and Salt Creek Conductivity Data

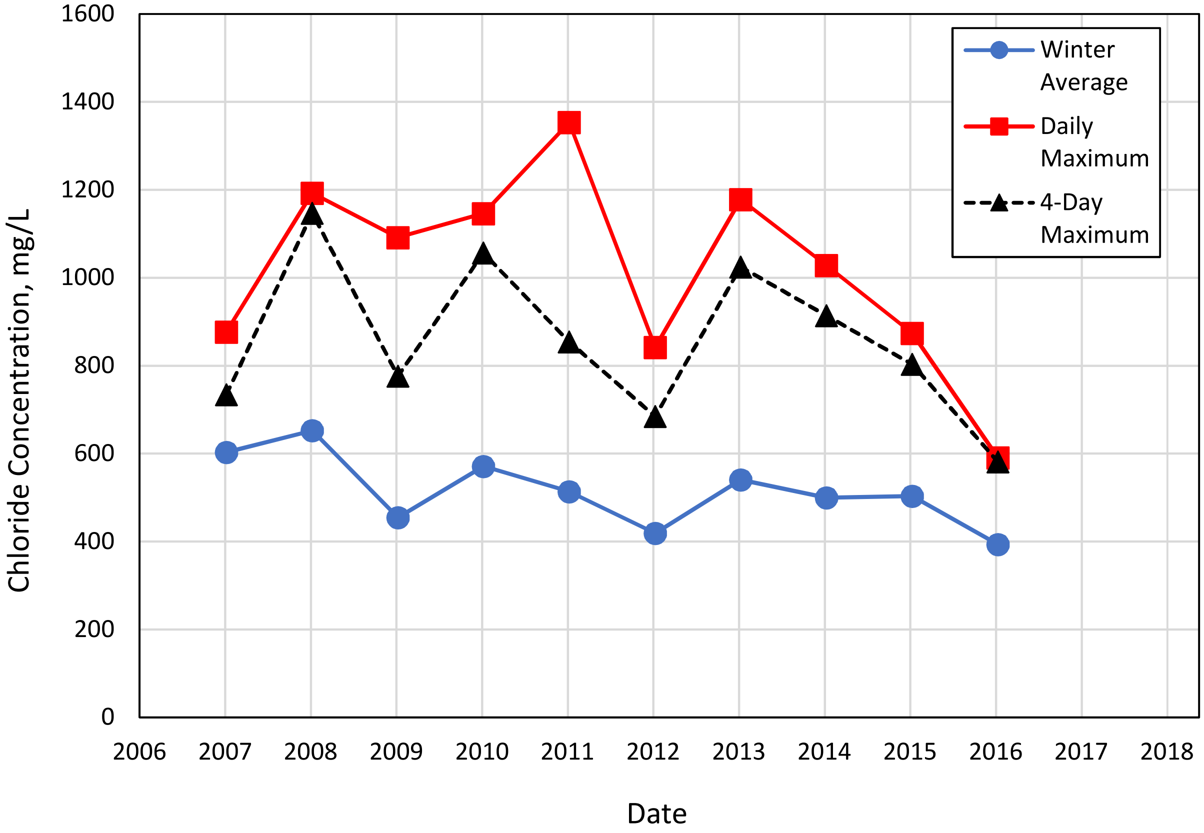
The DuPage River/Salt Creek Workgroup (DRSCW) has monitored conductivity and chlorides on its waterways since 2007, and their data provide an excellent picture of intermediate-sized streams in urban areas. Salt Creek at Wolf River reflects closer to the downstream end, and below the Fullersburg Woods Dam, so the chloride spikes are dampened to a minor degree at this location. This stream also receives considerable wastewater effluents from the municipalities along the waterway. The following table summarizes the winter days above 640 mg/L and the maximum estimated chlorides.

Salt Creek at Wolf Road

Event	Estimated Duration above 640 mg/L Chlorides, days	Maximum Chloride Concentration Recorded, mg/L
2016	0	591
3/25/2015	5	771
3/12/2015	13	861
2/9/2015	9	874
3/13/2014	10	1,028
1/11/2014	5+	931
12/21/2013	2	742
3/7/2013	14	1,178
2/13/2013	1	645
2/8/2013	5	811
2/27/2011	6	815
2/16/2011	4+	990
1/19/2011	1+	**1,353**
2/23/2010	21	1,146
2/11/2010	7	894
2/10/2009	2	670
3/1/2008	11	984
2/9/2008	26	1,193
2/12/2007	4	877

The durations at this station are longer than at the other stations tabulated, and this may be due to the dam immediately upstream, retarding the flushing of the chlorides. However, the dam would also dampen the peak chloride concentrations. Overall, there appears to be a declining trend in both peak chlorides and in the durations. However, some of this recent improvement may be attributed to the milder winters over the last two years. Figure 1 clearly depicts this trend, including in the winter average chloride concentrations.

**Figure 1: ANNUAL CHLORIDE CONCENTRATION - WINTER MONTHS (2007-2016)
SALT CREEK AT WOLF ROAD**



DRSCW also monitored the East Branch of the DuPage River at Hobson Road (HR) in Woodridge and Unincorporated DuPage County, Illinois from 2008 to 2015. The results are presented in the following table.

East Branch DuPage River at Hobson Road

Event	Estimated 4-Day Duration above 640 mg/L Chlorides, days	Maximum Chloride Concentration Recorded, mg/L
2/9/2015	4	819
3/22/2014	8	859
2/20/2014	2	987

2/20/2013	6	1,097
2012	0	788
1/19/2011	3	980
2/23/2010	8	1,172
2/11/2010	3	782
2009	0	513
2/5/2008	17	1,017

The peak recorded chloride was 1,172 mg/L at Hobson Road, and the longest duration above 640 mg/L was 17 consecutive days, back in 2008. Since 2008, eight consecutive days is the longest duration above 640 mg/L.

On the West Branch of the DuPage River, the DRSCW has monitored at Arlington Drive (AD) in Hanover Park, DuPage County, Illinois from 2007 to 2015, with the following chloride winter spikes.

West Branch DuPage River at Arlington Drive		
Event	Estimated 4-Day Duration above 640 mg/L Chlorides, days	Maximum Chloride Concentration Recorded, mg/L
2015	0	589
3/8/2014	1	696
2/19/2014	2	812
3/8/2013	3	758
2012	0	612
1/29/2011	3	731
2010	0	719
2009	0	750
2/9/2008	6	826
2007	0	599

The West Branch is the furthest west, and the least urbanized compared to the East Branch and Salt Creek. The peak chloride over the nine years was 826 mg/L, so no exceedances of the proposed acute standard of 1,010 mg/L. Five events exceeded 640 mg/L for a 4-day period, or less than one event annually.

5. DERIVATION OF WATER QUALITY STANDARDS

Under federal regulation, 40CFR131.5(c), one of the minimum requirements for water quality standards is that the criteria be sufficient to protect the designated uses. In 1985, the United States Environmental Protection Agency (USEPA) published *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (Stephan, et al., 1985). This document outlines the methodology for deriving water quality criteria, and notes for the chronic criteria, that a four-day averaging period is appropriate, and that a 20 to 30-day period is unacceptable. The four-day period was intended to "prevent increased adverse effects on sensitive life stages by limiting the durations and magnitudes of exceedances of the CCC." (CCC is the Criterion Continuous Concentration, or chronic criteria.) (pg. 5.)

Stephan, et al., goes on to note that, "most bodies of water could tolerate exceedances once every three years on the average. In situations in which exceedances are grouped, several exceedances might occur in one or two years, but then there will be, for example, 10 to 20 years during which no exceedances will occur." (pg 6). This is consistent with the data presented in the previous section, where there are years with no exceedances and other years when exceedances are more frequent. This USEPA document notes that, "Whenever adequately justified, a national criterion may be replaced by a site-specific criterion," (pg 6).

Stephan (2009) updated the chloride database and derived a Criterion Maximum Concentration (CMC) of 602.4 mg/L. Since that time, there have been additional toxicity tests. Adding to Stephan's (2009) database, Table 1 was prepared. The data in Table 1 are limited to studies where hardness and sulfate data are presented. In the case of Jackson and Funk (2019), the LC_{50} results for all temperature tests were *normalized* to 25°C using the slopes presented for each species in the paper, and a geometric mean was utilized for the normalized LC_{50}.

Table 1 presents the data utilized with the normalized acute values (NAV), the species mean acute values (SMAV), and genus mean acute values (GMAV), again starting with Stephan's (2009) list and adding to it the more recent results, including our results (INHS 2018 and NEB 2018).

Table 2 presents the USEPA protocol for deriving acute standards, by ranking the GMAVs, and utilizing the four most sensitive species to compute the normalized CMC. The CMC is computed to be **518.3 mg/L** at 25°C, 300 mg/L hardness, and 65 mg/L sulfate.

Table 3 presents the acute water quality standard at various temperatures, hardness, and sulfate levels, based upon the normalized CMC, the linear relationship with temperature normalized to 4.5 percent per degree decline from 25°C, and the accepted hardness and sulfate correlations. This is provided just as an example of the typical water quality standards.

Table 4 presents the derivation of the Acute:Chronic Ratio (ACR) for both invertebrates and vertebrates, and includes the results from Dr. Soucek's work, as well as the New England Bioassay work. A separate ACR is then applied to the vertebrates and invertebrates based on the data where both test results are reported. A Final ACR (FACR) of 3.45 is calculated as the geometric mean of the available Species Mean ACRs (SMACR) for invertebrates and an FACR of 7.33 is calculated for vertebrates as the geometric mean of the two available SMACRs. The chronic values are then derived following the USEPA *Guidelines* and Stephan's 2009 "Derivation of Alternative Chronic Value" method from "*Calculation of Aquatic Life Criteria for Chloride*" and placed in the second part of Table 4 in ascending order of sensitivity. The four most sensitive species are presented, and the Criterion Continuous Concentration (CCC) of **300.5 mg/L** is computed based on the normalized values of 25ºC, 300 mg/L hardness, and 65 mg/L sulfate. Three mayflies and the fingernail clam are the most sensitive species based on Table 4, with the mayfly data dominated by the Stroud 2015 study.

Table 5 presents examples of the chronic water quality standard based on varying temperature, hardness, and sulfate levels, again simply for illustrative purposes.

6. COMPARISON OF PROPOSED WINTER STANDARDS TO RESEARCH RESULTS

Four species were selected for chloride toxicity evaluation at winter temperature, and the results were presented in Section 5 of the original Technical Support Document.

As described in Section 5, extended exposure tests were conducted. The *C. dubia* test, where the organisms were exposed to elevated chlorides for 35 days, yielded a No Observable Effects Concentration (NOEC) of 782 mg/L chlorides at 10ºC, above the range of computed chronic water quality standards presented in Table 5. The laboratory testing of *C. dubia* demonstrates that this sensitive species would readily be protected if the proposed criterion was adopted.

For the Fingernail clam toxicity testing, run for 28 days of exposure to various chloride concentrations, no chronic effects were detected with concentrations of 1,000 mg/L chlorides at 10ºC. This result also demonstrates that a chronic water quality standard illustrated in Table 5 would be protective of this sensitive species.

The third sensitive species, the Mayfly *Neocloeon triangulifer,* showed no chronic effects found in chloride concentrations up to 750 mg/L. At the highest dose of exposure, 1,500 mg/L chlorides, no chronic effects were observed, but survival declined to 55 percent (compared to 0 percent survival at 25ºC). Again, the chronic standards illustrated in Table 5 would be protective of this sensitive species.

The final species evaluated, the amphipod *Hyalella azteca* (Burlington strain) did not survive well at 10ºC, with only about a 50% survival rate at 7 to 10 days, independent of the chloride concentration. However, based on the acute testing at the two temperatures, at 25ºC the 96-hour LC_{50} was 1,733 mg/L versus 2,185 mg/L at 10ºC, or a ratio of 1.26. Applying this same ratio to the 28-day chronic test at 25ºC result of 516 mg/L yields an estimated chronic value at 10ºC of 650 mg/L from 28 days of exposure to chlorides. Again, this sensitive species will be protected with a 4-day chronic water quality standard illustrated in Table 5.

In summary, using the acute-to-chronic ratio, as described in the previous section, results in a conservative chronic water quality criterion, which will be protective of the most sensitive species.

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TABLES

Table 1

SMAV and GMAV in mg/L Chloride for Updated and Complete Literature Values for Chloride Criteria (Aquatic Life)

SMAVs and GMAVs are normalized to hardness of 300 mg/L, sulfate of 65 mg/L, and temperature of 25 °C

								Normalized to 25 °C Hardness = 300 mg/L Sulfate = 65 mg/L		
Genus	**Species**	**Common Name**	**Temperature of Toxicity Testing, °C**	***Reference***	**Hardness, mg/L**	**Sulfate, mg/L**	**Acute Value (LC50)**	**Normalized Acute Value**	**SMAV**	**GMAV**
Anguilla	rostrata	American eel	22 [a/]	Hinton, Eversole 1978	42.7	40.7	11,880	17,343.4	17,343.4	17,343
Cambarus	sp.	Crayfish	25	Clemens, Jones 1954	22	15	10,557	16,203.2	16,203.2	16,203
Fundulus	kansae	Plains kiliefish	25	Clemens, Jones 1954	22	15	9,706	14,897.1	14,897.1	14,897
Libellulidae	spp.	Dragonfly	25	Clemens, Jones 1954	22	15	9,671	14,843.4	14,843.4	14,843
Gasterosteus	aculeatus	Threespine stickleback	25	Garibay, Hall 2004	84.8	81.4	10,200	13,452.6	13,452.6	13,453
Gambusia	affinis	Mosquitofish	25	Clemens, Jones 1954	22	15	6,472	9,933.4	9,933.4	9,933
Lepomis	cyanellus	Green sunfish	25	Clemens, Jones 1954	22	15	6,499	9,974.9	9,974.9	9,157
Lepomis	macrochirus	Bluegill	25	Birge et al. 1985; et al. 1954-1968	---	---	---	---	8,406.5	---
Lepomis	macrochirus	Bluegill	25	Birge et al. 1985	84.8	81.4	5,840	7,702.3	---	---
Lepomis	macrochirus	Bluegill	25	Acad of Nat Sci 1960; Patrick 1968; Trama 1954	44.3	15.5	7,853	10,461.6	---	---
Notropis	lutrensis	Red shiner	25	Clemens, Jones 1954	---	---	---	---	8971.12	8,971
Notropis	lutrensis	Red shiner	25	Clemens, Jones 1954	22	15	5,771	8,857.5	---	---
Notropis	lutrensis	Red shiner	25	Clemens, Jones 1954	22	15	5,920	9,086.2	---	---
Oncorhynchus	*mykiss*	*Rainbow trout*	*25*	*Spehar 1986-1986; Elphick et al. 2011*	*40*	*58.5*	*---*	*---*	*8,921.0*	*8,921*
Oncorhynchus	*mykiss*	*Rainbow trout*	*12* [a/]	*Spehar 1986-1987*	*46*	*3.9*	*6,743*	*8,786.8*	---	---
Oncorhynchus	***mykiss***	***Rainbow trout***	***25***	***Elphick et al. 2011***	***40***	***58.5***	***6,030***	***9,057.2***	---	---
Ameiurus	melas	Black bullhead	25	Clemens, Jones 1954	22	15	4,849	7,442.4	7,442.4	7,442
Pimephales	*promelas*	*Fathead minnow*	*25*	*USEPA 1991, Birge 1985, Elphick 2011*	---	---	---	---	*4,994.5*	*4,995*
Pimephales	*promelas*	*Fathead minnow*	*25*	*USEPA 1991*	*39.2*	*39.2*	*2,790*	*3,481.7*	---	---
Pimephales	*promelas*	*Fathead minnow*	*25*	*USEPA 1991*	*39.2*	*39.2*	*2,123*	*2,649.3*	---	---
Pimephales	*promelas*	*Fathead minnow*	*25*	*USEPA 1991*	*339.0*	*325.4*	*2,244*	*2,467.3*	---	---
Pimephales	*promelas*	*Fathead minnow*	*25*	*Birge et al. 1985*	*84.8*	*81.4*	*6,570*	*8,665.1*	---	---
Pimephales	*promelas*	*Fathead minnow*	*25*	*Clemens, Jones 1954*	*22*	*15*	*5,288*	*8,116.2*	---	---
Pimephales	*promelas*	*Fathead minnow*	*25*	*Clemens, Jones 1954*	*22*	*15*	*5,431*	*8,335.7*	---	---
Pimephales	*promelas*	*Fathead minnow*	*25*	*Mount et al. 1997*	*84.8*	*81.4*	*3,876*	*5,112.0*	---	---
Pimephales	*promelas*	*Fathead minnow*	*25*	*WISLOH 2007*	*84.8*	*81.4*	*4,167*	*5,495.8*	---	---
Pimephales	*promelas*	*Fathead minnow*	*25*	*WISLOH 2007*	*169.5*	*162.7*	*4,127*	*4,970.0*	---	---
Pimephales	***promelas***	***Fathead minnow***	***25***	***Elphick et al. 2011***	***90***	***58.5***	***4,079***	***5,185.0***	---	---
Tubifex	*tubifex*	*Tubificid worm*	*25*	*GLEC, INHS 2007; Elphick 2011*	---	---	---	---	*6,523.7*	*6,524*
Tubifex	*tubifex*	*Tubificid worm*	*25*	*GLEC, INHS 2008*	*52*	*57.9*	*4,278*	*6,083.2*	---	---
Tubifex	*tubifex*	*Tubificid worm*	*25*	*GLEC, INHS 2008*	*220*	*58.9*	*6,008*	*6,357.1*	---	---
Tubifex	***tubifex***	***Tubificid worm***	***25***	***Elphick et al. 2011***	***90***	***58.5***	***5,648***	***7,179.5***	---	---
Hyalella	*azteca*	*Amphipod*	*25*	*INHS and Lasier et al (1997)*	*96.36*	*69.57*	---	---	*2,684.7*	*2,685*
Hyalella	*azteca*	*Amphipod*	*25*	*Lasier et al. 1997*	*102.5*	*98.4*	*3,947*	*5,077.7*	---	---
Hyalella	***azteca***	***Amphipod***	***25***	***Elphick et al. 2011***	***90***	***58.5***	***1,382***	***1,756.7***	---	---
Hyalella	***azteca***	***Amphipod***	***25***	***IHNS 2017***	***97***	***58.5***	***1,733***	***2,169.2***	---	---

Table 1

SMAV and GMAV in mg/L Chloride for Updated and Complete Literature Values for Chloride Criteria (Aquatic Life)

SMAVs and GMAVs are normalized to hardness of 300 mg/L, sulfate of 65 mg/L, and temperature of 25 °C

								Normalized to 25 °C Hardness = 300 mg/L Sulfate = 65 mg/L		
Genus	**Species**	**Common Name**	**Temperature of Toxicity Testing, °C**	***Reference***	**Hardness, mg/L**	**Sulfate, mg/L**	**Acute Value (LC50)**	**Normalized Acute Value**	**SMAV**	**GMAV**
Pseudacris	sp.	Chorus frog	25	Garibay, Hall 2004	84.8	81.4	3,553	4,686.0	4,686.0	4,686
Diaptomus	clavipes	Copepod	25	Clemens, Jones 1954	22	15	2,571	3,946.1	3,946.1	3,946
Lirceus	fontinalis	Isopod	25	Birge et al. 1985	84.8	81.4	2,950	3,890.7	3,890.7	3,891
Gyraulus	parvus	Snail	25	GLEC, INHS 2008	---	---	---	---	3,727.7	3,728
Gyraulus	parvus	Snail	25	GLEC, INHS 2008	56	60.9	3,078	4,326.9	---	---
Gyraulus	parvus	Snail	25	GLEC, INHS 2008	212	59.7	3,009	3,211.4	---	---
Physa	gyrina	Snail	25	Birge et al. 1985	84.8	81.4	2,540	3,350.0	3,350.0	3,350
Villosa	delumbis	Mussel	25	Bringolf et al. 2007	169.5	162.7	3,173	3,821.1	3,821.1	3,086
Villosa	iris	Mussel	25	Wang 2007	169.5	162.7	2,069	2,491.6	2,491.6	…
Lampsilis	*fasciola*	*Mussel*	*25*	*Bringolf et al. 2007; Gillis 2011*	---	---	---	---	*874.3*	*1,419*
Lampsilis	*fasciola*	*Mussel*	*25*	*Bringolf et al. 2007*	*169.5*	*162.7*	*2,414*	*2,907.1*	---	---
Lampsilis	***fasciola***	***Mussel***	***21***	***Gillis 2011***	***95***	***58.5***	***113***	***383.4***	---	---
Lampsilis	***fasciola***	***Mussel***	***21***	***Gillis 2011***	***95***	***58.5***	***285***	***599.6***	---	---
Lampsilis	*siliquoidea*	*Mussel*	*25*	*Wang et al. 2018; Bringolf et al. 2007*	---	---	---	---	*2,302.3*	---
Lampsilis	*siliquoidea*	*Mussel*	*25*	*Bringolf et al. 2007*	*169.5*	*162.7*	*1,905*	*2,294.1*	---	---
Lampsilis	*siliquoidea*	*Mussel*	*25*	*Wang 2007*	*169.5*	*162.7*	*2,766*	*3,331.0*	---	---
Lampsilis	***siliquoidea***	***Mussel***	***25***	***Wang et al. 2018***	***299***	***67***	***1,597***	***1,597.0***	---	---
Daphnia	ambigua	Cladoceran	25	Harmon et al. 2003	67.1	64.4	1,213	1,649.7	1,649.7	2,327
Daphnia	*magna*	*Cladoceran*	*25*	*USEPA, Wisloh, Davies, Seymour, Elphick et al.*	---	---	---	---	*3,779.7*	---
Daphnia	*magna*	*Cladoceran*	*25*	*Hoke et al. 1992*	*39.2*	*4.6*	*3,038*	*3,791.1*	---	---
Daphnia	*magna*	*Cladoceran*	*25*	*Hoke et al. 1992*	*39.2*	*4.6*	*2,726*	*3,401.8*	---	---
Daphnia	*magna*	*Cladoceran*	*25*	*Hoke et al. 1992*	*39.2*	*4.6*	*2,053*	*2,561.9*	---	---
Daphnia	*magna*	*Cladoceran*	*25*	*Dowden & Bennett 1965*	*41.5*	*31.2*	*3,563*	*5,068.2*	---	---
Daphnia	*magna*	*Cladoceran*	*25*	*Seymour et al. 1997*	*169.5*	*162.7*	---	*3,906.7*	---	---
Daphnia	*magna*	*Cladoceran*	*25*	*USEPA 1991*	*46*	*3.9*	*1,880*	*2,242.3*	---	---
Daphnia	*magna*	*Cladoceran*	*25*	*WISLOH 2007*	*169.5*	*162.7*	*3,944*	*4,749.6*	---	---
Daphnia	*magna*	*Cladoceran*	*25*	*Valenti et al. 2007*	*84.8*	*81.4*	*3,009*	*3,968.5*	---	---
Daphnia	*magna*	*Cladoceran*	*25*	*Davies & Hall 2007*	*106*	*102*	*3,136*	*4,017.4*	---	---
Daphnia	*magna*	*Cladoceran*	*25*	*Davies & Hall 2007*	*106*	*102*	*3,222*	*4,127.5*	---	---
Daphnia	*magna*	*Cladoceran*	*25*	*Davies & Hall 2007*	*106*	*102*	*3,137*	*4,018.6*	---	---
Daphnia	***magna***	***Cladoceran***	***25***	***Elphick et al. 2011***	***90***	***58.5***	***3,630***	***4,614.3***	---	---
Daphnia	pulex	Cladoceran	25	Birge et al. 1985, Palmer et al. 2004	---	---	---	---	2,020.5	---
Daphnia	pulex	Cladoceran	25	Birge et al. 1985	84.8	81.4	1,470	1,938.8	---	---
Daphnia	pulex	Cladoceran	25	Palmer et al. 2004	84.8	81.4	1,159	1,528.6	---	---
Daphnia	pulex	Cladoceran	25	Palmer et al. 2004	84.8	81.4	1,775	2,341.0	---	---
Daphnia	pulex	Cladoceran	25	Palmer et al. 2004	84.8	81.4	1,805	2,380.6	---	---
Daphnia	pulex	Cladoceran	25	Palmer et al. 2004	84.8	81.4	2,242	2,956.9	---	---

Table 1

SMAV and GMAV in mg/L Chloride for Updated and Complete Literature Values for Chloride Criteria (Aquatic Life)

SMAVs and GMAVs are normalized to hardness of 300 mg/L, sulfate of 65 mg/L, and temperature of 25 °C

								Normalized to 25 °C Hardness = 300 mg/L Sulfate = 65 mg/L		
Genus	**Species**	**Common Name**	**Temperature of Toxicity Testing, °C**	***Reference***	**Hardness, mg/L**	**Sulfate, mg/L**	**Acute Value (LC50)**	**Normalized Acute Value**	**SMAV**	**GMAV**
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC, INHS 2008; NEB 2018; Elphick 2011*	*---*	*---*	*---*	*---*	*1,567.9*	*1,568*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*Mount et al. 1997*	*84.8*	*81.4*	*1,189*	*1,568.2*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*Mount et al. 1997*	*84.8*	*81.4*	*1,042*	*1,374.3*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*USEPA 1991*	*39.2*	*4.6*	*1,395*	*1,740.8*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*USEPA 1991*	*39.2*	*4.6*	*1,638*	*2,044.1*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*USEPA 1991*	*39.2*	*4.6*	*1,274*	*1,589.8*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*USEPA 1991*	*39.2*	*4.6*	*1,395*	*1,740.8*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*USEPA 1991*	*339*	*325.4*	*1,698*	*1,867.0*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*WISLOH 2007*	*84.8*	*81.4*	*1,677*	*2,211.8*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*WISLOH 2007*	*169.5*	*162.7*	*1,499*	*1,805.2*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*Valenti et al. 2007*	*84.8*	*81.4*	*1,413*	*1,863.6*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*Harmon et al. 2003*	*67.1*	*64.4*	*964*	*1,311.1*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*30*	*78.7*	*947*	*1,542.9*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*44*	*75.9*	*955*	*1,434.1*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*96*	*73.7*	*1,130*	*1,442.1*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*180*	*67.7*	*1,609*	*1,792.8*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*400*	*78.7*	*1,491*	*1,425.5*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*570*	*76.2*	*1,907*	*1,690.9*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*800*	*75.5*	*1,764*	*1,457.7*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*25*	*69.9*	*1,007*	*1,688.4*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*49*	*67.8*	*767*	*1,117.1*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*95*	*70.3*	*1,369*	*1,744.7*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*194*	*69.9*	*1,195*	*1,314.3*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*375*	*68.9*	*1,687*	*1,618.3*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*560*	*68.3*	*1,652*	*1,458.2*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*792*	*70.9*	*1,909*	*1,573.5*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*280*	*28.1*	*1,400*	*1,334.0*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*280*	*59.6*	*1,720*	*1,733.4*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*280*	*117*	*1,394*	*1,477.2*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*280*	*239*	*1,500*	*1,676.5*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*280*	*482*	*1,109*	*1,306.0*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*280*	*729*	*1,206*	*1,464.7*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*279*	*22.9*	*1,311*	*1,231.2*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*276*	*49.7*	*1,258*	*1,254.4*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*283*	*107*	*1,240*	*1,302.5*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*281*	*229*	*1,214*	*1,351.5*	*---*	*---*
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*290*	*461*	*1,199*	*1,397.2*	*---*	*---*

Table 1

SMAV and GMAV in mg/L Chloride for Updated and Complete Literature Values for Chloride Criteria (Aquatic Life)

SMAVs and GMAVs are normalized to hardness of 300 mg/L, sulfate of 65 mg/L, and temperature of 25 °C

								Normalized to 25 °C Hardness = 300 mg/L Sulfate = 65 mg/L		
Genus	**Species**	**Common Name**	**Temperature of Toxicity Testing, °C**	***Reference***	**Hardness, mg/L**	**Sulfate, mg/L**	**Acute Value (LC50)**	**Normalized Acute Value**	**SMAV**	**GMAV**
Ceriodaphnia	*dubia*	*Cladoceran*	*25*	*GLEC & INHS 2008*	*278*	*694*	*1,179*	*1,428.8*	---	---
Ceriodaphnia	***dubia***	***Cladoceran***	***25***	***Elphick et al. 2011***	***90***	***58.5***	***1,068***	***1,357.6***	---	---
Ceriodaphnia	***dubia***	***Cladoceran***	***25***	***Mount et al. 2016***	***84.4***	***81***	***2,004***	***2,644.7***	---	---
Ceriodaphnia	***dubia***	***Cladoceran***	***25***	***NEB 2018***	***90***	***58.5***	***1,920***	***2,440.6***	---	---
Sphaerium	*simile*	*Fingernail clam*	*25*	*GLEC, INHS 2009*	---	---	---	---	*1,386.3*	*1,386*
Sphaerium	*simile*	*Fingernail clam*	*25*	*GLEC, INHS 2009*	*51*	*59.9*	*740*	*1,059.2*	---	---
Sphaerium	*simile*	*Fingernail clam*	*25*	*GLEC, INHS 2009*	*192*	*61.7*	*1,100*	*1,201.1*	---	---
Sphaerium	***simile***	***Fingernail clam***	***25***	***INHS 2017***	***97***	***58.5***	***1,673***	***2,094.1***	---	---
Procloeon	**fragile**	**Mayfly**	**25**	**Jackson and Funk 2018**	**97**	**17.3**	**763**	**872.2**	**872.2**	**872**
Leptophlebia	**cupida**	**Mayfly**	**25**	***Jackson and Funk 2018***	**97**	**17.3**	**1,949**	**2,227.8**	**2,227.8**	**2,228**
Maccaffertium	**modesum**	**Mayfly**	**25**	***Jackson and Funk 2018***	**97**	**17.3**	**1,004**	**1,147.6**	**1,147.6**	**1,148**
Cyprinella	**leedsi**	**Bannerfin shiner**	**25**	***Environ 2009; CCME 2011***	**296**	**---**	**6,070**	**6,111.0**	**6,111.0**	**6,111**
Rana	**catesbeiana**	**Bullfrong (tadpole)**	**25**	***Environ 2009; CCME 2011***	**300**	**---**	**5,846**	**5,897.0**	**5,897.0**	**5,897**
Nephelopsis	**obscura**	**Leech**	**25**	***Environ 2009; CCME 2011***	**290**	**---**	**4,310**	**4,369.0**	**4,369.0**	**4,369**
Lumbriculus	**variegatus**	**Blackworm**	**25**	***Elphick et al. 2011***	**90**	**58.5**	**3,100**	**3,940.6**	**3,940.6**	**3,941**
Chironomus	**dilutus**	**Midge**	**25**	***Elphick et al. 2011***	**90**	**58.5**	**5,867**	**7,457.8**	**7,457.8**	**7,458**
Brachionus	**calyciflorus**	**Planktonic rotifer**	**25**	***Elphick et al. 2011***	**90**	**58.5**	**1,645**	**2,091.0**	**2,091.0**	**2,091**
Neocloeon	***triangulifer***	***Mayfly***	***25***	***INHS (2015 & 2017); Jackson/Funk 2018***					***1,293.7***	***1,294***
Neocloeon	***triangulifer***	***Mayfly***	**25**	***INHS 2017***	**97**	**58.5**	**1,359**	**1,701.1**		
Neocloeon	***triangulifer***	***Mayfly***	**25**	***Soucek, Mount, Dickinson, Hockett 2017***	**90**	**58.5**	**837**	**1,064.0**		
Neocloeon	***triangulifer***	***Mayfly***	**25**	***Soucek, Mount, Dickinson, Hockett 2017***	**205**	**58.5**	**1,116**	**1,197.5**		
Neocloeon	***triangulifer***	***Mayfly***	**25**	***INHS 2015***	**93**	**58.5**	**910**	**1,149.0**		
Neocloeon	***triangulifer***	***Mayfly***	**25**	***INHS 2015***	**93**	**58.5**	**1,140**	**1,439.4**		
Neocloeon	***triangulifer***	***Mayfly***	**25**	***INHS 2015***	**93**	**58.5**	**1,153**	**1,455.8**		
Neocloeon	***triangulifer***	***Mayfly***	**25**	***Jackson and Funk 2018***	**97**	**17.3**	**1,017**	**1,162.5**		

[a/] SMAV was recalculated for the temperature adjustment to 25 °C, using the following equation: $[AV + (25 - T)*48]*[(300/Hardness)^{0.205797}]*[(65/Sulfate)^{-0.07452}]$.

*New SMAVs were calculated as the geometric mean of the Normalized Acute Values (NAV) for the *italicized* genus/species using NAVs taken from "*Summary of Data Concerning the Acute Toxicity of Sodium Chloride To Aquatic Animals* " (Stephan, 2009).

Bold cells signify data added from additional studies to accompany Stephan 2009 data.

Table 2
Ranked GMAV in mg Cl/L
Calculation of Final Acute Value (FAV) and Criterion Maximum Concentration (CMC)
SMAVs and GMAVs are normalized to hardness of 300 mg/L, sulfate of 65 mg/L, and temperature of 25 ^{o}C

Rank	GMAV	Genus	Species	Common Name	SMAV	Cumulative Probability, P
33	17,343	Anguilla	rostrata	American eel	17,343.43	0.971
32	16,203	Cambarus	sp.	Crayfish	16,203.20	0.941
31	14,897	Fundulus	kansae	Plains kiliefish	14,897.10	0.912
30	14,843	Libellulidae	spp.	Dragonfly	14,843.40	0.882
29	13,453	Gasterosteus	aculeatus	Threespine stickleback	13,452.60	0.853
28	9,933	Gambusia	affinis	Mosquitofish	9,933.40	0.824
27	9,157	Lepomis	cyanellus	Green sunfish	9,974.90	0.794
---	---	Lepomis	macrochirus	Bluegill	8,406.50	---
26	8,971	Notropis	lutrensis	Red shiner	8,971.12	0.765
25	8,921	Oncorhynchus	mykiss	Rainbow trout	8,920.97	0.735
24	7,458	Chironomus	dilutus	Midge	7,457.84	0.706
23	7,442	Ameiurus	melas	Black bullhead	7,442.40	0.676
22	6,524	Tubifex	tubifex	Tubificid worm	6,523.70	0.647
21	6,111	Cyprinella	leedsi	Bannerfin shiner	6,111.00	0.618
20	5,897	Rana	catesbeiana	Bullfrong (tadpole)	5,897.00	0.588
19	4,995	Pimephales	promelas	Fathead minnow	4,994.52	0.559
18	4,686	Pseudacris	sp.	Chorus frog	4,686.00	0.529
17	4,369	Nephelopsis	obscura	Leech	4,369.00	0.500
16	3,946	Diaptomus	clavipes	Copepod	3,946.10	0.471
15	3,941	Lumbriculus	variegatus	Blackworm	3,940.57	0.441
14	3,891	Lirceus	fontinalis	Isopod	3,890.70	0.412
13	3,728	Gyraulus	parvus	Snail	3,727.65	0.382
12	3,350	Physa	gyrina	Snail	3,350.00	0.353
11	3,086	Villosa	delumbis	Mussel	3,821.10	0.324
---	---	Villosa	iris	Mussel	2,491.60	---
10	2,685	Hyalella	azteca	Amphipod	2,684.67	0.294
9	2,327	Daphnia	ambigua	Cladoceran	1,649.70	0.265
---	---	Daphnia	magna	Cladoceran	3,779.70	---
---	---	Daphnia	pulex	Cladoceran	2,020.50	---
8	2,228	Leptophlebia	cupida	Mayfly	2,227.85	0.235
7	2,091	Brachionus	calyciflorus	Planktonic rotifer	2,091.04	0.206
6	1,568	Ceriodaphnia	dubia	Cladoceran	1,567.92	0.176
5	1,419	Lampsilis	fasciola	Mussel	874.32	0.147
---	---	Lampsilis	siliquoidea	Mussel	2,302.31	---
4	1,386	Sphaerium	simile	Fingernail clam	1,386.28	0.118
3	1,294	Neocloeon	triangulifer	Mayfly	1,293.73	0.088
2	1,148	Maccaffertium	modesum	Mayfly	1,147.64	0.059
1	872	Procloeon	fragile	Mayfly	872.16	0.029

Table 2
Ranked GMAV in mg Cl/L
Calculation of Final Acute Value (FAV) and Criterion Maximum Concentration (CMC)
SMAVs and GMAVs are normalized to hardness of 300 mg/L, sulfate of 65 mg/L, and temperature of 25 °C

Rank	GMAV	Type, *Genus species* (Common Name)	Cumulative Probability, P	$Ln(GMAV)^2$	Ln(GMAV)	$P^{1/2}$
4	1,386	*Sphaerium simile* (Fingernail clam)	0.118	52.34	7.234	0.343
3	1,294	*Neocloeon triangulifer* (Mayfly)	0.088	51.34	7.165	0.297
2	1,148	*Maccaffertium modesum* (Mayfly)	0.059	49.64	7.045	0.243
1	872	*Procloeon fragile* (Mayfly)	0.029	45.85	6.771	0.171

ΣP	$\Sigma (\ln(GMAV)^2)$	$\Sigma \ln(GMAV)$	$\Sigma P^{1/2}$
0.294	**199.16**	**28.216**	**1.054**

$(\Sigma \ln(GMAV))^2/4$	$(\Sigma P^{1/2})^2/4$
199.037	**0.278**

$S^2 = [\Sigma (\ln(GMAV)^2) - (\Sigma \ln(GMAV))^2/4] / [\Sigma P - (\Sigma P^{1/2})^2/4]$

S^2 = [199.162 - 199.037] / [0.294 - 0.278]

S^2 = 7.652

S = 2.766

$L = [\Sigma \ln(GMAV) - S*(\Sigma P^{1/2})] / 4$

L = [28.216 - 2.766*1.054] / 4

L = 6.325

$A = S*(0.05)^{1/2} + L$

A = 2.766*0.05^(1/2) + 6.325

A = 6.944

$FAV = e^A = \exp(A)$

FAV = exp(6.944)

FAV = 1,037

Criterion Max Concentration (CMC) = FAV/2= 518.3 mg/L

Table 3
Calculation of Acute Water Quality Standard for Chloride at Varying Water Temperature, Hardness, and Sulfate

Equation: Acute Water Quality Standard (WQS) for Chloride:

$$\text{Acute WQS} = [\text{CMC} + 1.045*\text{CMC}*(25 - \text{T})]*(\text{Hardness}/300)^{0.205797} * (\text{Sulfate}/65)^{-0.07452}$$

From Table 2, the Criterion Max Concentration (CMC) = 518.3 mg/L
which simplifies to:

$$\textbf{Acute WQS} = [1+(0.045)(25-\text{T})]*[518.3*(\text{Hardness}/300)^{0.205797} * (\text{Sulfate}/65)^{-0.07452}]$$

where Acute WQS is in mg Cl/L, T is in ^{o}C, Hardness is in mg/L as $CaCO_3$, and Sulfate is in mg SO_4^{2-}/L

Temperature ^{o}C	Hardness mg/L	Sulfate mg/L	Acute WQS mg/L
25	300	65	518
25	287	65	514
25	250	65	499
25	200	65	477
25	150	65	449
20	300	65	635
20	287	65	629
20	250	65	612
20	200	65	584
20	150	65	551
15	300	65	752
15	287	65	745
15	250	65	724
15	200	65	691
15	150	65	652
10	300	65	868
10	287	65	860
10	250	65	836
10	200	65	799
10	150	65	753
5	300	65	985
5	287	65	976
5	250	65	949
5	200	65	906
5	150	65	854

Table 3 (continued)
Calculation of Acute Water Quality Standard for Chloride at Varying Water Temperature, Hardness, and Sulfate

Equation: Acute Water Quality Standard (WQS) for Chloride:

$$\text{Acute WQS} = [\text{CMC} + 1.045*\text{CMC}*(25 - T)]*(\text{Hardness}/300)^{0.205797} * (\text{Sulfate}/65)^{-0.07452}$$

From Table 2, the Criterion Max Concentration (CMC) = 518.3 mg/L

which simplifies to:

$$\textbf{Acute WQS} = [1+(0.045)(25-T)]*[518.3*(\text{Hardness}/300)^{0.205797} * (\text{Sulfate}/65)^{-0.07452}]$$

where Acute WQS is in mg Cl/L, T is in °C, Hardness is in mg/L as $CaCO_3$, and Sulfate is in mg SO_4^{2-}/L

Temperature °C	Hardness mg/L	Sulfate mg/L	Acute WQS mg/L
25	300	86.8	507
25	287	86.8	503
25	250	86.8	489
25	200	86.8	467
25	150	86.8	440
20	300	86.8	621
20	287	86.8	616
20	250	86.8	598
20	200	86.8	572
20	150	86.8	539
15	300	86.8	736
15	287	86.8	729
15	250	86.8	708
15	200	86.8	677
15	150	86.8	638
10	300	86.8	850
10	287	86.8	842
10	250	86.8	818
10	200	86.8	782
10	150	86.8	737
5	300	86.8	964
5	287	86.8	955
5	250	86.8	928
5	200	86.8	887
5	150	86.8	836

Table 4
Ranked Predicted Genus Mean Chronic Values (pGMCV) in mg Cl/L
Calculation of Final Acute Chronic Ratio (FACR) and Final Chronic Value (FCV), or Criterion Continuous Concentration (CCC)

Using Stephan 2009 "Derivation of Alternative Chronic Value" method from "*Calculation of Aquatic Life Criteria for Chloride* ", Soucek/Dickinson (INHS, 2015), and the sources listed, the available ACRs are:

Genus	Species	Common Name	Type	SMACR	Source
Oncorhynchus	mykiss	Rainbow trout	Vertebrate	6.99	Stephan 2007 & 2009; Elphick 2011
Pimephales	promelas	Fathead minnow	Vertebrate	7.69	Birge et al 1985; Elphick 2011
Daphnia	ambigua	Cladoceran	Invertebrate	4.73	Stephan 2009; Harmon 2003
Daphnia	magna	Cladoceran	Invertebrate	4.12	Stephan 2009; Elphick 2011
Daphnia	pulex	Cladoceran	Invertebrate	3.95	Stephan 2009
Ceriodaphnia	dubia	Cladoceran	Invertebrate	2.40	Stephan 2007; NEB 2018; Elphick 2011
Neocloeon	triangulifer	Mayfly	Invertebrate	4.79	Soucek/Dickinson 2015 & Stroud 2015
Anafroptilum	semirufum	Mayfly	Invertebrate	8.30	Stroud 2015
Procloeon	fragile	Mayfly	Invertebrate	6.10	Stroud 2015
Lumbriculus	variegatus	Blackworm	Invertebrate	3.76	Elphick et al. 2011
Tubifex	tubifex	Tubificid worm	Invertebrate	9.31	Elphick et al. 2011
Chironomus	dilutus	Midge	Invertebrate	2.27	Elphick et al. 2011
Hyalella	azteca	amphipod	Invertebrate	2.07	Soucek 2018; Elphick 2011
Brachionus	calyciflorus	Planktonic rotifer	Invertebrate	1.09	Elphick et al. 2011
Sphaerium	simile	fingernail clam	Invertebrate	1.22	Soucek 2018

	FACR
Vertebrate	**7.33**
Invertebrate	**3.45**

NOTES:

1 The SMACR for N. triangulifer is the geometric mean from (Soucek, Dickinson / INHS; 2015) and (Stroud 2015), which represents four ACRs between the lower and upper chronic limits for the chloride testing from INHS and one ACR from Stroud (8.4).

2 The SMACR for Oncorhynchus mykiss is the geometric mean from (Stephan 2007 and 2009) and (Elphick 2011), which represents three ACRs for the species (7.308, 9.1, and 5.14).

3 The SMACR for Pimephales promelas is the geometric mean from (Birge et al 1985) and (Elphick 2011), which represents two ACRs for the species (10.2 and 5.80).

4 The SMACR for Daphnia amigua is the geometric mean from (Stephan2009) and (Harmon et al 2003), which represents two ACRs for the species (4.148 and 5.4).

5 The SMACR for Daphnia magna is the geometric mean from (Stephan2009) and (Elphick 2011), which represents two ACRs for the species (1.97 and 8.62).

6 The SMACR for Hyalella azteca is the geometric mean from (Soucek 2018) and (Elphick 2011), which represents two ACRs for the species (3.65 and 1.17).

7 The FACR for invertebrates is the geometric mean of the SMACRs for invertebrates. Per the *"Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* ", the SMACRs for invertebrates do not vary more than a factor of ten and therefore the FACR calculated as the geometric mean of the SMACRs is appropriate.

8 The calculated FACRs for vertebrates and invertebrates are used to calculate the pGMCVs in the below table, as: pGMCV = GMAV/FACR.

Table 4
Ranked Predicted Genus Mean Chronic Values (pGMCV) in mg Cl/L
Calculation of Final Acute Chronic Ratio (FACR) and Final Chronic Value (FCV), or Criterion Continuous Concentration (CCC)

Rank	pGMCV	GMAV	Genus	Species	Common Name	Type	Cumulative Probability, P
33	5,028.06	17,343	Anguilla	rostrata	American eel	invertebrate	0.971
32	4,697.43	16,203	Cambarus	sp.	Crayfish	invertebrate	0.941
31	4,303.15	14,843	Libellulidae	spp.	Dragonfly	invertebrate	0.912
30	2,162.11	7,458	Chironomus	dilutus	Midge	invertebrate	0.882
29	2,031.38	14,897	Fundulus	kansae	Plains kiliefish	vertebrate	0.853
28	1,891.30	6,524	Tubifex	tubifex	Tubificid worm	invertebrate	0.824
27	1,834.47	13,453	Gasterosteus	aculeatus	Threespine stickleback	vertebrate	0.794
26	1,354.48	9,933	Gambusia	affinis	Mosquitofish	vertebrate	0.765
25	1,266.62	4,369	Nephelopsis	obscura	Leech	invertebrate	0.735
24	1,248.69	9,157	Lepomis	cyanellus	Green sunfish	vertebrate	0.706
---	---	---	Lepomis	macrochirus	Bluegill	vertebrate	---
23	1,223.32	8,971	Notropis	lutrensis	Red shiner	vertebrate	0.676
22	1,216.48	8,921	Oncorhynchus	mykiss	Rainbow trout	vertebrate	0.647
21	1,143.99	3,946	Diaptomus	clavipes	Copepod	invertebrate	0.618
20	1,142.42	3,941	Lumbriculus	variegatus	Blackworm	invertebrate	0.588
19	1,128.04	3,891	Lirceus	fontinalis	Isopod	invertebrate	0.559
18	1,080.69	3,728	Gyraulus	parvus	Snail	invertebrate	0.529
17	1,014.80	7,442	Ameiurus	melas	Black bullhead	vertebrate	0.500
16	971.20	3,350	Physa	gyrina	Snail	invertebrate	0.471
15	894.54	3,086	Villosa	delumbis	Mussel	invertebrate	0.441
---	---	---	Villosa	iris	Mussel	invertebrate	---
14	833.31	6,111	Cyprinella	leedsi	Bannerfin shiner	vertebrate	0.412
13	804.13	5,897	Rana	catesbeiana	Bullfrog (tadpole)	vertebrate	0.382
12	778.32	2,685	Hyalella	azteca	Amphipod	invertebrate	0.353
11	681.06	4,995	Pimephales	promelas	Fathead minnow	vertebrate	0.324
10	674.59	2,327	Daphnia	ambigua	Cladoceran	invertebrate	0.294
---	---	---	Daphnia	magna	Cladoceran	invertebrate	---
---	---	---	Daphnia	pulex	Cladoceran	invertebrate	---
9	645.88	2,228	Leptophlebia	cupida	Mayfly	invertebrate	0.265
8	638.99	4,686	Pseudacris	sp.	Chorus frog	vertebrate	0.235
7	606.22	2,091	Brachionus	calyciflorus	Planktonic rotifer	invertebrate	0.206
6	454.56	1,568	Ceriodaphnia	dubia	Cladoceran	invertebrate	0.176
5	411.32	1,419	Lampsilis	fasciola	Mussel	invertebrate	0.147
---	---	---	Lampsilis	siliquoidea	Mussel	invertebrate	---
4	401.90	1,386	Sphaerium	simile	Fingernail clam	invertebrate	0.118
3	375.07	1,294	Neocloeon	triangulifer	Mayfly	invertebrate	0.088
2	332.71	1,148	Maccaffertium	modesum	Mayfly	invertebrate	0.059
1	252.85	872	Procloeon	fragile	Mayfly	invertebrate	0.029

Table 4

Ranked Predicted Genus Mean Chronic Values (pGMCV) in mg Cl/L

Calculation of Final Acute Chronic Ratio (FACR) and Final Chronic Value (FCV), or Criterion Continuous Concentration (CCC)

Rank	GMCV	Type, *Genus species* (Common Name)	Cumulative Probability, P	$Ln(GMCV)^2$	Ln(GMCV)	$P^{1/2}$
4	402	*Sphaerium simile* (Fingernail clam)	0.118	35.95	5.996	0.343
3	375	*Neocloeon triangulifer* (Mayfly)	0.088	35.13	5.927	0.297
2	333	*Maccaffertium modesum* (Mayfly)	0.059	33.72	5.807	0.243
1	253	*Procloeon fragile* (Mayfly)	0.029	30.61	5.533	0.171
			Σ P	**$\Sigma (\ln(GMCV)^2)$**	**Σ ln(GMCV)**	**$\Sigma P^{1/2}$**
			0.294	**135.42**	**23.263**	**1.054**
					$(\Sigma \ln(GMCV))^2/4$	**$(\Sigma P^{1/2})^2/4$**
					135.296	**0.278**

$S^2 = [\Sigma (\ln(GMCV)^2) - (\Sigma \ln(GMCV))^2/4] / [\Sigma P - (\Sigma P^{1/2})^2/4]$

$S^2 =$ [135.421 - 135.296] / [0.294 - 0.278]

$S^2 =$ 7.652

S = 2.766

$L = [\Sigma \ln(GMCV) - S^*(\Sigma P^{1/2})] / 4$

L = [23.263 - 2.766*1.054] / 4

L = 5.087

$A = S^*(0.05)^{1/2} + L$

A = 2.766*0.05^(1/2) + 5.087

A = 5.705

$FCV = e^A = \exp(A)$

FCV = exp(5.705)

FCV = 300

Criterion Continuous Concentration (CCC) = FCV = 300.5 mg/L

Table 5
Calculation of Chronic Water Quality Standard for Chloride at Varying Water Temperature, Hardness, and Sulfate

Equation: Chronic Water Quality Standard (WQS) for Chloride:

$$\text{Chronic WQS} =[1+(0.045)(25-T)]*[CCC*(\text{Hardness}/300)^{0.205797} * (\text{Sulfate}/65)^{-0.07452}]$$

From Table 4, the Criterion Continuous Concentration (CCC) = 300.5 mg/L

which simplifies to:

$$\textbf{Chronic WQS} = [1+(0.045)(25-T)]*[300.5*(\text{Hardness}/300)^{0.205797} * (\text{Sulfate}/65)^{-0.07452}]$$

where Chronic WQS is in mg Cl/L, T is in ^{o}C, Hardness is in mg/L as $CaCO_3$, and Sulfate is in mg SO_4^{2-}/L

Temperature ^{o}C	Hardness mg/L	Sulfate mg/L	Chronic WQS mg/L
25	300	65	301
25	287	65	298
25	250	65	289
25	200	65	276
25	150	65	261
20	300	65	368
20	287	65	365
20	250	65	355
20	200	65	339
20	150	65	319
15	300	65	436
15	287	65	432
15	250	65	420
15	200	65	401
15	150	65	378
10	300	65	503
10	287	65	499
10	250	65	485
10	200	65	463
10	150	65	436
5	300	65	571
5	287	65	566
5	250	65	550
5	200	65	525
5	150	65	495

Table 5 (continued)
Calculation of Chronic Water Quality Standard for Chloride at Varying Water Temperature, Hardness, and Sulfate

Equation: Chronic Water Quality Standard (WQS) for Chloride:

Chronic WQS $=[1+(0.045)(25\text{-}T)]*[CCC*(Hardness/300)^{0.205797} * (Sulfate/65)^{-0.07452}]$

From Table 4, the Criterion Continuous Concentration (CCC) = 300.5 mg/L

which simplifies to:

Chronic WQS = $[1+(0.045)(25\text{-}T)]*[300.5*(Hardness/300)^{0.205797} * (Sulfate/65)^{-0.07452}]$

where Chronic WQS is in mg Cl/L, T is in ^oC, Hardness is in mg/L as $CaCO_3$, and Sulfate is in mg SO_4^{2-}/L

Temperature ^oC	Hardness mg/L	Sulfate mg/L	Chronic WQS mg/L
25	300	86.8	294
25	287	86.8	291
25	250	86.8	283
25	200	86.8	271
25	150	86.8	255
20	300	86.8	360
20	287	86.8	357
20	250	86.8	347
20	200	86.8	331
20	150	86.8	312
15	300	86.8	426
15	287	86.8	423
15	250	86.8	411
15	200	86.8	392
15	150	86.8	370
10	300	86.8	493
10	287	86.8	488
10	250	86.8	474
10	200	86.8	453
10	150	86.8	427
5	300	86.8	559
5	287	86.8	554
5	250	86.8	538
5	200	86.8	514
5	150	86.8	484

ATTACHMENT 1

LITERATURE

Soucek Progress Report 2017

Progress update 12/04/17

Fingernail clams:

-Collected ~300 adult clams in April 2017 from Spring Creek, near Loda, IL, Iroquois County.

-Juvenile clams released in laboratory were acclimated to reconstituted water and appropriate test temperature (25 and 10 °C).

-For each temperature, the test system provided for two water volume additions per day for the duration of the test to ensure sufficient dissolved oxygen and minimized ammonia.

-Because of limited juveniles for testing, acute and chronic tests were conducted concurrently with mortality data from day 4 used to estimate median lethal concentrations (LC50s).

-Overall average % measured Cl/nominal Cl for both tests combined was 96.5 (min = 90.6; max = 101.4)

-25 °C test:

Fingernail clam (*Sphaerium simile)* sodium chloride acute and chronic data (25 C)

nominal Cl	measured Cl acute	measured Cl chronic	% surv. acute	% surv. chron.	dry weight chron. (mg)	96-h LC50 (mg Cl/L)	28-d LC50 (mg Cl/L)
27.56	27	26	100	100	10.048	1673	1672
100	98	98	100	100	9.928	(unreliable)	(unreliable)
300	292	290	100	100	9.480		
600	573	581	100	100	8.700		
1000	989	988	100	100	8.692		
3000	2831	2831	0	0			

-For the chronic dry weight data, ANOVA detected a significant difference among treatments, but post-hoc pair-wise comparisons failed to detected significant differences from the control. In addition weight did not decrease relative to controls sufficiently in Cl treatments to permit calculation of a 20% effect concentration (EC20).

-10 °C test:

Fingernail clam (*Sphaerium simile)* sodium chloride acute and chronic data (10 C)

nominal Cl	measured Cl acute	measured Cl chronic	% surv. acute	% surv. chron.	dry weight chron. (mg)	96-h LC50 (mg Cl/L)	28-d LC50 (mg Cl/L)
27.56	27	26	100	100	9.240	>2920	1664
100	99	97	100	100	9.160		(unreliable)
300	289	290	100	100	8.476		
600	568	572	100	100	9.656		
1000	978	970	100	100	9.488		
3000	2920	2855	100	0			

-Controls grew less at 10 °C than at 25 °C, and no dose dependent response was observed for weight.

-While temperature appeared to affect the acute response of *S. simile* to NaCl, we were unable to detect an influence of temperature on chronic response.

Mayflies:

-Conducted acute and chronic (14-d) tests at both temperatures (25 and 10 °C).

- For acute tests, overall average % measured Cl/nominal Cl for both tests combined was 99.65 (min = 92.2; max = 104.2)

-- For chronic tests, overall average % measured Cl/nominal Cl for both tests combined was 99.0 (min = 94.2; max = 110.6)

-Acute data:

Mayfly (*Neocloeon triangulifer)* sodium chloride acute data (25 C)

nominal Cl	measured Cl acute	# dead (of 20)	96-h LC50 (mg Cl/L)	95% C.L.
27.6	27.7	0	1359	1249 - 1478
560	543	0		
750	783	0		
1053	1063	2		
1492	1503	14		
2120	2140	20		

Mayfly (*Neocloeon triangulifer)* sodium chloride acute data (10 C)

nominal Cl	measured Cl acute	# dead (of 20)	96-h LC50 (mg Cl/L)	95% C.L.
27.6	25.8	1	1960	1640 - 2343
276	268	0		
524	533	1		
1021	1029	1		
2014	2003	9		
4000	4071	20		

Chronic data:

Mayfly (*Neocloeon triangulifer)* sodium chloride chronic data (25 C)

nominal Cl	measured Cl acute	% survival	dry weight/ind (mg)	weight std. dev	14-d LC50 (mg Cl/L)
27.6	27	95	0.069	0.037	998
100	100	100	0.09	0.034	(905 - 1101)
200	202	100	0.095	0.039	weight EC20 (mg Cl/L)
400	395	95	0.049	0.022	326
750	732	95	0.03	0.017	(201 - 529)
1500	1484	0	na		

Mayfly (*Neocloeon triangulifer)* sodium chloride chronic data (10 C)

nominal Cl	measured Cl acute	% survival	dry weight/ind (mg)	weight std. dev	14-d LC50 (mg Cl/L)
27.6	27	100	0.004	nc	>1466
100	99	89	0.006	nc	
200	198	75	0.080	nc	weight EC20 (mg Cl/L)
400	391	95	0.010	nc	nc
750	723	90	0.010	nc	
1500	1466	55	0.046		

Amphipods:

-We culture two genetically distinct “strains” of *Hyalella azteca*. Most ecotox labs culture and test “US Lab” strain. This is a southern species, and it did not do well at 10 °C in initial experiments. A couple of labs in Canada culture and test with the “Burlington” strain. This is a northern species.

-We conducted control tests with Burlington strain at 10 °C and they survived well (but see below). Acute and chronic testing were/will be conducted with the Burlington strain for this project.

-We used 23 °C as the upper temperature for *Hyalella* because that is the typical acute test temperature for this species.

-Both acute tests have been completed (data below).

- For acute tests, overall average % measured Cl/nominal Cl for both tests combined was 102.3 (min = 96.9; max = 113.1)

-Acute data:

Amphipod (*Hyalella azteca (* Burlington strain)) sodium chloride acute data (23 C)				
nominal Cl	measured Cl acute	# dead (of 20)	96-h LC50 (mg Cl/L)	95% C.L.
27.6	29.0	1	1733	1592 - 1887
741	785	1		
1047	1098	0		
1484	1550	5		
2108	2173	18		
3000	3084	20		

Amphipod (*Hyalella azteca (* Burlington strain)) sodium chloride acute data (10 C)				
nominal Cl	measured Cl acute	# dead (of 20)	96-h LC50 (mg Cl/L)	95% C.L.
27.6	27.0	0	2185	2013 - 2372
741	745	0		
1047	1055	0		
1484	1492	1		
2108	2100	7		
3000	3008	20		

-Chronic data:

-We have conducted a 28-d chronic at 23 °C.

-For the chronic tests at 23°C, overall average % measured Cl/nominal Cl was 96.5% (min = 91.7; max = 109.2)

-Data for 23 °C test are below.

-We have been having difficulty with control survival for *Hyalella* at 10 °C. Two tests we have started have had ~50% survival within 7-10 days. Therefore we will attempt to use older organisms (~14-d) to start a test to allow young amphipods to grow stronger prior to acclimation to cold temperature and testing.

Amphipod (Hyalella azteca) sodium chloride chronic data (23 C)				
nominal Cl	measured Cl	% survival	dry weight/ind (mg)	28-d LC50 (mg Cl/L)
27.56	26	82	0.204	949 (829 - 1087)
187	177	76	0.167	28-d EC20 (survival; mg Cl/L)
375	364	82	0.185	744 (617 - 897)
750	745	63	0.079	28-d EC20 weight mg Cl/L)
1500	1480	6	0.038	516 (357 - 745)
2000	1956	0	na	

Elridge et. al. 2010

Review of the 2012 proposed water quality criteria for chloride for the protection of aquatic life in Pennsylvania

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1. Introduction

The Environmental Quality Board of Pennsylvania on July 7, 2012 proposed amending 25 Pennsylvania Code Chapter 93.7 relating to the water quality standard for chloride. The Board proposed adopting the Iowa equation-based aquatic life criteria for chloride based on the best available sound science (PA Bulletin 12-1292). This review is in response to the proposed standard for chloride in surface waters and builds on a previous report (Stroud Water Research Center 2010) that evaluated the water quality standard for chloride proposed by the EQB in 2010.

Chloride criteria for aquatic organisms are needed in Pennsylvania to protect aquatic life in our surface waters. Chloride can enter surface water via road salt runoff (e.g., following brine application for dust suppression, or following deicer application or storage), or through wastewater or other industrial discharge. In 2010, the EQB of Pennsylvania proposed adopting the recommended criteria from the US EPA (EPA 1988) for Aquatic Life Uses for Cold Water Fishes (CWF), Warm Water Fishes (WWF), Migratory Fishes (MF), and Trout Stocking (TSF). Those criteria were an acute criterion of 860 mg chloride/L and a chronic criterion of 230 mg chloride/L.

We found a number of faults with the criteria proposed in 2010 and the conclusions of the previous report are attached as **Appendix 1** at the end of this document. The changes proposed in 2010 were not adopted by the EQB, in part because they did not incorporate the latest available science. Some of the concerns identified by us in 2010 have been addressed by the proposed Iowa equation based criteria. However, the Iowa equation based criteria do not adequately address some critical scientific gaps which we feel will leave some species at risk of harm. In this comment, we suggest some options that the EQB may consider. Due to the large amount of uncertainty that remains, one option that may be rapidly incorporated would be to re-derive the chronic criterion and apply a safety factor to provide an enhanced level of protection.

The acute and chronic criteria equations proposed by the EQB of Pennsylvania are based on reports by Stephan (2009 a,b,c,d,e,f,g,h). We will use these reports as the basis of our review and critique.

2. Strengths of the proposed standard

2.1. EPA has not officially adopted new national criteria since 1988 (EPA 1988). The derivation of the 2009 Iowa criteria incorporated data from recent chloride toxicity studies.

2.2. The most significant development with the 2009 Iowa Criteria is an acknowledgement that the toxicity of chloride to aquatic organisms varies depending upon the other ions present. Specifically, the criteria use equations to account for changes in toxicity due to water hardness (i.e., cation content [primarily calcium and magnesium, but could also include iron and manganese] of water) and sulfate concentrations.

2.3. The 2009 Iowa criteria also clarified rules of data inclusion or exclusion. The 2009 criteria included static tests that were excluded in 1988 (Stephan 2009a). The approach was to include a test unless there was an obvious reason to exclude it (Stephan 2009a).

3. Weaknesses and concerns resulting from implementing the proposed standard

3.1. These proposed criteria are based on toxicity studies of dissolved chloride that has dissociated from sodium chloride (NaCl), although chlorides dissociated from calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), or potassium chloride (KCl) may be present in surface water and can be more toxic to aquatic organisms (e.g., Mount et al. 1997).

3.1.1. In 1988, the EPA noted that "the chlorides of potassium, calcium, and magnesium are generally more toxic to freshwater species than sodium chloride" (EPA 1988 p. 7), but there was insufficient data on the toxicity of the chlorides of calcium, magnesium, or potassium to derive criteria.

3.1.2. The relationship between the toxicity of the chloride of sodium and the toxicity of the chlorides of potassium, calcium and magnesium has held over time. Below are the *<u>ratios</u>* of the LC_{50} concentrations for the chloride of calcium (Ca), magnesium (Mg), or potassium (K) to the LC_{50} concentration for the chloride of sodium (Na) for the same species and test water (Stephan 2009a p. 10):

- Ca/Na (n=5): 0.57-0.98
- Mg/Na (n=3): 0.34-0.55
- K/Na (n=5): 0.11-0.25

Note: a ratio <1 indicates that the chlorides dissociated from calcium, magnesium, or potassium caused mortality at a lower concentration.

3.1.3. The chlorides of calcium, magnesium, or potassium may be present in the environment in such a way that they threaten surface waters.

- Potassium, magnesium, or calcium chlorides are used as deicers (Salt Institute 2004, Chang 2009).
- Potassium chloride can be present in the effluent from hydraulic fracturing for natural gas extraction (URS Corporation 2011), and is also commonly used as a water softener.
- Use of liquid brine salts as dust suppressants on roadways and at construction sites (Piechota et al. 2002)

3.2. The proposed chronic criteria may be above the level that causes adverse impacts to aquatic organisms in Pennsylvania.

3.2.1. The proposed criteria are based solely on studies of animals and do not consider toxicity to aquatic plants. Stephan (2009a, 2009b, 2009c, 2009d, 2009g) did not indicate why plants were not considered in the derivation of the Iowa Criteria. In 1988, the EPA noted that the alga *Spirogyra setiformis* was extremely sensitive to the effects of chloride (71 mg/L; growth, chlorophyll, C^{14} fixation; 10d; Shitole and Joshi 1984) as was the desmid *Netrium* *digitus* (200 mg/L; growth inhibition; 21d; Hosiaisluoma 1976). However, the 1988 criteria did not include plant species in the derivation because "a Final Plant Value, as defined in the Guidelines, cannot be obtained because no test in which the concentrations of chloride were measured and the endpoint was biologically important has been conducted with an important aquatic plant species" (EPA 1988). These concentrations for plants are below the SMCV observed for vertebrate and invertebrate animals (Table 1) suggesting that plants may be more sensitive to chloride than are animals.

3.2.2. Recent research with freshwater mussels suggests that the glochidia of some species may be more sensitive to chloride than the current suite of aquatic organisms for which data is available (Gillis 2011, Pandolfo et al. 2012). Stephan included data from juvenile freshwater mussels or freshwater mussels that do not have a glochidia stage (i.e., *Villosa delumbis* and *Lampsilis fsciola*, Bringolf et al. 2007; *Villosa iris* and *Lampsilis siliquoid,* Wang 2007; *Sphaerium simile,* GLEC and INHS 2008), but Stephan excluded all studies with glochidia because of their unique life-history which requires that they attach to a fish host in order to survive (Stephan 2009a p. 7[d]). The unique life history of most freshwater mussel species makes it difficult to design toxicology studies with them. However, freshwater mussels are among the most imperiled organisms in Pennsylvania (PNHP 2012), therefore it is important that water quality criteria be protective of them. One "very important question is 'What species-specific toxicity-test duration is ecologically relevant for glochidia?'" (Stephan 2009a p. 7[d]).

3.3. The proposed chronic criterion is not robust.

3.3.1. The genus mean chronic values (GMCV) should not have been calculated directly from the species mean chronic values (SMCV) without first correcting for hardness and sulfate.

3.3.1.1. The SMCV from different experiments were not normalized for hardness and sulfate (Stephan 2009c). As a result, the SMCV are not directly comparable because the toxicity of chloride varies depending upon the chemical composition of the water in which the test was done (e.g., Mount et al. 1997, Soucek 2007, Elphick et al. 2011). Therefore, calculating the GMCV as the geometric mean of the SMCV for a given species is not appropriate. It should be noted that the species mean acute values (SMAV) were corrected for hardness and sulfate before calculating the GMAV (Stephan 2009g).

3.3.2. There is inconsistency in the meaning of the species mean chronic value (SMCV). The SMCV determined by Stephen (2009c) refer to different levels of impairment for different species.

3.3.2.1. Stephen (2009c) used the geometric mean of the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) to determine the SMCV. The NOEC and LOEC refer to test concentrations used in the experiments, but the amount of impairment at the NOEC and LOEC varied among experiments (Table 1). Therefore the SMCV determined by Stephen (2009c) refer to different levels of impairment for different species.

3.3.3. Different researchers analyzing the same data have arrived at different results and different conclusions.

3.3.3.1. The guidelines that different researchers have used to determine which studies should be included and the interpretation of the EPA 1985 guidelines differ between researchers (e.g., EPA 1988, Stephan 2009a). As a result the rules to determine the appropriate studies and data to use to derive chloride criteria are not interpreted in a consistent manner and researchers have differed in the tests they included or excluded. For example, Stephan (2009c, 2009e) excluded an acute and chronic study of *Rana sylvatica* by Sanzo and Hecnar (2006) because they used technical grade sodium chloride, but Elphick et al. (2011) included this study when deriving a chronic criterion.

3.4. The proposed chronic criterion does not account for uncertainty in data and methods.

3.4.1. The proposed chronic criterion equation may allow for chloride concentrations in surface waters of Pennsylvania above the concentration shown to cause harm to aquatic organisms during laboratory experiments. For example, the SMCV for some species in Table 1 are near or exceed the normalized chronic criterion of 389 mg chloride/L which Iowa adopted for surface waters where sulfate and hardness are not known. The SMCV in Table 1 would need to be corrected for hardness and sulfate to confirm that the chronic criterion would exceed the SMCV. In 1988, the EPA affirmed that the proposed chronic criterion was below the three SMCV available at that time (EPA 1988). Stephan in 2009 did not affirm that the proposed chronic equation was below the level shown in laboratory experiments to impair aquatic organisms.

3.4.2. The proposed chronic criterion equation includes a correction for hardness and sulfate although the exponents for hardness and sulfate are based on studies in two labs (GLEC and INHS 2008, p29 & 36) of only one species (*C. dubia*) under acute conditions (Stephan 2009f). Stephan (2009f) presents evidence that "supports the concept" that "the sulfate exponent might be more negative than indicated by the GLEC and INHS (2008) data" (Stephan 2009f p. 4). A negative exponent for sulfate means that a higher sulfate concentration lowers the LC_{50} for chloride. Thus, reliance on the 2009 Iowa equations may not offer the intended level of protection to aquatic organisms in Pennsylvania.

3.4.3. The endpoints of chronic tests conducted under laboratory conditions (e.g., survival, reproduction) may not reflect the most sensitive response in nature. In nature, a stress response may occur at lower concentrations than what are observed under controlled laboratory settings. A similar pattern is seen with behavioral responses such as avoidance, coughing or rapid breathing by fish, or increased activity (Atchison et al. 1987, Scott and Sloman 2004, Hellou 2011). Behavioral responses have been poorly documented or not measured in most laboratory experiments of chloride toxicity, therefore it is unknown how the behavior of aquatic organisms in nature would be affected by elevated chloride.

3.4.4. There has been no attempt to account for the fact that the available data represents only a small percentage of the species found in Pennsylvania. Including studies conducted since the 2009 Iowa criteria were derived results in a different acute-to-chronic ratio (ACR) and acute and chronic criteria (e.g., Elphick et al. 2011). It is to be expected that additional data may change the criteria, such as occurred between the derivation of the 1988 criteria and the 2009 criteria (e.g., ACR= 7.594, EPA 1988; ACR = 3.187, Stephan 2009h; ACR = 3.50, Elphick et al. 2011). Focusing on species found in Pennsylvania may also alter the criteria.

3.4.5. Different methods to derive the chronic criterion may result in different criterion. Stephan (2009c, 2009h) used the ACR whereas Elphick et al. (2011) derived a chronic criterion directly from chronic studies. (chronic criterion = 307 mg/L, Elphick et al. 2011; chronic criterion = 428 mg/L, Stephan 2009h). It should also be noted that other factors, such as if how hardness and sulfate were accounted for, could also account for differences in the criterion.

3.4.6. The lack of robustness in the derivation of the chronic criterion is further evidence of uncertainty.

Table 1: Data used to derive the species mean chronic values (SMCV) by Stephan (2009c). The SMCV is the geometric mean of the NOEC and the LOEC. A subset of this data was used to calculate the genus mean chronic values and the acute-to-chronic ratio, which was used to derive the proposed chronic criterion.

Category	Species	Endpoint	NOEC (mg/L)	LOEC (mg/L)	SMCV(mg/L)	Reference
Fish – non-salmonid	Fathead minnow	33d; survival	352 (9% reduction)	533 (15% reduction)	433.1	(Birge et al. 1985)
Fish - salmonid	Rainbow trout	Early life stage; survival	643 (4% reduction)	1324 (46% reduction)	922.7	Spehar 1987[1]
Cladoceran	*Ceriodaphnia dubia*	7-9d; Reproduction	?[2]	? (EC50)	925	(Cowgill and Milazzo 1990)
Cladoceran	*Ceriodaphnia dubia*	7 d; ?	?	?	235	(Diamond et al. 1992)
Cladoceran	*Ceriodaphnia dubia*	6-7d; Reproduction	N/A[3]	442.2[4] (IC25)	<442.2	WISLOH 2007 (mod. Hard water)
Cladoceran	*Ceriodaphnia dubia*	6-7d; Reproduction	N/A	385.2 (IC25)	<385.2	WISLOH 2007 (Hard water)
Cladoceran	*Ceriodaphnia dubia*	7d; Reproduction	N/A	340 (IC25)	<340	Lasier et al. 2004[5]
Cladoceran	*Ceriodaphnia dubia*	7d; Reproduction (12 studies)	<152-303	346-685 (IC50)	<322	(Aragão and Pereira 2003)
Cladoceran	*Ceriodaphnia dubia*	7d; Survival	1092	1456	N/C[6]	(Cooney et al. 1992)
		7d; Reproduction	<455-819	455-1092	<629	
Cladoceran	*Ceriodaphnia dubia*	7d; Reproduction	N/A	370.6 (EC20)	370.6	(Harmon et al. 2003)
Cladoceran	*Daphnia ambigua*	10d; Reproduction	N/A	292.4 (EC20)	292.4	(Harmon et al. 2003)
Cladoceran	*Daphnia magna*	10d; Reproduction	2184	2597 (EC50)	2382	(Cowgill and Milazzo 1990)
Cladoceran	*Daphnia pulex*	21d; Reproduction	314 (0% reduction)	441 (27% reduction)	372	(Birge et al. 1985)
Frog	*Rana sylvatica*	90d; Survival	N/A	625 (62% reduction)	<625	(Sanzo and Hecnar 2006)

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[1] Unpublished memorandum sent directly to C. Stephan from R. L. Spehar on June 24, 1987. Data is not available on-line (scholar.google.com, search "chloride author:Spehar", Aug. 3, 2012).

[2] Data not presented in Stephan (2009c) and document not available for download (Aug. 3, 2012)

[3] Stephan (2009c) did not use the NOEC to calculate the SMCV.

[4] WISLOH 2007 refers to an unpublished study that could not be found on the Wisconsin State Laboratory of Hygiene webpage (http://www.slh.wisc.edu/, search "chloride", Aug. 3, 2012). IDNR (2007) presents results from the WISLOH lab covering the period 2000-2007, however the results in that report (Table 4: *C. Dubia* chronic toxicity 703 mg Cl^-/L; Table 7: *C. dubia* chronic toxicity: 427 mg Cl^-/L) do not match those presented by Stephan (2009c). Corsi et al. (2010) present results from the WISLOH lab over the same time period, but the studies do not appear to be the same as the ones reviewed by Stephan (2009c) because the Corsi study focused on surface waters receiving road run-off.

[5] Data was presented in a poster at the SETAC meeting and is not available on-line (Aug. 3, 2012)

[6] The geometric mean for *C. dubia* survival in the study by Cooney et al. (1992) was not calculated by Stephan (2009c) because reproduction was more sensitive.

4. Point of Clarification

4.1. As written in PA Bulletin 12-1292 it appears that Pennsylvania will adopt the Iowa criteria *in toto*, including Iowa's criteria for waterbodies where sulfate and hardness are not known. Iowa defined normalized acute and chronic criteria to be applied to waterbodies where sulfate and hardness are not known that were based on the statewide background values for hardness (200 mg/L) and sulfate (63 mg/L). Average hardness and sulfate concentrations may be different in PA and therefore the normalized acute and chronic criteria for Iowa may not be appropriate for PA.

5. Summary and Recommendations

5.1. The chloride criteria proposed by the EQB on July 7, 2012 are an improvement over the criteria that were proposed in 2010. Specifically, the proposed criteria incorporate characteristics of the receiving waters that affect chloride toxicity. However, as was highlighted in our previous review, the newly proposed criteria based on the Iowa standard may not be protective of aquatic life in Commonwealth streams, rivers, and lakes. Examples of uncertainty are:

5.1.1. The proposed chronic criterion may allow for ambient chloride concentrations in surface waters in Pennsylvania above the concentrations shown to cause harm to aquatic organisms in laboratory experiments.

5.1.2. The criteria are based only on the chloride of sodium although the chlorides of calcium, magnesium or potassium may enter surface waters of Pennsylvania and are more toxic to aquatic organisms.

5.1.3. The proposed criteria are derived from only a few species found in Pennsylvania.

5.1.4. There are only seven species (6 after excluding the frog, *Rana sylvatica* which Stephan [2009g] excluded because the sodium chloride used in the experiment was technical grade) for which there are acceptable chronic data (Table 1).

5.1.5. Glochidia and plants were not included in the derivation of the acute or chronic criteria.

5.1.6. The proposed criteria may not be protective of our more sensitive stream dwelling invertebrate species, particularly early life history stages (e.g., glochidia of mussels or early life stages of other invertebrates).

5.1.7. Exponents for hardness and sulfate in the acute and chronic criteria equations may be under-protective.

5.1.8. The species mean chronic values (SMCV's) were not corrected for hardness and sulfate concentrations.

5.1.9. The SMCV refer to different levels of impairment for the different experiments and species.

5.1.10. The SMCV are not corrected for hardness or sulfate.

5.1.11. The endpoints of laboratory toxicity studies do not include behavioral responses. Behavior may be affected at lower chloride concentrations than are survival, reproduction or growth.

5.2. Following are some recommendations on how the EQB may address uncertainty.

5.2.1. Include a safety factor in the derivation of the chronic criterion. At a minimum, that safety factor should be sufficient to ensure that the chronic criterion is below the SMCV or GMCV. Following are some reasons that a safety factor should be used:

5.2.1.1. "Safety factors are used to provide an extra margin of safety beyond the known or estimated sensitivities of aquatic organisms" (EPA 1985 p 36).

5.2.1.2. The acute criterion incorporates a safety factor (i.e., 2) but the chronic criterion does not. The 1985 EPA guidelines indicate that a safety factor of 2 is always to be used when calculating the acute criterion (called the criterion maximum concentration in EPA 1985, p 54, item XI.B.) but does not give a rationale for this using this safety factor. Although the EPA did not include a safety factor when deriving the chronic criterion in 1988, the chronic value was below the level shown to cause harm to the three species for which data were available at that time (EPA 1988). It is unclear if the proposed chronic criterion is below the level shown to cause harm because the SMCV in Table 1 have not been corrected for hardness or sulfate.

5.2.1.3. The acute and chronic criteria are based solely on studies using the chloride of sodium, but the chlorides of potassium, magnesium or calcium may be present in surface waters of Pennsylvania and are more toxic to aquatic organisms than is the chloride of sodium.

5.2.1.4. Environmental impacts (including avoidance) may occur at lower concentrations then those that affect growth or survival.

5.2.1.5. British Columbia (Nagpal et al. 2003) used a safety factor of 5 in the derivation of the chronic guideline. Their justification for this safety factor was as follows:

- Chronic data available from the literature were scant;
- In a recent study, Diamond et al. (1992) found a LOEC/NOEC ratio for reproduction of 3.75 in *C. dubia* exposed to NaCl for 7 days. Also, LC_{50}/LC_0 of 3 and LC_{100}/LC_0 of 4 were obtained by Hughes (1973), whereas the DeGreave et al. (1991) data yielded LC_{50}/NOEC ratios that ranged from about 1.0 to 6.9;
- Additional protection may be required for those species that are more sensitive but have not yet been tested in the literature.

5.2.2. A new review of chloride toxicity studies should be conducted to generate a more complete and up-to-date list of species and genus mean acute and chronic values. The references sited at the end of this comment include a few studies that have been published since 2009. A new review should:

5.2.2.1. Resolve the controversy regarding aquatic plants and glochidia.
5.2.2.2. Clearly define rules to include or exclude a study and document the rationale for studies that are excluded;
5.2.2.3. The species mean acute values and species mean chronic values should be calculated using a consistent and biologically meaningful endpoint. For example,

Elphick et al. (2011) used probit regression to determine an endpoint that was consistent among species (e.g., the IC10).

5.2.2.4. Derive species mean chronic values normalized for hardness and sulfate;

5.2.2.5. Explore the possibility of deriving chronic criterion directly from the data rather than using the ACR (e.g., Elphick et al. 2011);

5.2.2.6. Include in the review toxicity studies with the chlorides of potassium, magnesium or calcium. Although conducting additional experiments with species found in Pennsylvania is the preferred approach, it may be appropriate to use the ratios cited above (3.1.2) to derive the SMAV or SMCV. For example, the chloride of potassium appears to be 4-10x more toxic to aquatic organisms than is the chloride of sodium.

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Conclusions

After reviewing four different approaches for deriving water quality chloride criteria to protect aquatic life (Stephan et al. 1985, Evans and Frick 2001, Nagpal et al. 2003, Iowa DNR 2009) and the data underpinning PA's proposed criteria (EPA 1988) and the Iowa criteria (Stephan 2009a,b,c), it is clear that:

1) All approaches set chloride criteria that are at least several times greater than natural baseline chloride concentrations, and therefore represent a measurable and significant change in the chemical composition of freshwater ecosystems in the NE United States. The question that the current evidence is unable to answer is: will these criteria result in significant biological change? There is limited evidence of the biological impact of previous elevated chloride levels in aquatic ecosystems in the U.S. or Canada. Past monitoring efforts (see introduction) suggest that some streams regularly reach the acute criterion, but there has not been a noted change in biota following these pulses, largely because of a dearth of biological data following these episodic events. One study has demonstrated that macroinvertebrate drift increases in response to pulsed chloride input (Blasius and Merritt 2002). Another study has demonstrated losses of species in stream fish communities with small changes in chloride levels across a regional-scale analysis (Meador and Carlisle 2007), and the composition of algal species has been observed to change when chloride concentrations increase (Evans and Frick 2001). Nonetheless, there are limited data on biological changes accompanying changing chloride concentrations in the natural environment. We could not find any studies evaluating the influence of chloride on vital stream functions such as primary production, stream metabolism, or nutrient uptake or processing, all of which are important indicators of water quality for aquatic ecosystems.

2) All of these criteria are based on data for invertebrate and fish species that are not a random subset of stream invertebrate and fish species. Rather, most of the species with chloride data are known to be not especially sensitive to changes in environmental condition, which is one reason they survived well in the laboratory and became standards in laboratory bioassay protocols. The most recent iteration of the taxa that qualify based on EPA standards (in Stephan 2009a,b,c) doesn't include any classically sensitive stream invertebrate species such as stoneflies, mayflies, and caddisflies, all of which are important indicators of stream condition and are integral in the regulatory definition of stream impairment. Our concern is that criteria intended to protect most (e.g., 90% or 95%) of the species with chloride data might actually protect a much smaller proportion of all species that occur in a natural community because the natural community includes many species known to be sensitive to environmental change while the laboratory studies are biased toward species known to be at least moderately tolerant of environmental change. This is one reason to approach the acute and chronic criteria with a strong safety factor.

3) Data available are primarily from acute toxicity studies, but the chronic criterion may be more important for long-term structuring stream communities and maintaining designated use for aquatic life. For example, fish tend to be moderately tolerant of acute chloride stress relative to macroinvertebrates, but they are one of the more sensitive taxa to chronic chloride stress. For example, fat head minnows (Birge et al. 1985) experienced the greatest mortality between days 9 and 21 and therefore had one of the highest acute-to-chronic ratios examined. The dearth of chronic studies on both invertebrates and fish is troubling. It is likely that, like some amphibians (e.g., spotted salamander), embryonic and early life stages of some fish will be more sensitive than is currently recognized.

4) The majority of chloride criteria developed to date are limited to or dominated by data on NaCl chloride toxicity, the least toxic salt. This point is routinely justified by the fact that NaCl is the most anthropogenically abundant of these four salts. However, no special guidance is given for permitting salt applications or industrial effluents known to include significant amounts of chloride derived from the more toxic non-sodium salts, including Marcellus Shale wastewater.

5) Using the data provided in Stephan 2009a (Table 2 herein), we have calculated both the acute (CMC) and chronic (CCC) criteria using the methods of the EPA (Stephan et al. 1985, EPA 1988), Evans and Frick (Evans and Frick 2001), British Columbia (Nagpal et al. 2003), and Iowa (Iowa DNR 2009) and have compared the range of values with the proposed PA values (Table 3). The range of acute values is 564 – 830 mg/l Cl- and the range of the chronic values is 91 – 428 mg/l Cl-. This comparison eliminates the variability in the choices each of the authors have made with regard to studies included or excluded. We note that the PA proposed acute value is the least protective criterion, primarily because it is not based on more recent acute toxicity studies. We recommend that PA adopt an acute criterion that is reflective of these new data. The method adopted by British Columbia is the most protective of aquatic life among these approaches. BC invoked a precautionary principle that acknowledged both the uncertainty of the available data and analyses and the importance of protecting their aquatic life. Since BC adopted their criteria, only new acute datasets have become available and the values in Table 3 utilize those data but use the BC approach to arrive at a final value (i.e., lowest SMAV/2[safety factor]). The BC use of a safety factor of 2 for the acute criteria was also consistent with what the EPA had done. However, BC was the only entity to apply a safety factor for the chronic criterion (5). We feel that the use of a safety factor for chronic criteria derived from the use of an ACR is clearly justified given the very limited number of chronic toxicity studies, and the desire to protect species that may be more sensitive than those used in the standard laboratory bioassays. We recommend that PADEP adopt the same methodology that BC has used for calculating both acute and chronic data. We feel that this is particularly important for the chronic criteria, as there is the potential for permitted discharges (particularly from the Marcellus Shale gas drilling industry) to raise chloride concentrations in streams to near the chronic criteria level. Given the paucity of data determining thresholds for chronic effects, this approach is warranted. At the very least, a safety factor should be applied to any of the other methods producing a chronic criterion.

We have a number of concerns that are specific to the actions and options available for PADEP:

6) Protecting CWFs and TSFs based on ACRs that included more chloride-tolerant *Daphnia* is not justified when it may expose rainbow trout to chloride concentrations approaching their chronic

levels (1,324 mg/l Cl^- killed 46% of individuals in an early life stage test and at 643 mg/l Cl^- killed <4%). Trout are an integral component in the definition of these two aquatic life uses. The proposed chronic value of 230 mg/l is potentially a concern for biotic assemblages in Pennsylvania. For example, Meador (2007) suggests that optimum Cl^- values are low (3-35 mg/l) and we infer that if those Cl^- concentrations are exceeded it may result in changes in fish community structure. Similarly, not having a temperature component also seems to invite season-specific impairments of macroinvertebrates in TSFs and WWFs based on the recent findings of Silver et al. (2009), based on the seasonal movement of organisms into and out of various life history stages, and based on variation in their metabolic rates in response to seasonal changes in water temperature. Adding a temperature component to the chloride criteria would require further research on temperature effects.

7) The Evans and Frick (2001) method has the benefit of being reproducible and open to interpretation. Their use of nearly all of the valid acute LC_{50} data in Fig. 7-2 (Evans and Frick 2001), and the calculation of a sigmoid curve function (including 95% confidence intervals) that describes the percent of genera affected versus chloride concentration, is readily digestible by the public. However, the sigmoid curve function can be generated using various numbers of terms (parameters) in the equation and/or various equations (e.g., sigmoid, logistic, Weibull). The result of choosing a slightly different function can result in differences in acute and chronic values. To use this approach requires a valid justification for the choices made in fitting the curve to these data. Furthermore, these data still represented a small subset of aquatic species, and were biased towards lab friendly species that are easiest to culture (e.g., *Daphnia*). Since the selection of taxa was not a random subset of the aquatic species at large, most criteria based on the animals selected are primarily protective of those species tested (e.g., being protective of 95% of those taxa might only be protective of 50% of all species). This point is not limited to Evans and Frick but is valid for all of the approaches we have reviewed. This is the primary reason that the application of a safety factor is needed. The Evans and Frick (2001) study did not apply a safety factor to either their acute LC_{50} relationship or the derived chronic relationship.

8) More data is generally better, but there is a need for more consideration of how data gets incorporated. The Stephan (2009a,b,c) approach of calculating a predicted genus mean chronic value from the species mean acute values does not seem justified in this case. The GMCVs are not much better than guesses, and there is no attempt to correct for this inherent uncertainty. Adding GMCV values above the lowest four gives the false sense of increased precision of the true distribution of the GMCV, which has the result of increasing the final chronic value (FCV). We feel it would be appropriate to apply a safety factor to the chronic criteria to acknowledge the uncertainty in the FCV.

9) The use of hardness and sulfate equations (Iowa DNR 2009) in PA will improve protections and application of the chloride criteria only to a limited extent since the range of criteria in PA would be narrow (based on EMAP site values for hardness and sulfate in PA). Secondly, the hardness and sulfate exponents in the Iowa criteria were based on data from an acute toxicity study of only one species (*C. dubia*), although four species were studied and three were sensitive to hardness. No data were available on the relationship between hardness or sulfate and chronic toxicity. In the end, Iowa uses a default value for hardness and sulfate if no other data are available. This is akin to setting a fixed criterion value but allowing site-specific deviations if one

gathers the appropriate data. Clearly, more species-specific data are needed to better understand the relationship between chloride toxicity and hardness or sulfate.

10) As noted above, previous reviews of chloride considered only NaCl and considered road salt to be the most likely source of chloride. We feel that the current proposed standard should explicitly acknowledged that these criteria are specific to NaCl derived chloride, and guidance should be given to address cases when significant chloride is derived from salts (i.e., KCl, $MgCl_2$ and $CaCl_2$) that have proven to be more toxic sources of chloride.

Our review of four approaches (Stephan et al. 1985, Evans and Frick 2001, Nagpal et al. 2003, Iowa DNR 2009) for deriving chloride criteria to protect aquatic life identified a number of weaknesses in the available data and the analyses used to derive criteria. We were especially concerned with (1) the near absence of important stream-inhabiting and stream-classifying species such as mayflies, stoneflies, and caddisflies, (2) the dependence on relatively few chronic studies, and (3) the choice of excluding some studies that were very important (e.g., fat head minnow Birge et al. 1985). We believe these weaknesses justify using a very conservative approach to assigning criteria. All four approaches to set acute and chronic criteria would result in chloride concentrations at least several times greater than base flow concentrations commonly observed in Pennsylvania streams in their most natural condition (i.e., Exceptional Value and High Quality waters). The lowest criteria for chloride were derived by the Canadian Province of British Columbia (Nagpal et al. 2003) – they acknowledged the weaknesses in available data, and applied safety factors of 2 for the acute criterion and 5 for the chronic criterion. Given the limits in the available data, and the potential that treated wastewaters from Marcellus Shale drilling may result in near-criterion chloride concentrations 356 days per year (versus the 30 days of a standard chronic bioassay), we believe the British Columbia criteria (either the originally adopted criteria or our re-calculated criteria in Table 2) would be the most protective of aquatic life for Pennsylvania streams, especially for the trout and many pollution-sensitive macroinvertebrate species that characterize Cold Water Fishes streams.

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Hazard/Risk Assessment

CHRONIC TOXICITY OF CHLORIDE TO FRESHWATER SPECIES: EFFECTS OF HARDNESS AND IMPLICATIONS FOR WATER QUALITY GUIDELINES

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Abstract—Toxicity tests using nine freshwater species (*Ceriodaphnia dubia*, *Daphnia magna*, *Oncorhynchus mykiss*, *Pimephales promelas*, *Lumbriculus variegatus*, *Tubifex tubifex*, *Chironomus dilutus*, *Hyallela azteca*, and *Brachionus calyciflorus*) were conducted to evaluate their sensitivity to chloride. Acute-to-chronic ratios (ACRs) from these tests indicate the ACR of 7.59 employed by the United States Environmental Protection Agency (U.S. EPA) in deriving its water quality guideline for chloride may be conservative; a revised ACR of 3.50 is presented here. The endpoints used to calculate the ACR included 24-h to 96-h median lethal concentrations (LC50s) for acute tests, and 48-h to 54-d inhibition concentration (IC*x*) values for growth or reproduction for chronic exposures. Data from the present chronic toxicity tests, and other investigators, were used to propose a water quality guideline for long-term exposure to chloride using a species sensitivity distribution (SSD) approach. The 5th percentile from the SSD was calculated as 307 mg/L and proposed as the water quality guideline. Cladocerans were the most sensitive species in the dataset. *Ceriodaphnia dubia* was used to evaluate the relationship between water hardness and sensitivity to chloride. A strong relationship was observed and was used to establish a hardness-related equation to modify the proposed water quality guideline on the basis of water hardness, resulting in values ranging from 64 mg/L chloride at 10 mg/L hardness to 388 mg/L chloride at 160 mg/L hardness (as $CaCO_3$). These data suggest that current water quality guidelines for chloride may be overly conservative in water with moderate-to-high hardness, and may not be sufficiently protective under soft-water conditions. Environ. Toxicol. Chem. 2011;30:239–246. © 2010 SETAC

Keywords—Chloride Aquatic toxicology Species sensitivity distribution Effluent Water quality guideline

INTRODUCTION

Chloride is ubiquitous in natural waters and is essential in a wide range of biological functions, including facilitating a variety of ion-exchange mechanisms through trans-membrane chloride channels. It forms salts with each of the major cations (Na, K, Ca, and Mg), but is highly soluble and exists primarily in the environment as a dissociated monovalent anion.

Freshwater organisms are generally hyperosmotic in their internal fluids relative to the external environment and maintain an active gradient of chloride across membranes through use of active pumps and/or bicarbonate exchange mechanisms at exterior surfaces such as the gill [1,2]. Increasing concentrations of chloride in the external environment may decrease this gradient and associated energy requirements; however, chloride can exhibit toxicity at elevated concentrations once homeostatic mechanisms are overwhelmed. Toxicity may result from osmotic stress related to overall ionic strength or disruption of individual cellular processes in which chloride plays a role [2].

The toxicity of chloride is of interest in aquatic environments as a result of its tendency to occur at elevated concentrations in effluents from industrial operations that involve subsurface mining (including coal, potash, metal, and diamond mines) [3–5], and oil and gas extraction [6]. In addition, chloride salts are widely used in road salts and, consequently, stormwater and snow-melt runoff often contain high concentrations of chloride in areas of application [7].

The EKATI Diamond Mine, operated by BHP Billiton Diamonds, is located in the southern Arctic ecoregion, 300 km northeast of Yellowknife in the Northwest Territories, Canada. The receiving environment at this mine is comprised of a series of low ionic-strength lakes. Chloride concentrations have increased in the receiving environment as a result of contributions primarily from groundwater and to a lesser extent from dissolution of solids from crushed kimberlite, and use of chloride-containing settling agents. Concentrations have increased from less than detection (<0.5 mg/L) to, in some cases, greater than 150 mg/L in receiving water bodies. Modeling of the discharge and receiving environment water, through the remainder of the mine life and into the closure period, has indicated the potential for the concentrations to continue to increase. Consequently, establishing water quality objectives for chloride for application at this site is a matter of interest.

Water quality guidelines for chloride are typically derived on the basis of toxicity tests using sodium chloride, because this cationic counter-ion contributes less toxicity than other major cations, such as K, Mg, and Ca [8]. Thus, the Na salt provides the most accurate measure for the toxicity of chloride itself by minimizing the toxicity contributed by the counter cation. The United States Environmental Protection Agency (U.S. EPA) published water quality criteria for chloride based on data for sodium chloride in 1988. The final acute value for chloride was 1,720 mg/L and the chronic criterion was 230 mg/L [9]. The chronic toxicity test data available were insufficient to calculate a chronic criterion directly and, consequently, the chronic value was calculated by dividing the final acute value by an acute-to-chronic ratio (ACR) of 7.594. This ACR was calculated as the geometric mean of ACR values from tests with three species: rainbow trout (7.308), fathead minnows (15.17), and *Daphnia pulex* (3.951). These individual estimates varied by more than

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fourfold, resulting in uncertainty in the final ACR estimate, particularly considering the small number of datapoints on which it was based.

No Canadian water quality guidelines for chloride presently exist; however, an evaluation conducted by Environment Canada has calculated a concentration of 212.6 mg/L chloride that is expected to be protective of at least 95% of species in long-term exposures [10]. This estimate was made using a species sensitivity distribution (SSD) approach using acute toxicity data, divided by the ACR value reported by U.S. EPA [9], and was performed as part of an evaluation of risk associated with application of road salts for control of snow and ice [7,10].

Considerable data are available on the acute toxicity of sodium chloride to aquatic organisms [7,9]; however, a general lack of chronic toxicity data exists for this anion. Consequently, guideline derivation and risk assessments for chloride have estimated effects thresholds for long-term exposure by applying an ACR to results from acute toxicity tests to derive a long-term exposure guideline [7,9,10]. However, this approach relies heavily on the accuracy of the ACR employed. The objectives of the present study were to develop additional acute and chronic toxicity data to derive a more robust estimate of the ACR, as well as obtain sufficient chronic data to calculate a chronic guideline directly. In addition, because increasing concentrations of chloride at EKATI are associated with increases in concentrations of other major ions, such as Ca, Mg, K, carbonate, and sulfate, the present study was also designed to evaluate whether increases in ionic strength modify the toxicity of chloride. Water hardness was used as a proxy for increased concentrations of other ions.

The present study was designed to provide information necessary to establish safe levels of chloride in the receiving environment at EKATI. However, the results of the testing conducted here are broadly applicable.

METHODS

Test species were chosen on the basis of providing a suitable representation of invertebrate and fish species for which both acute and chronic exposures could be conducted in the laboratory following standardized procedures. Additional considerations for species selection were: inclusion of organisms that have previously been shown to be sensitive to chloride (e.g., cladocerans and fathead minnows [9]); selection of species that the laboratory had previous experience working with and that were available; selection of invertebrate species that would occur in planktonic (e.g., cladocerans and rotifers) and benthic habitats (e.g., amphipods, chironomids, and oligochates); and selection of species that were either resident, or were suitable surrogates for species that occur in subarctic lakes. Algal species were not tested because toxicity test data were already available for a number of these species, and they had generally exhibited a low degree of sensitivity to this anion [10].

Acute and chronic toxicity tests were conducted using two cladocerans (*Ceriodaphnia dubia* and *Daphnia magna*), two oligochaetes (*Lumbriculus variegatus* and *Tubifex tubifex*), a chironomid (*Chironomus dilutus*), an amphipod (*Hyalella azteca*), a rotifer (*Brachionus calyciflorus*), and two fish (rainbow trout, *Oncorhynchus mykiss*, and fathead minnows, *Pimephales promelas*). The tests followed standardized methods published by the U.S. EPA [11–13], Environment Canada [14,15], American Society for Testing Materials (ASTM) [16–19], or American Public Health Association (APHA) Standard Methods [20], with the exception of the test using *Lumbriculus variegatus*, which was adapted from a U.S. EPA method designed for evaluation of bioaccumulation with this species [13] to incorporate a growth (biomass) endpoint. Test durations, endpoints, and references to the methods followed are provided in Table 1.

Exposures were conducted in constant environment rooms that maintained temperature within 1°C of the target temperature. Water used in the tests was prepared by supplementing deionized water with reagent grade salts according to procedures specified by U.S. EPA [12], to achieve a hardness of between 80 and 100 mg/L, as $CaCO_3$, with the exception of the rainbow trout tests which were conducted using dechlorinated municipal tapwater, supplemented with salts in the ratios specified by U.S. EPA [12] for hardness adjustment, to a hardness of approximately 40 mg/L, as $CaCO_3$. Test solutions incorporated five concentrations, in addition to the control, following a 0.5-fold dilution series, and were prepared by addition of sodium chloride. The highest test concentration in the tests was 16 g/L as NaCl for acute tests and 8 g/L as NaCl for chronic tests, with the exception of chronic tests using *C. dubia, D. magna,* and *B. calyciflorus* which used 3, 15, and 16 mg/L NaCl, respectively, as the highest test concentration.

Chloride concentrations were measured on subsamples from the test solutions. Subsamples were collected at the beginning and end of each of the tests, with the exception of tests using *B. calyciflorus,* which was only subsampled at test initiation, as well as at intermediate intervals during the longer-term chronic toxicity tests, typically at weekly intervals. Concentrations of chloride were averaged for each test solution and the mean measured chloride concentration was used for calculation of the test endpoints.

Chronic toxicity tests using *Tubifex, Lumbriculus, Hyalella,* and *Chironomus* were performed using clean sediment comprised of a beach-collected sand that was rinsed with laboratory control water and supplemented with peat at a rate of 2% by

Table 1. Duration and endpoints of toxicity tests used to determine acute to chronic ratios for chloride

Species	Common name	Acute test duration	Method	Chronic test duration	Method	Chronic test endpoints
Ceriodaphnia dubia	Water flea	48 h	[12]	7 ± 1 d	[14]	Survival, reproduction
Daphnia magna	Water flea	48 h	[12]	21 d	[19]	Survival, reproduction
Oncorhynchus mykiss	Rainbow trout	96 h	[12]	54 d	[15]	Survival, development, biomass
Pimephales promelas	Fathead minnow	96 h	[12]	33 d	[11]	Survival, development, biomass
Lumbriculus variegatus	California blackworm	96 h	[16]	28 d	[13]	Survival, reproduction
Tubifex tubifex	Sludge worm	96 h	[16]	28 d	[18]	Survival, reproduction
Chironomus dilutus[a]	Midge	96 h	[16]	20 d	[13]	Survival, biomass
Hyallela azteca	Amphipod	96 h	[16]	28 d	[13]	Survival, weight
Brachionus calyciflorus	Rotifer	24 h	[17]	48 h	[20]	Survival, reproduction

[a] Formerly *Chironomus tentans.*

weight. Test solutions were renewed daily three times a week throughout exposure with freshly prepared chloride-spiked water, at which time Tetramin (for *Chironomus, Lumbriculus,* and *Tubifex*) or digested yeast, cerophyll, and trout chow (YCT) (for *Hyalella*) was added as food. These tests were conducted using four replicates per concentration in glass jars containing 100 ml of sediment and filled to 275 ml with the test solutions. The exposures were conducted at 23 ± 1°C with a 16:8 h light:dark photoperiod. *Lumbriculus* and *Tubifex* tests were conducted using 5 test organisms per replicate, whereas *Hyalella* and *Chrionomus* tests used 10 and 12 organisms per replicate, respectively. *Hyalella* tests were initiated with 7- to 8-d-old amphipods, *Chironomus* tests with <24-h posthatch organisms; both of these test species were obtained from Aquatic Biosystems. *Tubifex* and *Lumbriculus* tests were initiated with adults obtained from Aquatic Research Organisms.

Chronic toxicity tests using *Ceriodaphnia* were conduced in 10-ml volumes in 15-ml glass test tubes. Each concentration comprised 10 replicates, each containing a single <24-h-old daphnid obtained from in-house cultures. Solutions were renewed daily, at which time they were fed with a mixture of *Pseudokirchneriella* cells and YCT. Exposures were conducted at 25°C under a 16:8 h light:dark photoperiod. Survival and reproductive output were recorded daily for the three brood, 7 ± 1-d test. Tests with this species were performed regularly as a reference toxicant test and, consequently, the long-term geometric mean (and 2 SD range) from 20 separate tests was used for this water type, because this reflects a more robust approach than using a single test, although these reference toxicant tests did not have analytical confirmation of chloride concentrations.

Daphnia magna tests were conducted in 100-ml volumes in 250-ml glass beakers. Exposures were initiated with <24-h-old organisms obtained from in-house cultures, with one daphnid in each of 10 replicates. Solutions were renewed three times per week, at which time the organisms were fed with a mixture of *Pseudokirchneriella* cells and YCT. Exposures were conducted at 20°C under a 16:8 h light:dark photoperiod. Survival and reproductive output recorded daily for the 21-d duration of the test.

Brachionus calyciflorus were exposed for 48 h in a culture plate using a 0.5-ml exposure volume and eight replicates per concentration, each containing one rotifer. The test was initiated with organisms that were <4-h posthatch, and the solutions were supplemented with *Pseudokirchneriella* as food at test initiation. Exposures were conducted at 25°C in the dark. This test was considered to be a chronic test despite its relatively short duration because of the short life-history of this organism and the fact that the method incorporated a reproductive endpoint within this timeframe. Rotifer cysts were supplied by Micro Bio Tests, and were hatched in control water prior to test initiation.

Chronic toxicity tests with rainbow trout and fathead minnows were initiated with embryo-stage fish; rainbow trout gametes were obtained from Trout Lodge and dry fertilized in the laboratory prior to initiation of exposure, and fathead minnow embryos were obtained from Aquatic Biosystems. In the case of rainbow trout, the exposures were initiated within 30 min, and for fathead minnows, within 36 h of fertilization. Rainbow trout were exposed at 14°C using four replicates of 30 organisms in 500-ml volumes. Once the fish reached the swim-up stage, the number of fish was thinned to 10 per replicate, the exposure volume was increased to 2 L, and the fish were fed daily with *Artemia* nauplii. Fathead minnows were exposed at 25°C using five replicates with 15 organisms per replicate and using 100-ml exposure volumes for the first week, 250-ml for the next two weeks, and 500-ml for the remainder of the exposure period. Fathead minnows were fed twice daily with *Artemia* following hatch.

In general, acute toxicity tests were conducted under the same exposure regime and initiated with the same lifestage as described for the chronic tests, with the exception of the following: Acute tests on sediment-dwelling species were conducted in the absence of sediment; acute tests with *Ceriodaphnia* and *Daphnia* were conducted using five organisms per replicate; and acute tests using rainbow trout and fathead minnows were initiated using juvenile fish. Acute toxicity tests were conducted using four replicates and were performed under static conditions for 96 h, with the exception of tests using *Daphnia* and *Ceriodaphnia*, which were exposed for 48 h, and *Brachionus* which was exposed for 24 h. Acute tests were conducted without feeding, with the exception of the *Hyalella* test, which was fed with YCT after 48 h of exposure.

In addition, a series of toxicity tests were conducted using *C. dubia* to evaluate the relationship between water hardness and chloride toxicity using 7-d survival and reproduction tests. In advance of the tests, the organisms were cultured in water with hardnesses of 10, 20, 40, 80, 160, and 320 mg/L, as $CaCO_3$, for a minimum of two generations (more than two weeks) in order for the cladocerans to acclimate to the water hardness. Test water was prepared by addition of reagent grade salts to deionized water to achieve the target hardnesses; characteristics of the water types are summarized in Table 2. After the acclimation period, toxicity tests using sodium chloride were conducted with waters at each hardness using the organisms acclimated to the corresponding hardness (i.e., 10, 20, 40, 80, 160, and 320 mg/L). The tests were conducted according to the procedures outlined previously for chronic toxicity tests with chloride-spiked water using this species. *Ceriodaphnia dubia* was selected for this evaluation because this species was among the most sensitive to chloride, and could be acclimated to the range of required water hardnesses, and because of its relatively short test duration (~7 d) which enabled acclimation and testing within a reasonable period.

Statistical analyses were conducted using Comprehensive Environmental Toxicity Information System (CETIS) statistical

Table 2. Characteristics of waters used to evaluate the effect of hardness on toxicity of chloride to *Ceriodaphnia dubia*[a]

Hardness (mg/L as $CaCO_3$)	pH	Chloride (mg/L)	Sulfate (mg/L)	Sodium (mg/L)	Potassium (mg/L)	Calcium (mg/L)	Magnesium (mg/L)
10	6.8	0.2	11.3	3.3	0.3	2.2	1.5
20	7.0	0.5	22.6	6.6	0.5	4.4	3.0
40	7.2	1.0	45.1	13.1	1.0	8.8	6.1
80	7.8	1.9	90.2	26.3	2.1	17.6	12.1
160	8.2	3.8	180.5	52.6	4.2	35.3	24.2
320	8.3	7.6	360.9	105.1	8.4	70.6	48.5

[a] Concentrations are nominal, based on the quantities of salts added.

software package (Tidepool Scientific Software) on the basis of measured concentrations of chloride. Analyses followed procedures recommended by U.S. EPA for statistical analyses of toxicological data [11,12]. Median lethal concentration (LC50) estimates were calculated using Probit regression or, if assumptions for this method were not met, with Trimmed Spearman–Karber. Inhibition concentration values (IC*x*) associated with 10, 25, and 50% responses from chronic toxicity tests were calculated using linear interpolation. ACR values were calculated by dividing the IC25 from the chronic test by the LC50 from the acute test with the same species.

An SSD was calculated for chloride according to procedures specified by Environment Canada [21]. This procedure involves calculating toxicological thresholds for available data, and plotting a cumulative distribution of the dataset. No-observed-effect concentration (NOEC) values have often been used in constructing SSDs [22]; however, these values are subject to constraints associated with statistical power of the tests and use of these endpoints has been criticized [23]. Thus, consistent with Environment Canada guidelines [21], toxicological thresholds were defined as the most appropriate IC*x* value reflecting the threshold for toxicity in the test. Ideally, the IC10 was considered to be the toxicological threshold; however, if the IC10 value was lower than the NOEC, the test was not considered to be sufficiently robust to provide a reasonable estimate of the IC10, in which case, the IC25 was used as the toxicological threshold. In the event that suitable point estimates were not available for a given test, the next most appropriate endpoint was selected based on the following order of preference: maximum-acceptable-toxicant concentration (MATC) > NOEC > lowest-observed-effect concentration (LOEC) > median effect concentration (EC50). Only the most sensitive endpoint from long-term tests (e.g., reproduction, growth) was included in the distribution. In cases where multiple endpoints were available from different studies, a geometric mean of the values was used in the distribution.

Nonlinear regression was conducted using CETIS to model the distribution and calculate the 5th percentile of the distribution; this value, referred to as the HC5, is considered by Environment Canada to be protective of overall ecosystem health and function [21]. Models tested included normal, log normal, logistic, log logistic, log Gompertz and Weibull; relative fit of the models was evaluated on the basis of corrected second order Akaike information criteria (AIC), and the fit of individual candidate models with the smallest and similar AIC values were inspected to select the curve that best described the distribution, with particular attention to the lower tail of the distribution where the HC5 is located. Normal distribution of the dataset was tested using a Shapiro–Wilk test for normality ($p < 0.01$).

RESULTS AND DISCUSSION

All of the acute and chronic toxicity tests conducted in the present study met control performance requirements, with the exception of the chronic test with *Hyalella*, which had 62.5% survival and was lower than the control acceptance criterion of 80% survival. Survival in the three lowest test concentrations in this test was consistent with the control performance, ranging from 62.5 to 75% and a typical dose–response curve was obtained with the entire dataset. Consequently, the data from this test appear to provide useful information, despite not meeting the control performance specifications of the method. Interestingly, the chronic test using *Hyalella* was less sensitive to chloride than the acute test with this species, suggesting that the presence of control sediment and peat may ameliorate the toxicity of chloride; organic carbon influences the toxicity of a number of metals [24,25], but it is not known if this might explain decreased toxicity in this case.

Results of the chronic toxicity tests are presented in Table 3, and acute and chronic toxicity test data and calculated ACR values for nine species tested in this investigation are summarized in Table 4. The ACRs calculated in the present study include a second measure of the ACR for each of the three genera that were previously used in the development of the U.S. EPA chloride water quality guideline. The values used by U.S. EPA are also provided in Table 4. The genus mean ACRs were recalculated for each of these three genera, on the basis of the geometric mean of the two values. The overall ACR, calculated as the geometric mean of the ACR values for all nine species, was 3.50. Thus, the results of these tests suggest that the estimate for the ACR provided in the U.S. EPA guideline (7.59) likely overestimated the actual value by approximately twofold. Since this ACR value was employed by both the U.S. EPA in deriving the 1988 chronic guideline for chloride and by Environment Canada in conducting a risk assessment for road salts, these studies likely resulted in benchmarks that were unnecessarily conservative to be protective of long-term ecosystem health.

Where sufficient data exist, it is preferable to calculate long-term exposure guidelines directly on the basis of chronic toxicity test results, rather than relying on use of an ACR to calculate this value. As a result of the toxicity testing conducted here, a number of additional chronic toxicity values are now

Table 3. Results of sublethal toxicity tests[a]

Species	Endpoint	IC10[b]	IC25[b]	IC50[b]	NOEC	LOEC
Ceriodaphnia dubia	Reproduction	NR	454 (251–819)[c]	697 (540–901)[c]	NC	NC
Daphnia magna	Reproduction	NR	421 (262–825)	1,037 (684–1,491)	<506	506
Oncorhynchus mykiss	Biomass	NR	1,174 (733–1,344)	1,559 (1,362–1,679)	1,104	2,327
Pimephales promelas	Biomass	NR	704 (486–973)	958 (700–1,582)	558	1,058
Lumbriculus variegatus	Biomass	NR	825 (549–1,256)	1,366 (1,199–1,541)	<366	366
Tubifex tubifex	Reproduction	519 (235–529)	606 (391–632)	752 (628–803)	462	964
Chironomus dilutus	Biomass	2,316 (NC)	2,590 (2,118–2,590)	3,047 (2,732–3,047)	2,133	3,960
Hyallela azteca	Biomass	NR	1,705 (440–1,907)	2,298 (1,852–2,937)	2,210	4,237
Brachionus calyciflorus	Reproduction	1,241 (211–1,345)	1,505 (540–1,670)	1,945 (1,631–2,263)	1,120	2,330

[a] NOEC = no-observed-effect concentration; LOEC = lowest-observed-effect concentration; NR = not reported, because the IC10 was lower than the NOEC. Thus, the test data were not considered sufficiently robust to calculate an IC10; NC = not calculated, since these point estimate data were derived from multiple tests (i.e., 20).

[b] IC = inhibition concentration values (IC*x*) associated with 10, 25, and 50%.

[c] Mean (±two standard deviations) for 20 tests conducted as reference toxicant tests.

Table 4. Results of toxicity tests and acute-to-chronic ratio calculations[a]

Genus	Acute test LC50 (mg/L Cl)	Chronic test IC25 (mg/L Cl)	Acute–chronic ratio from the present study	Acute–chronic ratio from U.S. EPA [9]	Combined acute–chronic ratio
Ceriodaphnia	1,068 (603–1,533)[b]	454 (251–819)[b]	2.35	NC	2.35
Daphnia	3,630 (3,172–4,154)	421 (262–825)	8.62	3.95	5.84
Oncorhynchus	6,030 (5,916–6,145)	1,174 (733–1,344)	5.14	7.31	6.13
Pimephales	4,079 (3,644–4,565)	704 (486–973)	5.80	15.17	9.38
Lumbriculus	3,100 (2,759–3,483)	825 (549–1,256)	3.76	NC	3.76
Tubifex	5,648 (5,219–6,111)	606 (391–632)	9.31	NC	9.31
Chironomus	5,867 (5,452–6,313)	2,590 (2,118–2,590)	2.27	NC	2.27
Hyalella	1,382 (1,276–1,496)	1,186 (693–1,516)[c]	1.17	NC	1.17
Brachionus	1,645 (1,588–1,703)	1,505 (540–1,670)	1.09	NC	1.09
Geometric mean acute-to-chronic ratio			3.40	7.59	3.50

[a] LC50 = median lethal concentration; IC25 = 25% inhibition concentration; NC = not calculated by the U.S. EPA [9].

[b] Mean (±2 SD) for 20 tests conducted as reference toxicant tests.

[c] The chronic test for *Hyalella* was less sensitive than the acute test and, consequently, for calculation of the acute-to-chronic ratio, the chronic test value was assumed to be the LC25 (25% lethal concentration) from the acute test. The actual IC25 for biomass of *Hyalella* was 1,705 mg/L.

available for the toxicity of chloride; these values, combined with those from the literature, provide sufficient data to calculate a long-term exposure guideline directly using an SSD approach. Additional data using in the SSD include data that were reported in the U.S. EPA water quality guideline for *D. pulex* [26], *P. promelas* [26], *Nitzschia linearis* [27], *Chlamydomonas reinhardtii* [28], and *Chlorella emersonii* [29], as well as other data from the literature for *Lemna minor* [30] and *Stenonema modestum* [31]. To meet the recommendations of Environment Canada [21], point estimates from these studies (i.e., IC10 or IC25 values) were used where possible; sufficient data were present in one of these documents to recalculate the threshold using point estimates, rather than relying on the hypothesis tests that were reported in that study [26]. The data used in calculation of the SSD are shown in Table 5.

A subset of data points that were used in the U.S. EPA water quality guideline development were excluded here. For example, data for rainbow trout, attributed to Spehar and cited by U.S. EPA [9], were not available for review because this study was apparently not published. In addition, data for the sensitivity of a number of unicellular freshwater algae were excluded from the SSD because these papers only reported tolerance to chloride, rather than evaluating inhibition of growth compared to control performance [32,33].

Differing opinions have been expressed in the literature on the number of species required to construct an SSD, with as few as six [21], and up to 15 to 55 species being considered ideal to achieve an HC5 estimate with minimal variance [34]. In addition, the composition of the species assemblage reflected in the dataset can alter the outcome, particularly in cases where the toxicological mode-of-action varies between species, and the dataset needs to reflect the ecosystem being protected [35]. The dataset shown here has 15 data points, including nine invertebrates, two fish, two algae, one plant, and one diatom. The cumulative distribution appears to fit a single distribution, without any indication of a bimodal distribution (Fig. 1). Furthermore, a Shapiro–Wilk test for normality demonstrated that the dataset was normally distributed ($p = 0.55$), indicating that no unusual patterns in the data occurred. Thus, it appears that the species reflected in the dataset provide a reasonable distribution from which to calculate an HC5.

The HC5 was calculated using a Weibull distribution to model the SSD dataset using nonlinear regression. Log logistic and log normal models produced a similar fit to the Weibull

Table 5. Chronic toxicity test data used for calculation of the species sensitivity distribution[a]

Species	Category	Threshold value (mg/L Cl)		Source
Daphnia pulex	Cladoceran	Reproduction; 21 d IC10[b]	368	[26]
Daphnia magna	Cladoceran	Reproduction; 21 d IC25	421	Present study
Ceriodaphnia dubia	Cladoceran	Reproduction; 7 d IC25	454	Present study
Tubifex tubifex	Oligochaete	Reproduction; 28 d IC25	519	Present study
Pimephales promelas	Fish (non-salmonid)	Survival; 33 d LC10[b]	598	[26][c]
		Biomass; 32 d IC25	704	Present study
		Geometric mean	649	
Lumbriculus variegatus	Oligochaete	Reproduction; 28 d IC25	825	Present study
Lemna minor	Plant	Growth; 96 h MATC	1,172	[30]
Oncorhynchus mykiss	Fish (salmonid)	Biomass; 56 d IC25	1,174	Present study
Nitzschia linearis	Diatom	Growth; 5 d EC50	1,482	[27]
Brachionus calyciflorus	Rotifer	Reproduction; 48 h IC25	1,505	Present study
Hyalella azteca	Amphipod	Growth; 28 d IC25	1,705	Present study
Chironomus dilutus	Midge	Growth; 20 d IC25	2,316	Present study
Chlamydomonas reinhardtii	Alga	Growth; 6 d EC∼50	3,014	[28]
Stenonema modestum	Mayfly	Survival; 14 d MATC	3,074	[31]
Chlorella emersonii	Alga	Growth; 8–14 d MATC	7,000	[29]

[a] IC = inhibition concentration values (IC*x*) associated with 10 and 25%; LC10 = 10% lethal concentration; MATC = maximum acceptable toxicant concentration; EC50 = median effect concentration.

[b] Point estimates were calculated using linear interpolation based on original data provided in Birge et al. [26].

[c] Point estimates were calculated using multiple linear estimation (Probit) based on original data provided in Birge et al. [26].

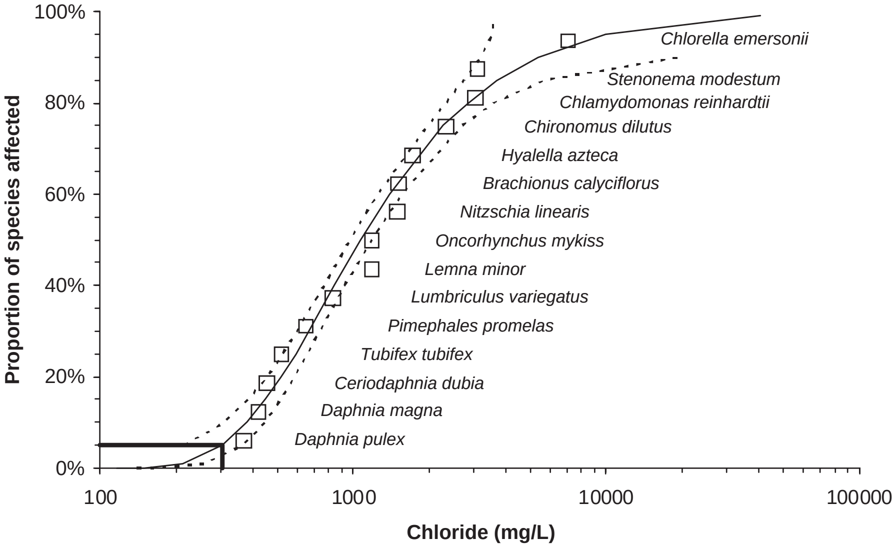

Fig. 1. Species sensitivity distribution for chronic toxicity test data using chloride. The squares represent the empirical data, the solid line represents the modeled distribution, and the dashed lines represent the 95% confidence limits.

distribution (i.e., produced similar AIC values); however, the Weibull model was selected because it provided a closer fit to the data in the lower tail of the distribution, by inspection. The HC5 (and 95% confidence intervals) calculated on this basis was 307 (217 to 369) mg/L (Fig. 1). This value is lower than all of the thresholds reported in Table 4 and appears to be appropriately protective to apply as a long-term objective for chloride, at least under moderately hard conditions.

The results of toxicity tests using *C. dubia* conducted at various hardnesses are provided in Table 6. A clear decrease in the toxicity of chloride was observed with increasing hardness across the range of 10 to 160 mg/L hardness. Lasier et al. [36] also reported lower chronic toxicity of chloride in higher hardness water with this species, and Mount et al. [8] reported decreased acute toxicity of chloride to *C. dubia* when tested as a combination of sodium chloride and calcium chloride (resulting in higher hardness), compared with sodium chloride alone. Interestingly, these authors also reported that the toxicity of the combined salts was lower than calcium chloride alone, which would be even higher in hardness than the mixture. These data suggest that decreased toxicity corresponding to increasing hardness may relate to maintenance of a tolerable balance in molar ratios of cations, rather than a mechanistic effect of hardness (i.e., Ca or Mg ions) itself. Regardless, the data presented here demonstrate a clear reduction in toxicity of sodium chloride in solutions with higher hardness, with water hardness potentially being a proxy for higher overall ionic strength or more balanced ionic ratios of major ions.

Table 6. Results of *Ceriodaphnia dubia* chronic toxicity tests conducted across a range of hardnesses[a]

Hardness (mg/L as $CaCO_3$)	Chloride toxicity endpoints (mg/L Cl)		
	Ceriodaphnia reproduction (IC25 [95% CL])	*Ceriodaphnia* reproduction (IC50 [95% CL])	*Ceriodaphnia* survival (LC50 [95% CL])
10	117 (94–169)	161 (126–211)	132 (107–161)
20	264 (104–280)	301 (275–362)	316 (268–373)
40	146 (82–277)	481 (207–541)	540 (460–633)
80	454 (251–819)[b]	697.4 (540–901)[b]	1,134 (858–1,410)[b]
160	580 (210–733)	895 (706–1,177)	1,240 (1,025–1,501)
320	521 (361–588)	700 (613–784)	1,303 (1,019–1,665)

[a] IC = inhibition concentration values (ICx) associated with 25 and 50%; CL = confidence limits; LC50 = median lethal concentration.

[b] Mean and 2 SD range of 20 data points for chronic toxicity tests using chloride.

A logarithmic regression of the data for hardnesses of 10 through 160 mg/L resulted in R^2 values of 0.95, 0.99, and 0.78 for LC50, IC50, and IC25 values, respectively, indicating a strong positive relationship between these parameters (Fig. 2). Above a hardness of 160 mg/L, an additional reduction in toxicity was not as apparent, with generally similar values for sensitivity to chloride at hardnesses of 160 and 320 mg/L.

The majority of toxicity data used to establish the SSD value of 307 mg/L were derived from toxicity tests conducted under moderately hard water conditions (80 to 100 mg/L, as $CaCO_3$). Consequently, this value may not be sufficiently conservative for soft-water conditions, and appears to be unnecessarily conservative at hardnesses exceeding 100 mg/L. Using the slope of the hardness toxicity relationship shown in Figure 2, the results from the SSD can be hardness-adjusted to accommodate this relationship in a similar manner to water quality guidelines for metals such as Zn, Cu, Cd, and Ni.

The relationship between IC25 values and hardness across a range of 10 to 160 mg/L resulted in a logarithmic trendline described by Equation 1.

$$\mathrm{IC25}_{(\mathrm{hardness}\,x)} = [161 \cdot \ln_{(\mathrm{hardness}\,x)}] - 281.73 \tag{1}$$

Thus, using the water quality benchmark of 307 derived from the SSD for a hardness of 80 mg/L, and the IC25 for *C. dubia* of 423.78 mg/L chloride (calculated from Eqn. 1, for a hardness of 80 mg/L), the objective can be linked to hardness by incorporating this equation into Equation 2.

$$\begin{aligned} \mathrm{WQO}_{(\mathrm{hardness}\,x)} &= [\mathrm{WQO}_{(\mathrm{hardness}\,80)}/\mathrm{IC25}_{(\mathrm{hardness}\,80)}] \\ &\cdot [161 \cdot \ln_{(\mathrm{hardness})} - 281.73] = (307/423.78) \\ &\cdot [161 \cdot \ln(\mathrm{hardness}) - 281.73] \\ &= [116.63 \cdot \ln(\mathrm{hardness})] - 204.09 \end{aligned} \tag{2}$$

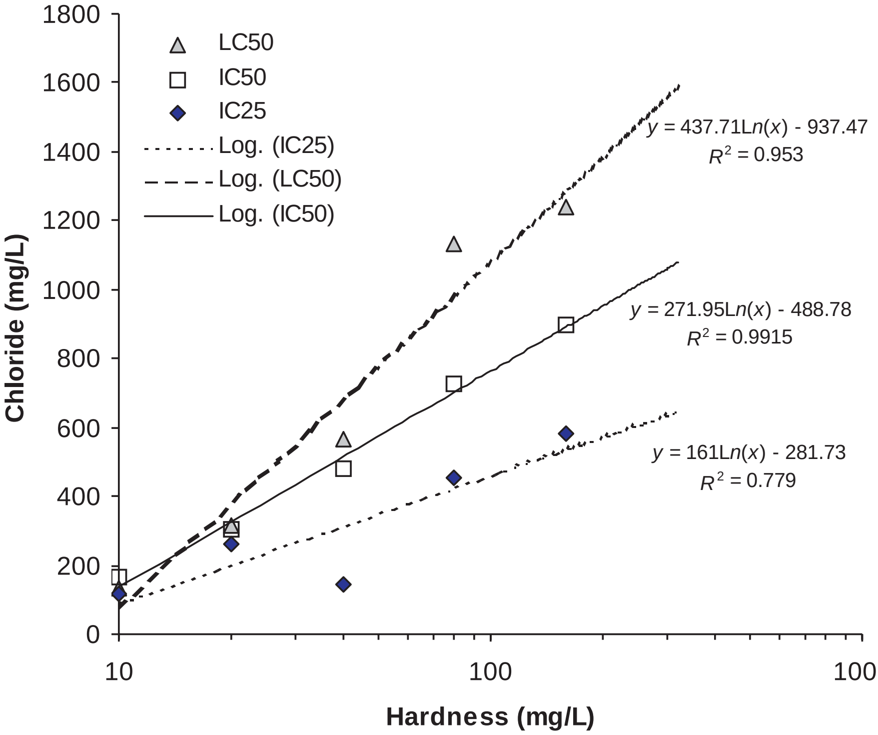

Fig. 2. Relationship between hardness and sensitivity to chloride for reproduction (IC25 and IC50 inhibition concentrations) and survival median lethal concentration (LC50) endpoints.

where: $WQO_{(hardness\ x)}$ = Water quality objective for chloride at hardness (*x*);

$WQO_{(hardness\ 80)}$ = Water quality objective for chloride at hardness 80 mg/L (i.e., the HC5 from the SSD); $IC25_{(hardness\ 80)}$ = Concentration resulting in a 25% reduction in reproduction of *C. dubia* at hardness 80 mg/L $CaCO_3$.

Thus, the hardness-specific WQO for chloride across a range of 10 to 160 mg/L hardness is established as

$$\mathrm{WQO} = [116.63 \cdot \ln(\mathrm{hardness})] - 204.09$$

Using the water quality benchmarks provided in Table 7should provide sufficient protection against adverse effects in receiving environments impacted by chloride.

Although data relating water hardness to the toxicity of chloride are only available for one species (i.e., *C. dubia*), it appears reasonable to assume that a similar response would also occur with other cladocerans, and potentially with other species as well, although uncertainly exists as to the extent to which that would be the case. Regardless, the range of water quality guidelines proposed in Table 7 (i.e., 64 to 388 for hardnesses ranging from 10 to 160 mg/L) is lower than the threshold for toxicity to any non-cladoceran species reported in Table 4. Thus, even if this phenomenon was limited to the cladocera, incorporation of hardness into a guideline would not appear to result in risk to other taxa, and takes account of the higher sensitivity of cladocerans to chloride under low hardness conditions.

The results presented here suggest that current U.S. EPA water quality guidelines for chloride may not be sufficiently protective of aquatic life under soft-water conditions. This finding has particular significance in areas of road salt use, because snow-melt runoff is very low in hardness and can contain significant concentrations of chloride. Use of road salt formulations that combine calcium chloride with sodium chloride would appear to result in lower risk for adverse effects in the environment because this would confer an increased hardness to runoff and, consequently, lower risk of adverse effects. Conversely, the data presented here suggest that current water quality guidelines for chloride may be unnecessarily conservative in waters with moderate or high hardness.

Table 7. Hardness-dependent water quality benchmarks for chloride, calculated on the basis of application of the relationship between IC25 (inhibition concentration value associated with 25%) for *Ceriodaphnia dubia* reproduction and water hardness to the 5th percentile of the species sensitivity distribution (HC5)

Water hardness (mg/L $CaCO_3$)	Water quality objective for chloride $WQO_{(hardness\ x)}$ (mg/L)
10	64
20	145
40	226
60	273
80	307
100	333
120	354
140	372
160	388
>160	Not established

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Assessing the toxicity of sodium chloride to the glochidia of freshwater mussels: Implications for salinization of surface waters

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Freshwater mussel larvae were acutely sensitive to sodium chloride, such that chloride levels in some Canadian rivers may pose a threat to the survival of this early life stage.

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ABSTRACT

Chloride concentrations in surface waters have increased significantly, a rise attributed to road salt use. In Canada, this may be a concern for endangered freshwater mussels, many with ranges limited to southern Ontario, Canada's most road-dense region. The acute toxicity of NaCl was determined for glochidia, the mussel's larval stage. The 24 h EC50s of four (including two Canadian endangered) species ranged from 113–1430 mg Cl L^{-1} (reconstituted water, 100 mg $CaCO_3$ L^{-1}). To determine how mussels would respond to a chloride pulse, natural river water (hardness 278–322 mg $CaCO_3$ L^{-1}) was augmented with salt. *Lampsilis fasciola* glochidia were significantly less sensitive to salt in natural water (EC50s 1265–1559 mg Cl L^{-1}) than in reconstituted water (EC50 285 mg L^{-1}). Chloride data from mussel habitats revealed chloride reaches levels acutely toxic to glochidia (1300 mg L^{-1}). The increased salinization of freshwater could negatively impact freshwater mussels, including numerous species at risk.

1. Introduction

The increase in the chloride concentration of North American surface waters over the past 30 years has been correlated with the increased application of de-icing salts on paved surfaces (Kaushal et al., 2005; Jackson and Jobbágy, 2005). Kaushal et al. (2005) demonstrated that chloride levels in rivers and streams were correlated with the percentage of impermeable surfaces in the watershed. This increased salinization of freshwater has implications for both human and ecosystem health. Chloride concentrations in some drinking water reservoirs now exceed the level for potable water (Kaushal et al., 2005) and numerous urban streams frequently exceed the levels considered harmful to aquatic life (Evans and Frick, 2001; Trowbridge et al., 2010). In addition to the seasonal influx of salt in snowmelt and runoff, groundwater (Howard and Haynes, 1993; Kelly et al., 2008; Roy and Bickerton, 2010) and soils (Kincaid and Findlay, 2009) can also act as reservoirs releasing chloride throughout the year. Therefore, it is quite probable that the full impact of freshwater salinization has yet to be realized, not only because millions of tons of road salt are applied each year (Environment Canada and Health Canada, 2001), but also because delayed and longer-term inputs of chloride from contaminated soils and groundwater are expected (Kelly et al., 2008; Kincaid and Findlay, 2009). Kaushal et al., (2005) suggested that baseline salinity in the Northeastern United States is approaching levels where significant changes in ecological communities and ecosystem function are expected. Recent studies suggest that such shifts may in fact already be occurring for some contaminant sensitive groups. For example, Collins and Russell (2009) concluded that exposure to road salt affects amphibian community structure and species richness by excluding salt-sensitive species from high chloride environments.

Freshwater mussels, one of the most imperiled groups of organisms (Ricciardi and Rasmussen, 1999; Lydeard et al., 2004), are also known to be particularly sensitive to some waterborne contaminants. In fact environmental pollution is considered to be one of the factors responsible for their decline (Strayer et al., 2004; Lydeard et al., 2004). Nearly 70% of North American freshwater mussels are designated as either threatened, endangered, or in decline (Williams et al., 1993; Neves et al., 1997). Recent studies have reported that for some contaminants, freshwater mussel larvae and juveniles are much more sensitive than standard test organisms, leading to concerns that water quality regulations may not protect freshwater mussels (Augspurger et al., 2003; Wang et al., 2007, 2009; March et al., 2007). In Canada, the geographical distribution of freshwater mussels is thought to be limited by temperature, either because the mussels themselves or their fish hosts reach their lower limit of thermal tolerance (Metcalfe-Smith et al., 1998). Many species reach the northern limit of their range in

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the lower Great Lakes Basin, with 40 of Canada's 53 freshwater mussels species found in this area (Metcalfe-Smith et al., 1998). Of particular concern is that the range of eight Canadian mussel species classified as federally endangered are limited to the heavily populated and road dense southern Ontario. However, it is unknown whether the contamination of mussel habitats by chloride will affect the mussels, particularly their sensitive early life stages. The parasitic larvae of freshwater mussels, called glochidia, are released from the brooding chambers (marsupia) in the female's gills into the water column in order to make contact with fish hosts. In Canada, most glochidia are released between May and October, depending upon species specific temperature cues for release. Fortunately, the typical release period does not coincide with the seasonal influx of chloride associated with snowmelt runoff, but the steady increase in baseline chloride levels along with periodic summer chloride pulses from stormwater runoff and groundwater upwelling (Howard and Haynes, 1993; Kincaid and Findlay, 2009) may pose a risk to this imperiled, but ecologically significant group of animals.

This study examined acute sodium chloride (NaCl) toxicity in glochidia and compared median effective concentrations (EC50s) to chloride concentrations in the mussel's natural habitat. Specifically, sensitivity was determined for five species of mussels, three of which are designated as federally endangered in Canada. Toxicity tests with glochidia and NaCl were conducted in both standard reconstituted waters and natural waters. Reconstituted water exposures were used to determine the sensitivity of glochidia to chloride in relation to other aquatic organisms and the effect of water hardness on chloride toxicity. Natural water exposures employed water collected from four southern Ontario rivers that support diverse mussel populations (9–34 species). The aim of the natural water exposures was to determine how glochidia would respond to an episodic pulse of chloride in their habitat.

2. Materials and methods

2.1. Mussel collection and laboratory care

Gravid female mussels were collected from streams and rivers in southern Ontario. The period of gravidity varied with species such that *Lampsilis siliquoidea* (Barnes 1823) (fatmucket) and *Lampsilis cardium* (Rafinesque 1820) (plain pocketbook) were collected in May, *Lampsilis fasciola* (Rafinesque 1820) (wavy-rayed lampmussel) in mid-July, and *Epioblasma torulosa rangiana* (Lea 1838) (northern riffleshell) and *Ptychobranchus fasciolaris* (Rafinesque 1820) (kidneyshell) in early September. The endangered *L. fasciola*, *P. fasciolaris* and *E. torulosa rangiana* were collected under Canadian Species at Risk Permits (SECT 08 SCI 007, SECT 73 SARA C&A 09-012). Because the availability of gravid females varied each year, toxicity tests were conducted over two field seasons (2008–2009). In addition, acute chloride sensitivity of *L. siliquoidea* glochidia was assessed using gravid females collected from two different watersheds (one in each of 2008 and 2009) and toxicity tests with *L. fasciola* were also conducted in both years but using different gravid females collected from the same field site. Although mussels for this study were collected in Ontario, all species examined are also found in the U.S. (Parmalee and Bogan, 1998).

Gravid mussels were held at the University of Guelph's Aqualab facility and maintained in a flow-through system with well water held at 10 ± 2 °C (to prevent the glochidia release). Mussels were fed approximately 1.2×10^{10} algae cells per mussel per day with a commercial shellfish diet (Instant Algae Shellfish Diet 1800®, Richmond Hill, ON). Glochidia for testing were collected by flushing the marsupia (i.e., brooding chambers) with a water-filled syringe. The viability of each mussel's glochidia was assessed (described below) prior to use. Prior to initiating an exposure, glochidia collected from gravid mussels held at 10 °C, were gradually (over 2–3 h) acclimated to the exposure temperature (21 °C) through dilutions with room temperature reconstituted water. Glochidia were pooled from a minimum of three gravid females for each experiment. For the endangered species, glochidia were only collected from one marsupium gill, and each mussel was returned to the location from which they were collected to facilitate the release of remaining glochidia in their natural habitat.

2.2. Toxicity testing

Acute toxicity tests with glochidia were modeled after the American Society for Testing and Materials' method for conducting toxicity tests with the early life stages of freshwater mussels (ASTM, 2006). Briefly, the viability of glochidia were evaluated after exposure to waterborne contaminants. In order to parasitize fish, glochidia must be viable, which means they must be able to close their valves and clamp down on a fish's gill in order to encyst. Glochidia viability (i.e., ability to close valves) was assessed prior to exposure and after 24 h of exposure in a sub-sample (100–200) of the glochidia (500–1000) through the addition of a saturated salt solution (NaCl 240 g L^{-1}). Viability was calculated using the following equation: Percent Viability = 100 × (Number of closed glochidia after NaCl addition − Number of closed glochidia before NaCl addition)/(Number of closed glochidia after NaCl addition + Number of open glochidia after NaCl addition). Results are expressed as (chloride) effective median concentrations (EC50) rather than median lethal concentrations (LC50), but as they are obligatory parasites, for practical purposes non-viable glochidia should be considered 'dead' because they would be unable to attach to a host fish and complete their life cycle.

The ASTM (2006) method indicates that glochidia control survival remain above 90%. Therefore, for toxicity tests conducted in reconstituted water, pre-exposure ($t = 0$) and post-exposure ($t = 24$ h) control survival (i.e. viability) were determined. In addition, for toxicity tests conducted in natural waters, 24 h control survival in each river water (without salt augmentation) was determined.

An aqueous stock made from certified ACS grade (Fisher Scientific) sodium chloride (NaCl) was used to create exposure solutions. Waters (reconstituted or field-collected) were spiked with NaCl (nominal, 0–10 g NaCl L^{-1}) and held in the dark at 4 °C for 48 h before initiation of an exposure. Exposures were conducted in 250 mL glass beakers, under a 16:8 light:dark cycle at 21 ± 2 °C. Water quality including dissolved oxygen (DO), pH, alkalinity, dissolved organic carbon (DOC), water hardness as well as the concentration of major ions (Na, K, Ca) and trace metals were assessed at exposure initiation. DO, pH and Cl were also measured upon completion of an exposure. Water analysis was conducted by the Canadian National Laboratory for Environmental Testing (Environment Canada, Burlington, ON). Chloride was measured by Ion Chromatography (detection limit (DL) 0.02 mg L^{-1}). Mean chloride recovery was 100.4% (STD 0.36) using the National Water Research Institute's (NWRI) certified reference material ION-915. Metals, including copper, were measured by ICP-SFMS (copper DL 0.02 μg L^{-1}). Mean copper recovery was 100% (STD 0.16) using the National Research Council of Canada's certified reference material SLRS-4. DOC (DL 0.1 mg L^{-1}) was measured by a UV Persulfate Total Organic Carbon Analyzer. Mean DOC recovery was 95.5% (STD 0.2) using NWRI's certified reference material WINN-02. Major ions (e.g. potassium, DL 0.01 mg L^{-1}) were analyzed by Atomic Absorption Spectrometry. Mean potassium recovery was 99.4% (STD 0.4) using VHG Labs (New Hampshire) certified reference material QWSMIN. Glassware was acid washed with 10% nitric acid (Reagent Grade, Fisher Scientific) prior to use and solutions were made with Millipore™ water.

2.3. Chloride sensitivity in reconstituted waters

A series of toxicity tests were conducted with NaCl and reconstituted waters (ASTM, 2003). For each species studied at least one acute toxicity test was conducted in moderately-hard reconstituted water (95–115 mg $CaCO_3$ L^{-1}). In addition, a series of exposures were conducted in reconstituted waters of varying hardness (range 47–322 mg $CaCO_3$ L^{-1}) using *L. siliquoidea* glochidia.

2.4. Chloride sensitivity in natural waters

A series of toxicity tests were conducted with water collected from four significant mussel habitats in southern Ontario (Table 1). River water (10 L) was collected just below the surface where the water was visibly flowing. Water samples were held in the dark at 4 °C until used in an exposure (maximum one week). Acute exposures in NaCl-spiked natural waters were conducted with *L. fasciola* glochidia as described above.

In addition to the *L. fasciola* natural water exposures, another natural water test was conducted with *P. fasciolaris* glochidia. Unlike the other species examined which release free glochidia, *P. fasciolaris* produces conglutinates. These small packets of glochidia (100–200) resemble fish prey and serve to enhance infection of host fish. Two intact conglutinates were used in each replicate test concentration. One conglutinate was opened (by gently tearing casing with fine forceps) after 24 h and the other after 48 h of exposure to assess the viability of the encased glochidia (24 h data shown). Because the number of conglutinates was limited, an exposure with chloride-spiked natural water was selected as the most ecologically relevant test to conduct with this endangered species.

2.5. Statistical analysis

Chloride EC50s and EC20s were determined by Probit Analysis (Statistical Package for the Social Sciences (SPSS)) version 11.0 using measured chloride concentrations and presented with 95% confidence intervals (CI) (e.g. EC50 (95% CI). EC50s and EC20s were considered to be significantly different when their 95% CI did not overlap (Environment Canada, 2005). Linear regression analysis was conducted (SigmaStat version 3.2) to examine the relationship between water hardness and chloride toxicity (EC50s). Note: Although EC50s and EC20s are reported with respect to the chloride component of NaCl, no attempt was made to determine the toxic

Table 1
Summary of selected water chemistry[a] parameters for four Canadian rivers used in acute sodium chloride exposures with *Lampsilis fasciola* glochidia, along with pre-exposure ($t = 0$) viability, and post-exposure ($t = 24$ h) reconstituted water and un-spiked river water control survival.

Water source[b]	Chloride (mg L^{-1})	Potassium (mg L^{-1})	Copper (μg L^{-1})	DOC (mg L^{-1})	pH	Water hardness (mg $CaCO_3$ L^{-1})	% Viability, $t = 0$	% Viability reconstituted water, $t = 24$	% Viability river water, $t = 24$ h
Sydenham River	34.1	4.6	6.0	4.5	8.25	292	91.5	91.7	92.8
Grand River	66.8	3.3	4.9	6.2	8.62	278	91.5	91.7	89.1
Maitland River	57.4	5.8	5.6	6.7	8.32	322	91.0	87.1	85.1
Thames River	75.2	4.7	4.9	4.4	8.29	306	91.0	87.1	78.1

[a] Measured chloride, potassium, copper, dissolved organic carbon (DOC), pH, and water hardness values represent background concentrations in un-spiked river water.
[b] Rivers located in Ontario, Canada.

mode of action of NaCl in glochidia, therefore this study cannot distinguish whether glochidia are responding to the chloride ion or the sodium ion.

2.6. *Chloride concentrations and mussel distribution data in southern Ontario*

To assess the potential threat that chloride poses to freshwater mussels, the chloride levels in key mussel habitats in southern Ontario were examined. In Ontario, watersheds are managed locally by Conservation Authorities. The Canadian Department of Fisheries and Oceans has produced distribution lists of endangered mussels and fish species for each Conservation Authority (CA). Therefore, mussel distribution data and chloride concentrations are presented according to CA. Four CAs were selected for in-depth analysis of field-measured chloride levels and laboratory toxicity tests with waters from these habitats. The CAs selected along with their main mussel habitat (i.e. river) were the Grand River CA (Grand River), St. Clair Region CA (Sydenham River), Maitland Valley CA (Maitland River), and Upper Thames River and Lower Thames Valley CAs (Thames River). For the purposes of this summary, data from the Upper and Lower Thames CAs were combined. Chloride concentrations measured from 1998 to 2008 at 105 sites across the CAs were determined by the (Ontario) Provincial Water Quality Monitoring Network (PWQMN) and provided by the Ontario Ministry of the Environment (PWQMN, 2009).

Individual chloride concentrations at each site were averaged over time. These 'site averages' were then averaged to determine an overall mean for each CA, referred herein to as a "CA Mean". Site averages, rather than individual readings were used to calculate each CA Mean to prevent skewing of the mean by differences in sampling frequency or extreme readings. The 'CA Range' demonstrates the maximum and minimum individual chloride concentrations across the CA over the 10 years examined. The number of endangered mussel species reported for each CA was obtained from Canadian Department of Fisheries and Oceans (DFO) maps (DFO, 2010).

3. Results

3.1. *Chloride sensitivity in reconstituted waters*

Glochidia control survival (24 h) for the four mussel species employed in NaCl exposures with moderately-hard reconstituted water is presented in Table 2. With one exception (*L. siliquoidea*, 2008), all tests met the ASTM (2006) requirement of less than 10% drop in control survival. The 24 h chloride EC50s ranged from 113 mg Cl L^{-1} for *L. fasciola* (2008) to 1430 mg Cl L^{-1} for *L. siliquoidea* (2009) (Fig. 1). In addition to interspecific variation, *L. siliquoidea* glochidia collected from different water bodies exhibited significantly different EC50s. Those collected from Cox Creek (2008) produced an EC50 of 168 (135–198) mg Cl L^{-1}, while those collected from the Maitland River (2009) produced an EC50 of 1430 (1350–2953) mg Cl L^{-1}. In contrast, both tests (2008, 2009) of *L. fasciola* glochidia from a single field site produced relatively similar EC50s (113 (63–163), 285 (163–451) mg Cl L^{-1}, respectively).

Table 2
Pre-exposure ($t = 0$) viability and post-exposure ($t = 24$ h) control survival for freshwater mussel glochidia as well as observed 24 h chloride EC50s (95% confidence intervals) from sodium chloride exposures conducted in reconstituted water.

Mussel species	% Viability ($t = 0$)	% Viability ($t = 24$)	EC50 (95% CI) (mg Cl L^{-1})
Lampsilis siliquoidea[a] (2008)	91.3	77.4	168 (135–189)
Lampsilis siliquoidea (2009)	93.4	93.2	1430 (1350–2953)
Lampsilis cardium	91.1	88.3	817 (770–869)
Lampsilis fasciola[b] (2008)	91.9	92.2	113 (63–163)
Lampsilis fasciola (2009)	93.8	91.4	285 (163–451)
Epioblasma torulosa rangiana	95.2	91.3	244 (230–260)

[a] Gravid *L. siliquoidea* were collected from different water bodies in 2008 and 2009.
[b] Gravid *L. fasciola* were collected from the same site in 2008 and 2009.

A series of exposures with *L. siliquoidea* glochidia demonstrated that chloride sensitivity is influenced by water hardness (Table 3). A linear relationship between the 24 h chloride EC50s and water hardness ($r^2 = 0.97$) was observed for water hardness between 47 and 172 mg $CaCO_3$ L^{-1}, but no further protection was afforded when hardness increased to 322 mg $CaCO_3$ L^{-1}.

3.2. *Chloride sensitivity in natural waters*

Control survival of *L. fasciola* glochidia in the field-collected waters was more variable (78–93%) than in reconstituted water (87–92%) (Table 1). The 24 h chloride EC50 values for *L. fasciola* glochidia were similar across the natural waters tested (1265–1559 mg Cl L^{-1}) (Table 4), but all were significantly higher than the EC50 (285 (163–451) mg Cl L^{-1}) produced in reconstituted water with glochidia from the same gravid females. The 24 h natural water control survival for *P. fasciolaris's* conglutinate encased glochidia was 95% and the EC50 was 3416 (3059–3835) mg Cl L^{-1}.

Fig. 1. Chloride EC50s (24 h) for glochidia (larvae) of four species of freshwater mussels. Exposures were conducted in reconstituted moderately-hard water (95–115 mg $CaCO_3$ mg L^{-1}). Error bars represent 95% confidence intervals around the EC50. Asterisks indicate Canadian endangered species. Toxicity tests with *Lampsilis siliquoidea* and *Lampsilis fasciola* were conducted in both 2008 and 2009. *L. siliquoidea* were collected from different water bodies. *L. fasciola* were collected from the same field site both years.

Table 3
Concentrations of chloride, potassium, and water hardness for reconstituted waters employed in acute sodium chloride exposures with *Lampsilis siliquoidea* glochidia as well the pre-exposure ($t = 0$) viability, post-exposure ($t = 24$ h) control survival, and observed 24 h chloride EC50.

Reconstituted water	Chloride (mg L^{-1})	Potassium (mg L^{-1})	Water hardness (mg $CaCO_3$ L^{-1})	% Viability ($t = 0$)	% Viability ($t = 24$)	EC50 (95% CI) (mg Cl L^{-1})
Soft	1.8	1.0	47	89.5	87.7	763 (523–1214)
Moderately-hard	2.8	2.4	99	93.4	93.2	1430 (1350–1518)
Hard	5.5	4.7	172	89.5	86.4	1962 (1447–2953)
Very hard	8.9	9.4	322	93.4	90.7	1870 (1595–2225)

3.3. *Chloride concentrations and mussel distribution in southern Ontario*

A summary of chloride concentrations in four rivers in southern Ontario, along with the number of mussels species found in each habitat is presented in Table 4. Water hardness for the selected rivers ranged from 278 to 322 mg $CaCO_3$ L^{-1}. Although the range in mean chloride concentration was narrow (38–58 mg Cl L^{-1}), the range of individual measured chloride concentrations over the 10 years examined was much broader, covering 2–1300 mg L^{-1}.

4. Discussion

4.1. *Chloride sensitivity in reconstituted waters*

Acute toxicity testing in reconstituted water revealed that glochidia were sensitive to chloride, although significant interspecific and in one case intraspecific variation was observed. The EC50 values for free glochidia of the four mussel species tested ranged from 113 to 1430 mg Cl L^{-1} (Fig. 1). This 13 fold difference in chloride sensitivity between mussel species was not unlike the variation observed by Wang et al. (2007) (12 fold for 9 species) and Gillis et al. (2008) (5 fold for 8 species) in the acute sensitivity of glochidia to copper. Although chloride toxicity data for glochidia is limited, NaCl has been used as a reference toxicant for glochidia toxicity tests. Bringolf et al. (2007) reported EC50s from 0.55 to 3.3 g NaCl L^{-1} (334–2008 mg Cl L^{-1}) for five species of mussel glochidia, Valenti et al. (2007) reported EC50s from 2.68 to 3.08 g NaCl L^{-1} (1625–1868 mg Cl L^{-1}) for three species, and finally Cope et al. (2008) reported EC50s of 2.0 and 2.7 g NaCl L^{-1} (1213–1638 mg Cl L^{-1}) for *L. siliquoidea* glochidia. In this study there also appears to be intraspecific variation in chloride sensitivity. Although *L. fasciola* collected from the same site (Grand River, ON) on two different occasions produced somewhat similar EC50s (113 and 285 mg Cl L^{-1}), *L. siliquoidea* glochidia from two separate water bodies produced EC50s that varied by eight fold (Maitland River, 1430 mg L^{-1}; Cox Creek 168 mg L^{-1}). Perhaps the discrepancy is simply due to the fact that one batch of glochidia was healthier (Maitland River, control survival 93.2%) than the other (Cox Creek, control survival 77.4%) or perhaps prior exposure or even acquired tolerance may alter the response of glochidia to contaminants. But regardless, these data indicate that mussels from different water bodies may respond differently to chloride. While this observation was only based on the chloride sensitivity of one mussel species from two watersheds, possible differences in contaminant sensitivity across watersheds should be considered when selecting gravid females for toxicity testing with glochidia.

Even taking the variability between species into account, glochidia are still notably more sensitive to chloride than most previously tested aquatic organisms. While a full review of chloride toxicity in freshwater organisms is beyond the scope of this paper, Table 5 illustrates that compared to other groups, freshwater mussel larvae, were more sensitive to chloride. Particularly interesting is that some species of mussel glochidia (Fig. 1) experience chloride toxicity at a fraction of the concentration required to kill *Daphnia* (Mount et al., 1997; Harmon et al., 2003), a standard test organism often used to assess the toxicity of chemicals and effluents. Implications of this sensitivity for natural populations of freshwater mussels are discussed below.

4.2. *Effect of water hardness*

Water hardness had a significant effect on the sensitivity of glochidia to chloride. A two fold increase in the EC50 was observed when hardness increased from 47 to 99 mg $CaCO_3$ L^{-1}, but further increases in hardness were less effective at protecting glochidia (Table 3). The ameliorating effect of water hardness on chloride toxicity has been previously documented, in fact the state of Iowa has recently (2009) updated water quality criteria for chloride to adjust for water hardness (Iowa Department of Natural Resources,

Table 4
Summary of chloride concentrations in four significant mussel habitats in southern Ontario, the total number of mussel species and species at risk found in each habitat, as well as the observed 24 h chloride EC50s and EC20s for *Lampsilis faciola* glochidia in toxicity tests conducted with salt-spiked samples of those waters.

Conservation authority	CA mean chloride (mg L^{-1})	CA range chloride (mg L^{-1})	Observed EC50 (mg L^{-1})	Observed EC20 (mg L^{-1})	Total mussel species	Mussels species at risk[a,b]
Grand River	53 (1), $n = 45$	2–507	1313 (1239–1394)	432 (365–496)	25[c]	9
St. Clair Region	42 (14), $n = 9$	8–149	1559 (1338–1824)	403 (155–617)	34[d]	12
Maitland Valley	38 (29), $n = 13$	7–212	1391 (1308–1481)	261 (174–342)	9[e]	2
Upper Thames River & LowerThames Valley	58 (38), $n = 38$	6–1300	1265 (1167–1372)	153 (34–258)	26[f]	11

Watersheds in Ontario are organized by Conservation Authority (CA). Chloride data provided by the Ontario Ministry of the Environment (2009). Mean chloride values and ranges are for data collected from 1998 to 2008. Values reported as 'Mean' are the average of all site averages (repeated sampling at one site over time) for each CA. The number of individual site averages used to determine a 'CA Mean' (with standard deviation) is reported as *n*.

[a] Endangered species in Canada are designated by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC, 2007).

[b] Endangered species data, Department of Fisheries and Oceans, 2010.

[c] Metcalfe-Smith et al., 2000.

[d] Jacques Whitford Environment Limited, 2004.

[e] D.J. McGoldrick, J.L. Metcalfe-Smith, Environment Canada, Burlington, ON, Canada, unpublished data.

[f] Morris and Edwards, 2007.

Table 5
Acute toxicity of chloride (LC50s or EC50s) to various aquatic organisms illustrating the range of previously reported[a] sensitivities for each group.

Taxonomic Group	Species	Exposure duration (h)	LC50 (mg Cl L^{-1})	Reference
Molluscs	Glochidia[b] (4 species)	24	113–1430	Current study
	Glochidia[b] (5 species)	24	334–2008	Bringolf et al. (2007)
	Glochidia[b] (3 species)	24	1625–1868	Valenti et al. (2007)
	Glochidia[b] (1 species)	24	1213–1638	Cope et al. (2008)
	Physa sp. (snail)	96	3257	Clemens and Jones (1954)
Cladocerans	*Daphnia ambigua*	48	1213	Harmon et al. (2003)
	Daphnia magna	48	2893	Mount et al. (1997)
Amphibians	*Ambystoma maculatum* (larvae)	96	1178	Collins and Russell (2009)
	Bufo americanus (larvae)	96	3926	Collins and Russell (2009)
Fish	*Pimephales promelas*	96	3876	Mount et al. (1997)
	Fundulus kansae	96	9706	Clemens and Jones (1954)

[a] Data were limited to peer-reviewed publications.
[b] Free glochidia (i.e. not encased in conglutinates).

2009). The protection provided by hard water is beneficial for the freshwater mussels of southern Ontario as many key mussel habitats have very hard water (Table 1).

4.3. Chloride sensitivity in natural waters

L. fasciola glochidia were significantly less sensitive to salt in natural water than in reconstituted water. Some of the discrepancy can be explained by difference in water hardness because all of the natural waters tested were much harder (278–322 mg $CaCO_3$ L^{-1}) than the moderately-hard reconstituted water (100 $CaCO_3$ L^{-1}) used in the *L. fasciola* exposures. However, the four fold difference in EC50s is much larger than would be expected based solely on the difference in hardness because the *L. siliquoidea* exposures with a similar increase (100–322 mg $CaCO_3$ L^{-1}) produced less than a 30% difference in the chloride EC50. These data suggest that in addition to the protection provided by elevated water hardness that other water chemistry factors contributed to the reduced toxicity of chloride in natural waters.

The EC50 (3416 mg Cl L^{-1}) of conglutinate encased *P. fasciolaris* glochidia exposed to the salt-augmented water of the Grand River is nearly three times that of *L. fasciola* glochidia in the same water. This could simply be another example of intraspecific variation in glochidia contaminant sensitivity, or it could indicate that the life history strategy of encasing glochidia in conglutinates not only facilitates host transfer, but may also provide protection for the encased glochidia from chloride and potentially other ionic waterborne contaminants. For the current study it is not possible to determine the reason for the higher EC50 in conglutinate encased glochidia, although a previous study demonstrated that *P. fasciolaris* conglutinate encased glochidia were four fold less sensitive to copper than glochidia released from their conglutinate (Gillis et al., 2008).

The advantage of using reconstituted waters in toxicity tests is that they provide consistency and permit comparison between studies and between species; the disadvantage is that EC50s produced in reconstituted water may not necessarily predict how an organism will respond to that contaminant in its natural environment. On the other hand, one disadvantage of natural water exposures is that other contaminants may be present which can contribute to toxicity. Perhaps the variable (78–93%) control survival in the natural waters examined was due to other contaminants. One such contaminant of concern is potassium which is much more toxic than chloride. Imlay (1973) observed that only 2 of 10 rivers in the United States with potassium concentrations greater than 4 mg L^{-1} supported freshwater mussels, whereas 28 of 39 rivers with levels less than 4 mg L^{-1} were found to support mussels. All four natural waters tested were at or near this apparent threshold (Table 1). Moreover, preliminary data (Gillis unpublished) indicate that glochidia are sensitivity to potassium (*L. fasciola* 24 h moderately-hard reconstituted water LC50, 10 mg K L^{-1}). The potential effect of elevated potassium on freshwater mussel recovery requires further study especially because potassium chloride is currently being used as an alternative to sodium chloride for winter road maintenance (Evans and Frick, 2001). There have also been concerns that in some water bodies copper may be negatively impacting freshwater mussels (March et al., 2007; Ward et al., 2007). Background copper levels in the natural waters tested ranged from 5 to 6 μg L^{-1} (Table 1), but considering the level of DOC in these waters (>4 mg C L^{-1}) it is unlikely that copper contributed to the observed toxicity (Gillis et al., 2010; Wang et al., 2009). Unfortunately no comment can be made on the potential contribution of organic contaminants (such as pesticides) to the variation in control survival because these water samples were not analyzed for organics.

4.4. Implications for native populations of mussels

The rivers and streams of the lower Great Lakes Basin contain the richest assemblage of freshwater mussels in Canada (Metcalfe-Smith et al., 1998). After surveying historic (pre-1960) and more recent (up to 1996) mussel distribution data for southern Ontario, Metcalfe-Smith et al. (1998) concluded that significant species losses (15–30%) had already occurred, thereby verifying that the freshwater mussel decline documented in the U.S. (Neves et al., 1997) is also occurring in Canada. Although, many factors from exotic species to habitat loss (Williams et al., 1993; Bogan, 1993; Gillis and Mackie, 1994) are thought to have contributed to the decline of freshwater mussels, the role of waterborne contaminants remains uncertain. The chloride levels in the mussel habitats examined along with the heightened sensitivity of glochidia to NaCl suggest that chloride may in fact be impacting freshwater mussels in the lower Great Lake Basin. Even though the 'Mean' chloride concentrations (10–50 mg Cl L^{-1}) indicate that during the majority of the year, chloride levels are below the EC50, there are many documented instances where chloride concentrations would be toxic to glochidia. Even considering the higher EC50s produced in field-collected water (1265–1559 mg Cl L^{-1}), some rivers such as the Thames River, a habitat that supports eleven federally endangered species of mussels can exceed (1300 mg L^{-1}) the level found to be toxic to 50% of the glochidia.

Based on the results of this study, it is clear that even short-term spikes in chloride during the period of glochidia release would have a negative impact on the successful reproduction of freshwater mussels. Fortunately for most species, timing appears to be in the glochidia's favor, because the largest chloride spikes typically coincide with snowmelt (PWQMN, 2009), months before glochidia are released into the water column. However, many mussel species are referred to as long term brooders. This means that glochidia are held in the marsupial gills throughout the winter and released the following spring (Barnhart et al., 2008). Although there is evidence that brooding glochidia are less sensitive to waterborne copper than those released to the water column (Jacobson et al., 1997), it is unknown whether brooding glochidia are affected by the chloride laden waters their mothers are exposed to in early spring. While glochidia are only present in the water column during the spring and summer months, juvenile mussels, which have also been shown to be sensitive to some waterborne contaminants (Wang et al., 2007) would be exposed to chloride throughout the year. Another potential and likely year-round source of chloride is contaminated groundwater (Kincaid and Findlay, 2009). Juvenile mussels, because they remain burrowed in the sediment for their first few years of life, would be most at risk from elevated chloride from groundwater upwelling. Although the present study examined the potential risk of chloride toxicity in lotic habitats of freshwater mussels, urban basin analysis suggests the potential for chloride toxicity may be even greater in lentic habitats. Chloride contributes to densimetric stratification of receiving waters (Marsalek, 2003; Eyles and Meriano, 2010) which results in higher chloride concentrations just above the sediment-water interface in static or slow moving water bodies. Such stratification could exacerbate the risk of acute chloride toxicity for freshwater mussels living in embayments and lakes subjected to road salt runoff.

5. Conclusion

This study has demonstrated that compared to most other aquatic organisms that glochidia are very sensitive to chloride. It has also been demonstrated that increased water hardness and natural river water offer 'protection' from acute chloride toxicity. But even considering these ameliorating factors, the level of chloride, likely from road salt runoff, in key mussel habitats in the lower Great Lakes Basin, may pose a threat to the successful reproduction and thus recovery of endangered freshwater mussels in this area.

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Temperature affects acute mayfly responses to elevated salinity: implications for toxicity of road de-icing salts

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Salinity in freshwater ecosystems has increased significantly at numerous locations throughout the world, and this increase often reflects the use or production of salts from road de-icing, mining/oil and gas drilling activities, or agricultural production. When related to de-icing salts, highest salinity often occurs in winter when water temperature is often low relative to mean annual temperature at a site. Our study examined acute (96 h) responses to elevated salinity (NaCl) concentrations at five to seven temperature treatments (5–25°C) for four mayfly species (Baetidae: *Neocloeon triangulifer*, *Procloeon fragile*; Heptageniidae: *Maccaffertium modestum*; Leptophlebiidae: *Leptophlebia cupida*) that are widely distributed across eastern North America. Based on acute LC50s at 20°C, *P. fragile* was most sensitive ($\mathrm{LC50} = 767\ \mathrm{mg\ l^{-1}}$, $1447\ \mu\mathrm{S\ cm^{-1}}$), followed by *N. triangulifer* ($2755\ \mathrm{mg\ l^{-1}}$, $5104\ \mu\mathrm{S\ cm^{-1}}$), *M. modestum* ($2760\ \mathrm{mg\ l^{-1}}$, $5118\ \mu\mathrm{S\ cm^{-1}}$) and *L. cupida* ($4588\ \mathrm{mg\ l^{-1}}$, $8485\ \mu\mathrm{S\ cm^{-1}}$). Acute LC50s decreased as temperature increased for all four species ($n = 5$–7, $R^2 = 0.65$–0.88, $p = 0.052$–0.002). Thus, acute salt toxicity is strongly temperature dependent for the mayfly species we tested, which suggests that brief periods of elevated salinity during cold seasons or in colder locations may be ecologically less toxic than predicted by standard 20 or 25°C laboratory bioassays.

This article is part of the theme issue ‘Salt in freshwaters: causes, ecological consequences and future prospects’.

1. Introduction

Salinity in fresh waters is naturally variable, primarily reflecting differences in concentrations of dissolved inorganic cations calcium, magnesium and sodium, and anions carbonate, sulfate and chloride [1,2]. The differences in ion concentrations among fresh waters primarily reflect the weathering of soil and bedrock underlying a watershed, atmospheric deposition, and the evaporation–precipitation cycle. Sodium is generally less common than calcium and magnesium, and chloride is generally less common than carbonate or sulfate in natural waters. Elevated Na and Cl concentrations have been observed in effluents from wastewater treatment plants that reflect use of water softeners, table salt in the human diet, and disinfection before discharge [3,4], in wastewaters from some industrial, coal mining, and oil and gas production activities [5–7], in runoff and groundwater associated with various agricultural practices [8], and in road runoff following applications of de-icing products such as rock salt and anti-icing brines [9–13]. Recent analyses of multi-year data have found that sodium and chloride concentrations in surface waters have been increasing over the last two to five decades, at multiple locations (e.g. [14–18], and more recently [19–21]). This increase in sodium and chloride is part of a worldwide trend for increasing salinity along with pH and alkalinity [22–27], which was recently labelled the Freshwater Salinization Syndrome [28,29].

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With these increases in ambient salinity, there has been renewed interest in the toxicity of salt in our aquatic ecosystems. Building on early toxicity tests [30], researchers have again begun examining salt toxicity by focusing on specific ions such as sodium, magnesium, chloride, carbonate, and sulfate (e.g. delivered as $NaCl$, $MgCl_2$, Na_2SO_4) for a variety of aquatic algae [31], insects and other macroinvertebrates [32–41], mussels [42–46], zooplankton [47,48], amphibians [49,50], and fish [32,51–53]. Other researchers have approached salt toxicity as a function of total salinity (as salt concentration or electrical conductivity), rather than as an ion-specific issue (e.g. [54–57]). The challenge in both cases is general applicability of findings as it is well known that ion composition is important to overall salt toxicity [30,58–62]. Additional references can be found in review articles [63–69]. Salt toxicity has been found to vary greatly among aquatic species, with recent data showing that some mayflies and juvenile mussels are among the most sensitive species tested [34,52,56,70]. The combination of salt sensitivity and elevated ambient salinity suggests that, at least at times, salt may reach levels that may have a negative affect on aquatic organisms [10,31,71–76].

There are two challenges in understanding the potential salinity toxicity under field conditions in colder climates where de-icing salts can increase salinity dramatically during snow/ice storms. First, most salt toxicity studies have been conducted at constant 17–25°C, which are the recommended test temperatures for standard acute and chronic toxicity tests for many species, [77,78]. However, in colder climates where de-icing salts are frequently used, water temperature can vary naturally across seasons, with winter lows of 0–10°C versus summer highs of 20–30°C (e.g. figure 1). In addition, there can be significant differences among years (e.g. an interannual range of 10°C or more; figure 1). It has been found that temperature can affect toxicity of many chemicals [79–86]. For most toxins and species, the relationship between temperature and toxicity is positive—increases in temperature result in increased toxicity (i.e. a lower LC50). Mayer & Ellersieck [80] summarized the relationship as a 10°C increase in temperature results in a two- to fourfold decrease in the LC50. Second, streams and rivers that exhibit a long-term increase in Na and Cl concentrations also often exhibit a strong seasonal cycle that includes frequent, short-term snow and ice events when salinity can be many times greater than at base flow (figure 2) [87,88]. This is a sharp contrast to streams with little urbanization (e.g. figure 1 and [88]). Unfortunately, the magnitude and duration of these events are often not well quantified in the historic data because these data are primarily periodic grab samples while snow and ice events are better described with a continuously recording sensor. While the recent studies of salt toxicity have addressed the range of conditions needed to set regulatory limits [47,89], they have not included seasonal temperature variation as part of their analyses.

This paper describes a series of experiments that examine lethal responses of mayfly (Ephemeroptera) larvae in acute (96 h) exposures to elevated salinity (i.e. NaCl added to moderately hard source water) at five to seven different temperatures. The results show how understanding the experimental relationship between temperature and salt toxicity can provide important insight into the toxicity of ambient salt concentrations, especially those originating from winter de-icing programmes.

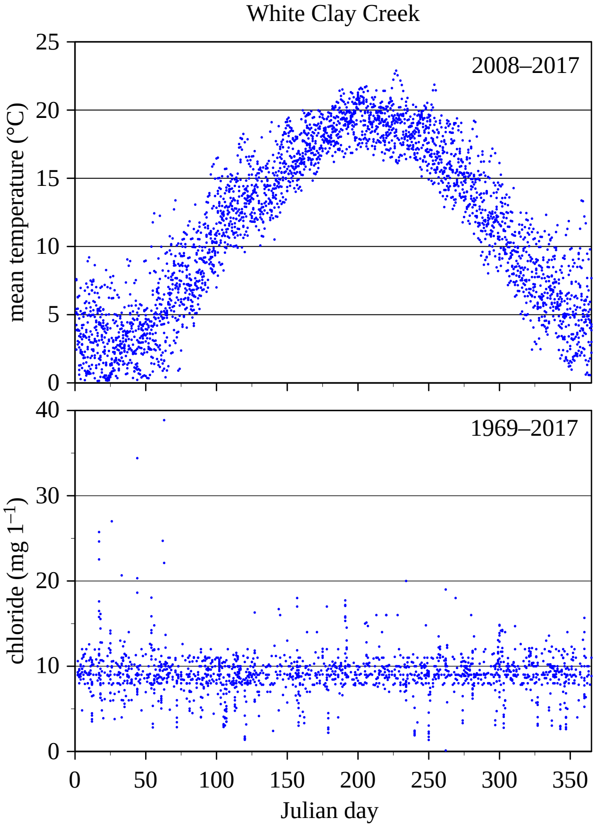

Figure 1. Long-term seasonal variation (date plotted as Julian day) in water temperature (mean daily from various continuous recorders, 2008–2017) and chloride concentration (from grab samples, 1969–2017) for White Clay Creek at the Stroud Water Research Center, 39°51′38.41″ N, 75°47′01.96″ W. Values greater than 20 mg Cl l^{-1} are presumably evidence of local de-icing efforts during winter. (Online version in colour.)

2. Methods

(a) Source water

Water for all tests was collected from White Clay Creek at the Stroud Water Research Center (39°51′38.41″ N, 75°47′01.96″ W), Chester Co. Pennsylvania, a limestone-influenced, headwater stream that drains a 7 km^2, rural (less than 0.5% developed) watershed and is moderately hard (mean 97 mg CO_3^{2-} l^{-1}) with relatively low salinity (143.8 mg l^{-1}, table 1). Seasonal patterns in temperature and chloride (as an indicator of de-icing salts affecting background salinity) from long-term data for White Clay Creek are shown in figure 1. The temperature treatments (see below) are representative of the range of conditions these test mayfly populations have experienced for generations in White Clay Creek. In contrast, the relatively low salinity in the historic data suggests that these wild mayfly populations from White Clay Creek have not been exposed to sodium or chloride concentrations similar to those in our experimental treatments in the last 50 years. Background concentrations on four dates when water was collected for laboratory bioassays averaged 6.6 mg l^{-1} for sodium and 12.3 mg l^{-1} for chloride (table 1).

To provide context for laboratory results, field data were collected every 5 min (30 Mar 2017–1 May 2018) with a Decagon CTD-10 (electrical conductivity or specific conductance corrected to 25°C, temperature, depth) sensor in Rocky Run, First State National Historic Park, New Castle County, Delaware, USA (39°49′00.45″ N and 75°33′02.84″ W), which drains a highly urbanized (60% developed), 2 km^2 watershed about 20 km from the Stroud Water Research Center. Salinity for Rocky Run was estimated from the conductivity : salinity relationship used in our experiments with White Clay Creek water, where salinity =

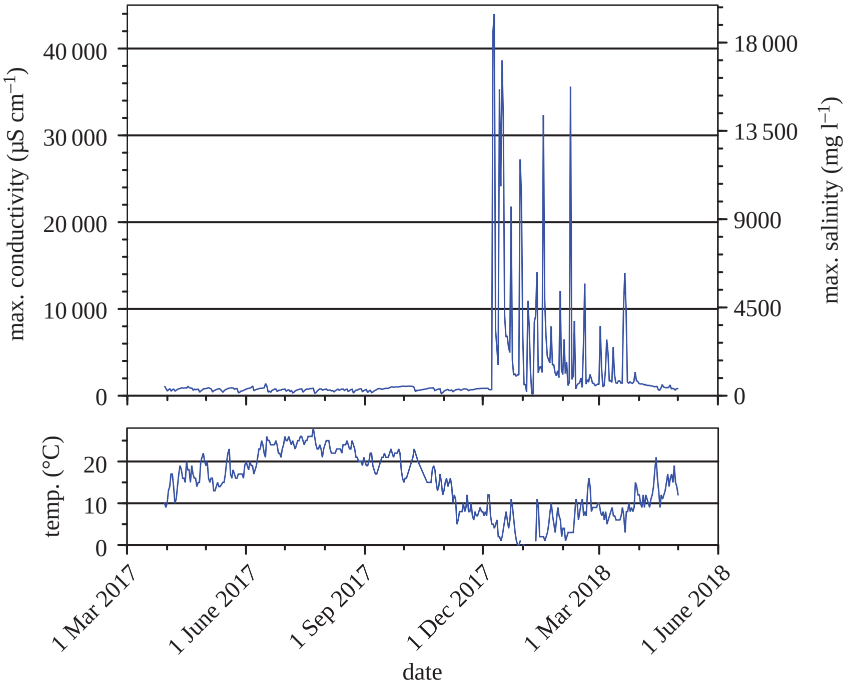

Figure 2. Seasonal variation (from 30 March 2017 to 1 May 2018) in maximum daily salinity as conductivity (µS cm^{-1}) and mg l^{-1}, and maximum daily temperature (°C) for Rocky Run, First State National Historic Park, New Castle County, Delaware. (Online version in colour.)

Table 1. Chemical characteristics of moderately hard water from White Clay Creek, PA used in acute toxicity tests in 2016. TDS, total dissolved salts.

date:	4 Apr 2016	16 Apr 2016	12 May 2016	5 June 2016	
time:	06.30	07.00	08.30	11.45	mean
pH	7.7	8.2	7.7	7.8	7.9
conductivity (µS cm^{-1})	238	232	239	241	238
alkalinity (mg l^{-1})	71.3	66.0	67.8	69.2	68.6
hardness (mg CO_3^{2-} l^{-1})	96	93	97	100	97
Ca^{2+} (mg l^{-1})	23.9	23.9	24.7	26.6	24.8
Mg^{2+} (mg l^{-1})	8.8	8.0	8.6	8.2	8.4
K^{+} (mg l^{-1})	2.0	1.6	1.7	1.9	1.8
Na^{+} (mg l^{-1})	7.1	6.1	6.5	6.5	6.6
Cl^{-} (mg l^{-1})	13.0	12.1	12.0	12.2	12.3
SO_4^{2-} (mg l^{-1})	17.0	17.6	17.5	17.2	17.3
TDS (mg l^{-1})	139	160	152	152	152.5

(electrical conductivity − 23.099)/1.844, where salinity is in mg l^{-1}, and electrical conductivity is in µS cm^{-1} at 25°C.

(b) Study species

Mayflies were chosen for this study because Ephemeroptera are ecologically significant in most streams and rivers, and they are considered pollution sensitive and have historically played important roles in water quality monitoring programmes [90–92]. We quantified acute responses to short-term (96 h) chloride exposures for four mayfly species that are common in White Clay Creek (where test species were collected) and widely distributed in eastern North America. *Neocloeon triangulifer* (McDunnough 1931) was until recently called *Centroptilum triangulifer* [93] and before that *Cloeon triangulifer* [94]. It is a parthenogenetic (clonal) mayfly species [95,96] that is most abundant during summer, when it has a relatively rapid larval development (egg hatch to adult in 25–30 days at 20°C). We worked with Stroud Water Research Center (SWRC) Clone WCC-2®, which occurs in low larval numbers during the winter, with minimal growth below 9.6°C. This specific clone has also been recently used in a number of experiments examining the toxic effects of cadmium, mercury, selenium and zinc [97–102], and chloride and sulfate salts [34,36,38–40]. *Procloeon fragile* (McDunnough 1923) was for many years called *Centroptilum fragile* [94]. It is a sexual mayfly species that exhibits a life history similar to that of *N. triangulifer* except that it has a winter egg diapause. *Maccaffertium modestum* (Banks, 1910) was long known as *Stenonema modestum*, but was recently reclassified [103]. It is a sexual

Table 2. Acute (96 h) salinity toxicity (LC50; geometric mean with 95% CI) expressed as mg l^{-1} and µS cm^{-1} for four mayfly species exposed to elevated NaCl in five to seven constant temperature (°C) treatments.

species	LC50 per temperature treatment (geometric mean with 95% CI, mg l^{-1} and µS cm^{-1})						
	5°C	7.5°C	10°C	12.5°C	15°C	20°C	25°C
salinity (mg l^{-1})							
N. triangulifer	9655 (8751 – 10 653)	10 462 (9655 – 11 337)	6719 (5541 – 8148)	5101 (4843 – 5373)	2573 (389 – 17 017)	2755 (2018 – 3762)	364 (171 – 773)
P. fragile			6874 (4595 – 10 284)	4115 (3111 – 5443)	3239 (1662 – 6312)	767 (187 – 3146)	766 (748 – 784)
L. cupida	10 086 (9037 – 11 257)	10 152 (9299 – 11 083)	10 439 (8551 – 12 743)	11 908 (11 650 – 12 172)	8368 (6955 – 10 067)	4588 (3108 – 6774)	3216 (1821 – 5678)
M. modestum		7236 (6005 – 8719)	5429 (2442 – 12 067)	7792 (5483 – 11 075)	7808 (3742 – 16 293)	2760 (1436 – 5303)	1656 (680 – 4035)
electrical cond. (µS cm^{-1})							
N. triangulifer	17 829 (16 161 – 19 668)	19 317 (17 829 – 20 929)	12 414 (10 241 – 15 049)	9430 (8953 – 9932)	4788 (739 – 31 042)	5104 (3745 – 6958)	698 (335 – 1453)
P. fragile			12 700 (8495 – 18 986)	7612 (5759 – 10 060)	5997 (3086 – 11 656)	1447 (367 – 5707)	1435 (1402 – 1469)
L. cupida	18 623 (16 688 – 20 782)	18 744 (17 172 – 20 461)	19 273 (15 792 – 23 522)	21 983 (21 506 – 22 470)	15 454 (12 849 – 18 587)	8485 (5754 – 12 514)	5955 (3381 – 10 487)
M. modestum		13 366 (11 096 – 16 102)	10 036 (4525 – 22 260)	14 393 (10 133 – 20 444)	14 425 (6923 – 30 053)	5118 (2676 – 9788)	3081 (1274 – 7451)

species that exhibits a bivoltine or multivoltine life history at White Clay Creek, with larval development of about 80 days at 20°C. *Leptophlebia cupida* (Say 1923) is a sexual mayfly species that exhibits a univoltine life history that begins with eggs hatching in mid-June and adult emergence the following April.

(i) Experimental treatments

We quantified acute responses of four mayfly species in short-term (96 h) exposures to elevated NaCl (A.C.S. reagent; J.T. Baker 33624-05). NaCl was chosen for these experiments because it represents 90–98% of the rock salt (halite) used for de-icing roads [104]. We conducted 100 temperature-specific acute tests (each test had one replicate of 20 individuals for each salinity treatment), with 20 newly hatched 1st instar larvae for *N. triangulifer*, *P. fragile*, *L. cupida* or *M. modestum* placed in a 30 ml beaker containing 15 ml of treatment solution. Newly hatched larvae were chosen because younger/smaller individuals are often more sensitive than older/larger individuals of the same species [55,105–107].

Each toxicity test had six treatments: a control (0 mg NaCl l^{-1} added to White Clay water) and five elevated salinity treatments that represented a 50% dilution series (i.e. 412, 824, 1649, 3297, 6594 mg NaCl l^{-1} added to White Clay water for *N. triangulifer* and *P. fragile*, and 824, 1649, 3297, 6594, 13 188 mg NaCl l^{-1} added to White Clay water for *L. cupida* and *M. modestum*). These were static (no renewal) experiments, conducted at five to seven constant (±0.1°C) temperature treatments (i.e. 10, 12.5, 15, 20, 25°C for all species, with the addition of a 7.5°C treatment for *M. modestum* and 5 and 7.5°C treatments for *N. triangulifer* and *L. cupida*). A diatom slurry (i.e. *ca* 20 µl of biofilm scrapings suspended in White Clay water) was provided as food in each test vessel for *N. triangulifer* and *P. fragile*. Food was not provided in *L. cupida* and *M. modestum* tests. Four replicate tests were run for each temperature treatment. Photoperiod (light : dark) was 16 : 8 h during the tests. Temperature in the rearing system was recorded every 5 min, and calibrated with a certified thermometer. Salinity across treatments was monitored with a calibrated conductivity meter.

Mayfly response was reported as survivorship after 96 h, and summarized as the lethal salinity associated with 50% mortality (or LC50) estimated using the nonparametric trimmed-Spearman–Karber method [76,108] of test population at a specific temperature. The relationship between temperature and LC50 for each species was assessed with a simple linear regression of geometric means. Linear regressions were used because it was a simple assessment of the relationship between five to seven temperature treatments and salinity toxicity, and because regression slope was consistent across the temperature range, which facilitates interpretation and incorporation into regulatory standards.

3. Results and discussion

(a) Interspecific differences in mayfly sensitivity to elevated salinity

Control survival was greater than 90% in most of the acute toxicity tests reported for *P. fragile*, *N. triangulifer* and *L. cupida*, and those tests with slightly higher control mortality were still included in these analyses as their dose-responses were similar to other tests. Survival was less than 90% for many tests with *M. modestum* (suggesting this species should be fed during 96 h tests), but the response to temperature was similar to the other mayfly species and is included in this report. However, because of low control survival, the LC50s for *M. modestum* should be used with caution until further verification.

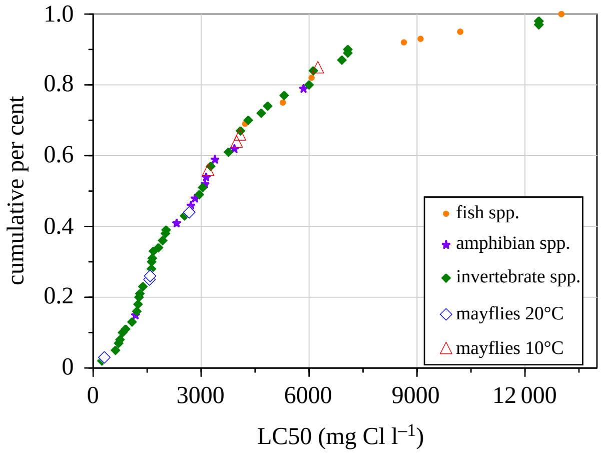

Figure 3. Relative sensitivities for the mayflies *N. triangulifer*, *P. fragile*, *M. modestum*, and *L. cupida* based on LC50s (expressed as mg Cl l^{-1}, electronic supplementary material, table S1) for 10 and 20°C (table 2) plotted with fish, amphibian and invertebrate data included in fig. 3 from [52].

Mean LC50s estimated by the nonparametric trimmed-Spearman–Karber method are expressed as salinity (mg l^{-1}) and electrical conductance (µS cm^{-1}) in table 2. We prefer to compare toxicities among mayflies at 20°C because it appears in some mayfly species we have examined that 25°C is physiologically stressful, independent of the chemical stressor being evaluated. Based on acute LC50s at 20°C, *P. fragile* was most sensitive (LC50 = 767 mg l^{-1}, 1447 µS cm^{-1}), followed by *N. triangulifer* (2755 mg l^{-1}, 5104 µS cm^{-1}) and *M. modestum* (2760 mg l^{-1}, 5118 µS cm^{-1}), and finally *L. cupida* (4588 mg l^{-1}, 8485 µS cm^{-1}) (table 2). NaCl toxicity for *N. triangulifer* has been examined in earlier studies [34,36,39], but all at 25°C. The acute LC50 for *N. triangulifer* at 25°C in our study was markedly lower than LC50 we observed at 20°C as well as the LC50s estimated by Soucek & Dickinson [34], Struewing *et al.* [36], and Soucek *et al.* [39]. Our LC50s for 25°C for all four mayfly species were not out of line with LC50s from colder temperature treatments, and the temperature versus LC50 regressions fitted the data relatively well (see below), so we do not currently have an explanation for differences observed among the studies of *N. triangulifer*. When salinity was expressed as electrical conductivity (µS cm^{-1} or mS cm^{-1}), the LC50s we observed for the baetids *P. fragile* and *N. triangulifer* (1447–5104 µS cm^{-1}) were similar to those observed for the baetid *Centroptilum* sp. (1.8–5.6 mS cm^{-1} in [59], and 10 mS cm^{-1} in [57]), and less than was observed for the baetid *Cloeon* sp. (21 mS cm^{-1} in [57]).

Mayflies are generally considered pollution sensitive, and are important contributors to metrics used to assess pollution impacts [90–92]. When we compared the LC50s for our mayflies at 20°C (expressed as mg Cl l^{-1}, electronic supplementary material, table S1) relative to the acute LC50s included in fig. 3 of [52], *P. fragile* was among the most sensitive species, *M. modestum* and *N. triangulifer* was moderately sensitive (*ca* 25th percentile) and *L. cupida* was average (45th percentile) (figure 3). Relative sensitivity for mayflies in our study would be even higher if we used LC50s from the common test temperature of 25°C (table 2)—*P. fragile*, *N. triangulifer* and *M. modestum* would be among the most sensitive, and *L. cupida* would be moderately sensitive.

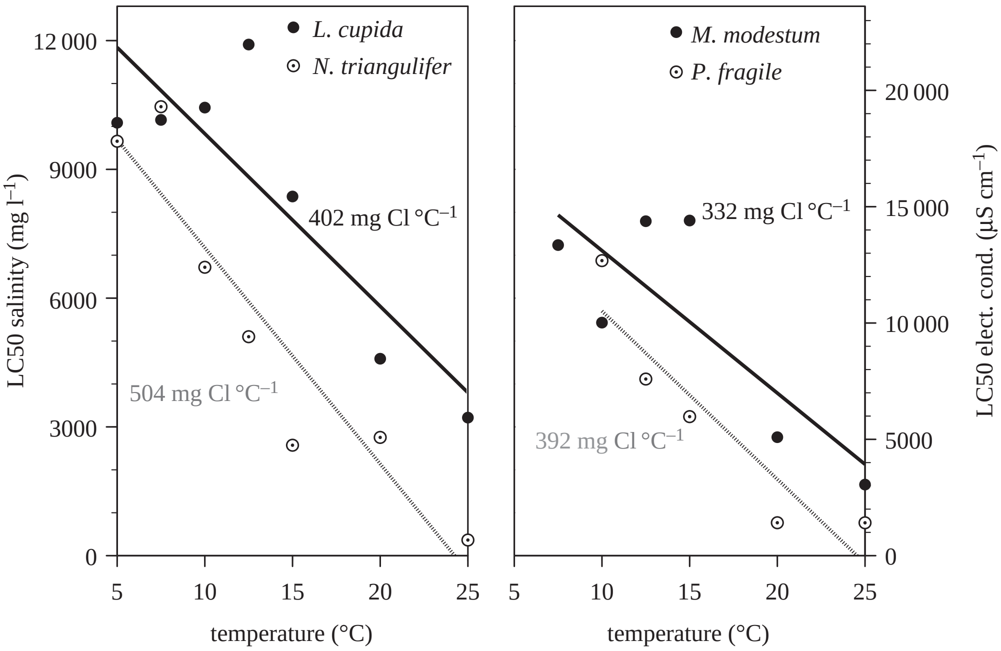

Figure 4. Simple linear regressions describing the relationship between temperature and acute salinity LC50s for the mayflies *L. cupida*, *N. triangulifer*, *M. modestum* and *P. fragile*.

Table 3. Simple linear regression results for figure 4 describing the relationship between temperature and acute salinity toxicity expressed as LC50 (geometric means, mg l^{-1}).

species	*N*	Pr > F	R^2	intercept	slope
N. triangulifer	7	0.002	0.881	12 211.2	−503.7
P. fragile	5	0.026	0.850	9616.0	−391.8
L. cupida	7	0.011	0.756	13 851.5	−402.2
M. modestum	6	0.052	0.653	10 425.3	−331.9

Conversely, the mayflies in our study would not be considered sensitive if we used LC50s from the 10°C test temperature (figure 3) We saw similar relative sensitivity when our study mayflies were compared to the mayflies and other macroinvertebrates presented in Wang *et al.* [70], and in the broader global survey of salinity sensitivity for mayflies and other macroinvertebrates in Kefford *et al.* [56].

The four mayfly species included in our study were not selected based on presumed or known pollution sensitivity. In fact, it is possible there are mayfly species that are as or more sensitive to elevated salinity than the species we examined. Our data, in combination with other published observations such as Wang *et al.* [70] and Kefford *et al.* [56], support the general belief that mayflies as a group are relatively sensitive to elevated salinity, although the physiological mechanisms surrounding mayfly sensitivity to salt remain to be determined [109]. Cormier *et al.* [110] defined a maximum acute benchmark of 680µS cm^{-1} for salinity derived from field observations of occurrence for 142 stream macroinvertebrate genera and annual chemistry data. While this hypothetical benchmark might not be directly comparable with our laboratory studies (Cormier *et al.* [110] eliminated several sites with high chloride), 680 µS cm^{-1} (=369 mg l^{-1} in our study) would appear to be over-protective for all species based on the LC50s at 5–10°C, and protective for *N. triangulifer*, *M. modestum* and *L. cupida*, and possibly *P. fragile*, based on the LC50s at 20°C. The benchmark might not be protective for *P. fragile* and *N. triangulifer* based on the LC50s at 25°C.

It is important to note that salinity toxicity is known to vary among salts and dilution waters tested [30,39,41,47,58–61], so our toxicities for elevated salinity that is predominately NaCl must be used with caution when referring to other de-icing and anti-icing salts such as $MgCl_2$, $CaCl_2$, KCl or calcium magnesium acetate ($CaMg_2(CH_3COO)_6$), to the ‘chemical cocktail’ that characterizes the Freshwater Salinization Syndrome [29], or to ambient waters with natural salinities that are markedly lower or higher than in White Clay Creek (e.g. a soft-water stream or a limestone stream).

(b) Changes in salinity toxicity in response to temperature

The relationship between salinity toxicity and temperature is important because, in regions where de-icing salts are frequently used, water temperature can change significantly with seasons (figure 1). Moreover, salinity from de-icing efforts peaks following snow and ice events when stream temperature is often nearest its lowest level, and well below the 20 or 25°C temperature used in standard bioassays (figure 2). We observed a significant or nearly significant ($n = 5–7$, $R^2 = 0.65–0.88$, $p = 0.052–0.002$) decrease in toxicity (i.e. acute LC50s increased) as temperature decreased for all four species (figure 4 and table 3). Based on the

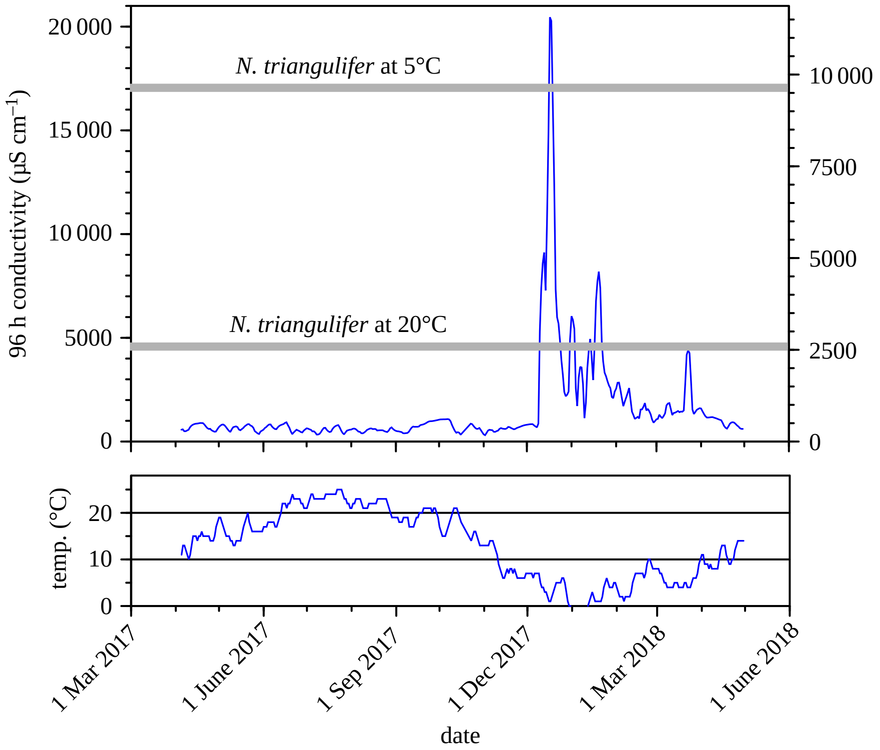

Figure 5. Plot showing mean 96 h LC50 for *N. triangulifer* at 5 and 20°C with seasonal variation (from 30 March 2017 to 1 May 2018) in 96 h running salinity as conductivity (µS cm^{-1}) and mg l^{-1}, and 96 h mean running daily temperature (°C) for Rocky Run, First State National Historic Park, New Castle County, Delaware, USA. (Online version in colour.)

regression slopes, the rate of change was similar for *P. fragile*, *M. modestum* and *L. cupida*. Their LC50s decreased between 332 and 402 mg l^{-1} for each 1°C increase in temperature. The response for *N. triangulifer* was somewhat stronger and its LC50s decreased 504 mg l^{-1} for each 1°C increase in temperature. The LC50s for *L. cupida* and *M. modestum* increased 1.7–1.9-fold for each 10°C decrease in temperature while the LC50s for *N. triangulifer* and *P. fragile* increased 3.5–3.6-fold for each 10°C decrease. This difference between *L. cupida* and *M. modestum* versus *P. fragile* reflects the estimated LC50s relative to the rate of change per °C. The species with lowest LC50 (*P. fragile*) increased proportionally more per °C than species with higher LC50s (*L. cupida* and *M. modestum*). The higher proportional change for *N. triangulifer* reflects a moderately low LC50 with a higher rate of change per °C. Our results almost match the summarization by Mayer & Ellersieck [80] that a 10°C increase in temperature results in a two- to fourfold decrease in the LC50. There are a few studies where reduced salt toxicity has been observed at lower versus higher temperature [73,111,112], but the relationship between acute salt toxicity and temperature has not been quantified in a manner that can be applied to water quality criteria (table 3).

To illustrate how the interaction between temperature and salinity toxicity provides important perspective to understanding aquatic ecosystems receiving de-icing salts, we took the raw data used to generate figure 2 and calculated 96 h (i.e. the duration of the acute toxicity tests) running mean values for conductivity, salinity (from conductivity) and temperature (figure 5). We then added the LC50 for *N. triangulifer* at 5°C and 20°C to figure 5. Based on the LC50 at 20°C, there were 21 dates that were preceded by 96 h with an average salinity that exceeded the LC50 at 20°C. In contrast, based on the LC50 at 5°C (which is more representative of thermal conditions at the time of elevated salinity), there were only two dates that were preceded by 96 h with an average salinity that exceeded the LC50 at 5°C. Thus, accounting for lower salt toxicity for an acute exposure at low temperature can change one's perspective on the apparent toxicity of ambient conditions during winter. However, it is important to note that, even after accounting for lower toxicity at 5–10°C, salinity in Rocky Run still appears to have been acutely toxic (i.e. ≥50% mortality in a 96 h period) for all four mayflies we examined. This suggests that elevated salinity (e.g. averaging 9500–11 500 mg l^{-1} for 96 h) during winter when snow and ice management programmes are being implemented may contribute to the overall impairment of the macroinvertebrate assemblage in Rocky Run, and probably other small urban streams that receive salt-laden runoff from roads, car parks and pavements. However, this is not to suggest that elevated (but not peak) salt concentrations during winter are not contributing to overall impairment. These non-peak exposures are more frequent (i.e. exposure time can be longer), and based on results for polar marine invertebrates, exposure time must be considered in the evaluation and interpretation of potential impact of toxicants at cold temperature [113,114].

(c) Regulatory and management implications of the relationship between salinity toxicity and temperature

As salinization of freshwater ecosystems resulting from de-icing and anti-icing salts continues, the regulatory and management challenge for winter road maintenance programmes will be to balance the need to protect public safety and reduce the economic costs of winter storms with the need to protect environmental health and infrastructure integrity related to excess salt, and to address potential drinking water/public health related to increased dietary intake of sodium [4,21,115,116]. Our study found low temperature can reduce the frequency or intensity of salt-related toxic events expected based on winter de-icing activities that increase NaCl concentrations. But it also shows that NaCl concentrations during winter can be so high that NaCl-related toxic events may still occur even after accounting for low temperature. Our results can also be applied to other activities that result in acute exposure to elevated salt. For example, spills or discharges of high salinity wastewaters such as oil and gas brine [6,7] may have more of an impact in summer, when both the stored wastewater and receiving stream water are seasonally warmer, than in winter, when both are cool. The negative relationship between temperature and salt toxicity we observed highlights the potential importance in considering water temperature when interpreting current environmental conditions or events, or setting regulatory standards for salinity or NaCl.

Data accessibility. Data are available as electronic supplementary material.

Authors' contributions. Overall project and experimental design: J.K.J. and D.H.F.; experimental set-up and data collection: D.H.F.; data analyses and interpretation: J.K.J. and D.H.F.; drafted manuscript: J.K.J.; edited manuscript: J.K.J. and D.H.F.

Competing interests. We declare we have no competing interests.

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THE ACUTE TOXICITY OF MAJOR ION SALTS TO *CERIODAPHNIA DUBIA*: I. INFLUENCE OF BACKGROUND WATER CHEMISTRY

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Abstract: The ions Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, $SO_4{}^{2-}$, and $HCO_3{}^-/CO_3{}^{2-}$ (referred to in the present study as "major ions") are present in all freshwaters and physiologically required by aquatic organisms but can increase to harmful levels from a variety of anthropogenic activities. It is also known that the toxicities of major ion salts can vary depending on the concentrations of other ions, and understanding these relationships is key to establishing appropriate environmental limits. The authors present a series of experiments with *Ceriodaphnia dubia* to evaluate the acute toxicity of 12 major ion salts and to determine how toxicity of these salts varies as a function of background water chemistry. All salts except $CaSO_4$ and $CaCO_3$ were acutely toxic below saturation, with the lowest median lethal concentrations found for K salts. All 10 salts that showed toxicity also showed some degree of reduced toxicity as the ionic content of the background water increased. Experiments that independently varied Ca:Mg ratio, Na:K ratio, $Cl:SO_4$ ratio, and alkalinity/pH demonstrated that Ca concentration was the primary factor influencing the toxicities of Na and Mg salts, whereas the toxicities of K salts were primarily influenced by the concentration of Na. These experiments also indicated multiple mechanisms of toxicity and suggested important aspects of dosimetry; the toxicities of K, Mg, and Ca salts were best related to the chemical activity of the cation, whereas the toxicities of Na salts also reflected an influence of the anions and were well correlated with osmolarity. Understanding these relationships between major ion toxicity and background water chemistry should aid in the development of sensible risk-assessments and regulatory standards. *Environ Toxicol Chem* 2016;35:3039–3057. Published 2016 Wiley Periodicals Inc. on behalf of SETAC. This article is a US government work and, as such, is in the public domain in the United States of America.

Keywords: Aquatic toxicology Major ions *Ceriodaphnia dubia* Toxicity mechanism Dose–response modeling

INTRODUCTION

Inorganic ions generally present at the highest concentrations in freshwaters are Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, $SO_4{}^{2-}$, and $HCO_3{}^-/CO_3{}^{2-}$ (referred to as "major ions" herein) and are used to describe the basic chemistry of natural waters [1]. All have physiological roles and are actively regulated by aquatic organisms [2] but can also cause toxicity when present in sufficient excess [3]. Concentrations in natural waters are governed by a variety of atmospheric, geochemical, and biological processes [1], but these natural concentrations can be greatly increased by a wide variety of anthropogenic influences, such as mineral mining, oil and gas extraction, irrigation, road deicing, water softening, and wastewaters from various industrial processes.

A variety of studies have shown or implicated major ions as causes of aquatic toxicity in surface waters, with sources such as oil and/or gas production [4,5], irrigation return flows [6,7], mining [8,9], road salt [10], and industrial wastewater [11]. In fact, toxicity identification studies on industrial and municipal effluents have shown major ions to be among the more common causes of effluent toxicity [12]. Field studies in Appalachian streams have also found associations between changes in macrobenthic communities and increased major ion concentrations from mining activities [13–15].

Understanding the aquatic hazards posed by increased major ion concentrations presents a number of challenges. First, concentrations of major ions cannot be manipulated individually because charge balance demands that increased concentrations of any ion be offset by equal and opposite charge from other ions, making it more difficult to infer the effects of individual ions. Second, the relative concentrations of major ions vary widely across watersheds and anthropogenic inputs, and such differences are known to influence aquatic toxicity. For example, based on total salt concentration, a 1:1 mixture (by mass) of NaCl and $CaCl_2$ has substantially lower acute toxicity to *Ceriodaphnia dubia* than either salt alone [3], indicating that toxicity of this salt mixture is not simply additive. Third, the toxicity of a single salt can vary based on the characteristics of the water to which it is added, such as water hardness [16–20] and, more specifically, Ca [21]. Although relationships between water hardness and the toxicity of various other chemicals are often attributed, explicitly or implicitly, to the Ca and Mg ions that comprise most hardness, more detailed studies sometimes show that the concentrations of other ions covarying with hardness are playing important roles. For example, though "hardness" was long reported to influence toxicity of metals such as copper, later research demonstrated more detailed roles of specific ions; this enhanced understanding was incorporated into a more refined toxicity model, the biotic ligand model [22].

In previous work, Mount et al. [3] approached the toxicity of major ion mixtures by developing a multivariate regression model based on a large number of acute toxicity tests conducted with many different combinations of major ion salts. The resulting models predict the survival of 3 test species,

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cladocerans *Ceriodaphnia dubia* and *Daphnia magna*, and the fathead minnow (*Pimephales promelas*) based on concentrations of the 7 major ions. Although this model represented a step forward in addressing the complexities of evaluating ion mixtures and showed effectiveness as a predictive tool [5,23], there are important aspects of major ion toxicity that were not fully addressed. Notable among these was that all of the ion solutions tested were created by adding ions to a single base water. This issue underlies the failure of the model to represent some influences of background water chemistry on ion toxicity, such as the reduction of NaCl and Na_2SO_4 toxicity afforded by adding hardness within ranges common to natural waters [16–21]. Other aspects of the interactions among the ions (e.g., independent vs additive toxicity) also are incompletely addressed by this regression model.

The present study is the first of 3 articles that establish a better foundation for predicting the acute toxicity of elevated major ion concentrations to *C. dubia*. The present study describes a comprehensive study of the influence of background water chemistry that extends earlier work on hardness effects [16–21] to more water chemistry factors and to more major ion salts. Toxicities of major ion salts were evaluated using a wide range of dilution waters; some mimicked natural waters, while others were designed specifically to isolate different components of background water chemistry to better understand their roles in influencing major ion toxicity and thereby determine what is important to risk assessment of ions in natural systems.

These experiments also allowed preliminary consideration of exposure metrics that more effectively describe major ion toxicity to *C. dubia* than total salt concentration. For example, are there different toxicity mechanisms among the salts that need to be addressed? Should the toxicity of a salt be related to an individual ion or both ions, and how should concentrations of multiple ions be combined? Should reductions in toxicity attributable to the formation of complexes between ions and to the general effects of high ion concentrations on chemical reactivity be addressed?

The second article will present results of mixture tests with pairs of salts to more rigorously address the preceding questions. The third will address how the information from the other articles can be incorporated into a mathematical model applicable to any ion mixture and will test the predictions of that model for more complex mixtures relevant to field exposures.

The acute toxicity to *C. dubia* was selected as the endpoint for these efforts because this is a widely distributed organism with considerable sensitivity to ions and for which it was practical to conduct the large number of tests needed to adequately address the multiple factors and interactions of interest. The knowledge gained from the present study with *C. dubia* supports more informed testing and model development for other endpoints and species, which are now under way and will be the subject of additional publications. When combined with the efforts of other investigators, this body of information will support better assessment of the risks of major ions to aquatic communities.

MATERIALS AND METHODS

Test water composition and study design

Twenty-six experiments were conducted on the acute toxicity of individual major ion salts to *C. dubia*. Each experiment consisted of 3 to 8 simultaneous toxicity tests with different combinations of dilution waters and toxicants, for a total of 149 median lethal concentration (LC50) determinations. Test waters were developed from deionized water, sand-filtered and ultraviolet light-treated Lake Superior water (LSW), or a combination of both. Deionized water was produced from a Millipore Super-Q system configured as specified by the US Environmental Protection Agency (USEPA) [24]. Lake Superior water was obtained from an intake located offshore from our laboratory at 46.840°N, 92.004°W; typical hardness and alkalinity are 47 mg/L and 43 mg/L as $CaCO_3$, respectively, with conductivity of 104 μS/cm and pH approximately 7.5. The full ionic composition of LSW is provided in Table 1.

Table 1. Composition of dilution waters

Dilution water description	Abbreviation	Base water	Na (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)	Cl (mg/L)	SO_4 (mg/L)	Alkalinity (mg $CaCO_3$/L)	Hardness (mg $CaCO_3$/L)
Lake Superior water	LSW		1.62	0.60	14.0	2.92	1.50	3.40	43.0	47.0
Amended Lake Superior water	ALSW	LSW	6.48	1.51	14.6	4.09	7.66	14.9	43.0	53.3
1/3× strength ALSW	1/3× ALSW	1/3× LSW	2.16	0.50	4.87	1.36	2.55	4.97	14.3	17.8
			(4.77)^{a}	(1.18)^{a}	(4.87)^{a}	(1.36)^{a}	(5.04)^{a}	(7.88)^{a}	(14.4)^{a}	
3× strength ALSW	3× ALSW	LSW	19.4	4.52	43.8	12.3	23.0	44.7	129	160
			(16.7)^{a}	(2.74)^{a}	(43.9)^{a}	(12.3)^{a}	(19.0)^{a}	(46.0)^{a}	(125)^{a}	
Moderately hard reconstituted water	MHRW	Deionized water	26.3	2.10	14.0	12.0	1.90	81.0	57.2	84.4
One-third strength MHRW	1/3× MHRW	Deionized water	8.75	0.70	4.65	4.01	0.63	27.0	19.1	28.1
One-eighth strength MHRW	1/8× MHRW	Deionized water	3.28	0.26	1.75	1.50	0.24	10.1	7.2	10.6
ALSW with high Ca:Mg ratio	High Ca:Mg	0.214× LSW	6.48	1.51	19.0	1.40	7.66	14.9	43.0	53.3
ALSW with low Ca:Mg ratio	Low Ca:Mg	0.214× LSW	6.48	1.51	3.00	11.1	7.66	14.9	43.0	53.3
ALSW with high Cl:SO_4 ratio	High Cl:SO_4	LSW	6.48	1.51	14.6	4.09	14.3	3.40	43.0	53.3
ALSW with low Cl:SO_4 ratio	Low Cl:SO_4	LSW	6.48	1.51	14.6	4.09	1.50	20.7	43.0	53.3
ALSW with high Na:K ratio	High Na:K	0.333× LSW	7.65	0.20	14.6	4.09	7.66	14.9	43.0	53.3
ALSW with low Na:K ratio	Low Na:K	0.333× LSW	1.89	10.0	14.6	4.09	7.66	14.9	43.0	53.3
ALSW with high alkalinity	High Alk	0.233× LSW	28.3	1.51	14.6	4.09	7.66	14.9	90.0	53.3
ALSW with low alkalinity	Low Alk	0.233× LSW	28.3	1.51	14.6	4.09	7.66	14.9	10.0	53.3
ALSW with varying Na		LSW	1.62	1.51	14.6	4.09	1.50	14.9	43.0	53.3
			3.00				3.62			
			10.0				14.4			
			30.0				45.3			
			100				153			
			300				460			

[a] Parentheses denote estimated geometric average ion concentrations at designated hardness for selected U.S. waters.

ALSW = amended Lake Superior water; LSW = Lake Superior water; MHRW = moderately hard reconstituted water.

Table 2. Major salts tested within each set of experiments

	Experimental Set Description	NaCl	Na_2SO_4	$NaHCO_3$	KCl	K_2SO_4	$KHCO_3$	$MgCl_2$	$MgSO_4$	$MgCO_3$	$CaCl_2$	Na gluconate	Mg gluconate	Ca gluconate	Mannitol
1	1/3× ALSW culture vs 1× ALSW culture, tested in 1/3× ALSW 1/3× MHRW culture vs 1× MHRW culture, tested in 1/3× MHRW	X[a]						X							
2	1/8× MHRW culture vs 1× MHRW culture, tested in 1/8× MHRW	X[b]	X	X	X			X	X		X				
3	MHRW vs ALSW	X	X	X	X	X	X	X	X	X	X				
4	1/3× ALSW vs 3× ALSW	X[c]	X[c]	X	X	X		X	X	X	X				
5	Low Ca:Mg vs high Ca:Mg	X	X	X	X			X	X						
6	Low $Cl:SO_4$ vs high $Cl:SO_4$	X	X	X		X			X	X					
7	Low Na:K vs high Na:K	X	X	X				X	X		X				
8	Low vs high alkalinity	X	X		X			X	X						
9	ALSW with low vs ambient vs high pH	X						X							
10	ALSW with varying Na (0.07–13 mM)				X										
11	$CaCO_3$ precipitation effects, aged dilution water vs not aged, 1× ALSW vs 3× ALSW			X						X					
12	Organic salts and mannitol, 1× ALSW only	X										X	X	X	X

[a] Organisms from both amended ALSW cultures also tested in 1× ALSW.

[b] Organisms from both cultures also tested in 1× MHRW.

[c] Duplicate tests conducted which also included 1× ALSW.

ALSW = amended Lake Superior water; MHRW = moderately hard reconstituted water.

Some test waters were based on the commonly used reconstituted water formulas given by the USEPA [24] and originally proposed by Marking and Dawson [25], specifically the formula for "moderately hard reconstituted water" (MHRW), which was also used by Mount et al. [3]. While commonly used, MHRW (and other reconstituted waters based on this formula) has a chemistry (Table 1) that is atypical for surface waters of the United States: the Ca:Mg ratio is low, the $Cl:SO_4$ ratio is very low, and the Na concentration is extremely high relative to hardness. The high Na concentration is a consequence of using $NaHCO_3$ to impart alkalinity; in nature, alkalinity generally comes from dissolution of carbonate minerals of Ca and Mg, but these have solubilities and dissolution rates that are inconveniently low for water preparation in the laboratory. The low Ca:Mg and $Cl:SO_4$ ratios in MHRW are not a practical requirement, and the reason for their original selection is not clear.

To design a dilution water with chemistry more like natural waters, we analyzed data from the US Geological Survey national stream water-quality monitoring networks [26]. Data records were obtained for 425 sites in operation from 1983 to 1992. Records ($n = 32\,895$) were used that had 1) measurements for all major ions, 2) a charge imbalance of no more than 10%, 3) a hardness $\geq$10 and $\leq$400 mg/L as $CaCO_3$, and 4) pH no lower than 6.0. These data were subjected to regression analyses using Sigmaplot (v11.0; Systat Software) to establish relationships between log(hardness) and the logarithms of various other ion concentrations and ratios. A formula was then developed for an "amended Lake Superior water" (ALSW; Table 1) consistent with these relationships. Salts were added to LSW to increase hardness to the estimated geometric average (53.3 mg $CaCO_3$/L) in the US Geological Survey data for the alkalinity of LSW (43.0 mg $CaCO_3$/L) and to increase other ions to average values for this hardness. Benefits of modifying LSW to create this test dilution water, rather than creating a completely synthetic water from deionized water, are that it provides alkalinity without having to dissolve carbonate salts or add excessive Na and that it will contain trace constituents from the source water that make the water more realistic and possibly beneficial to the test organisms.

The present study was organized into 12 sets of 1 to 4 experiments addressing different aspects of dilution water chemistry or experimental procedure. These sets of experiments and the toxicants that were tested are summarized in Table 2, and the chemistries of the various dilution waters are provided in Table 1. The purpose and design of each experimental set were as follows.

Sets 1 and 2: Effects of acclimation to dilution water. Some previous studies of the effect of dilution water on major ion toxicity to *C. dubia* used organisms that were cultured in the dilution water prior to testing [16–20]. This was not done routinely in the present study because the logistics involved in culturing organisms in so many different dilution waters (Table 1) were prohibitive. However, because a preliminary comparison of NaCl toxicity in MHRW and MHRW diluted by a factor of 3 (1/3× MHRW) showed much less of an impact of this dilution than the 1×, 1/2×, 1/4×, and 1/8× MHRW series tested by Elphick et al. [19], we conducted experiments to determine whether observed salt toxicity in waters more dilute than MHRW or ALSW varied significantly depending on whether the organisms were cultured in these dilute test waters or in the standard-strength water formulations. Initially, organisms from our primary culture (in 1× MHRW) were used to create secondary cultures in 1× MHRW, 1/3× MHRW, 1× ALSW, and 1/3× ALSW, which were maintained for a minimum of 2 generations before being used in tests. The toxicities of NaCl and $MgCl_2$ in 1/3× MHRW and 1/3× ALSW were simultaneously tested using the cultures from both the dilute water and the corresponding full-strength water (Table 2, set 1). For NaCl, tests were also conducted in 1x ALSW using both the 1× ALSW and 1/3× ALSW cultures. To further explore this issue, we conducted additional acclimation studies (Table 2, set 2) comparing the toxicity of several salts in 1/8× MHRW using *C. dubia* cultured in MHRW and in 1/8× MHRW (the same composition as the 10 and 80 hardness waters in the Elphick et al. [19] study). For NaCl, tests were also conducted in 1× MHRW using both cultures.

Set 3: Salt toxicity in MHRW versus ALSW. Because ALSW was developed in response to concerns about the composition of MHRW and because much existing ion toxicity information was conducted in MHRW or similar waters, comparative toxicities in these 2 waters were of interest. Therefore, a set of experiments (Table 2, set 3) compared the toxicity of all major ion salts in MHRW and ALSW, except for $CaSO_4$ and $CaCO_3$, for which preliminary experiments showed no toxicity at saturation in these waters.

Set 4: Salt toxicity in different strengths of ALSW. Natural waters vary widely in total ion concentration, the 10th and 90th percentiles in our analysis of US Geological Survey data being approximately 1.5 mEq/L and 16 mEq/L for the sum of major cations and anions. To determine the effect of varying overall ion concentrations, another set of experiments (Table 2, set 4) compared toxicities of 9 salts in dilution waters that had one-third and 3 times the ion concentrations of ALSW (1/3× ALSW and 3× ALSW; Table 1). We note that, in natural waters, average concentrations of the various ions do not vary exactly in proportion with total ion concentrations, so 1/3× ALSW and 3× ALSW do not match our estimated field water averages at the same hardnesses, which are also provided in Table 1 for comparison purposes. We elected not to match the field chemistry so that all ions would be in the same proportions across the 1/3×, 1×, and 3× ALSW series but the ion ratios would still be well within the ranges observed in the US Geological Survey data.

The results of these tests were combined with separate data for 1× ALSW (from set 3) to compare across a 1/3×, 1×, and 3× ALSW dilution water series; for NaCl and Na_2SO_4 an additional experiment simultaneously tested this entire series to verify results. Preparation of 1/3× ALSW was simply by dilution of ALSW with deionized water. Creating the 3× ALSW chemistry required dissolution of $CaCO_3$ to provide higher alkalinity while maintaining the desired cation ratios, in addition to adding other more soluble salts. This was accomplished by adding the appropriate amounts of the various salts to LSW in a large graduated cylinder and bubbling CO_2 gas through the solution until pH stabilized near 5.0 and all salts were dissolved. The solution was then aerated with ambient air until pH was approximately 7.8.

Sets 5–7: Effects of modifying specific ion ratios. Most previous work on the effects of dilution water chemistry on major ion toxicity to daphnids has focused on water hardness [16–20] as a generalized parameter without parsing the influence of other ions that covaried with the hardness, although Davies and Hall [21] did identify Ca as being more important than Mg for the effects of hardness on Na_2SO_4 toxicity. Regarding other ions in dilution water, Soucek [17] reported an ameliorative effect of low concentrations of added NaCl on the toxicity of Na_2SO_4 to *Hyalella azteca* (attributed to the chloride requirements for this species) but no such effect on *C. dubia*. At higher NaCl concentrations, there was an exacerbation of Na_2SO_4 toxicity for both species, attributed to additive toxicity of the 2 salts. Soucek et al. [18] also reported a small increase in apparent NaCl toxicity when Na_2SO_4 was added, again attributed to additive toxicity of the 2 salts.

To more completely evaluate the influence of particular dilution water characteristics on the toxicity of major ion salts, we tested selected salts in ALSW modified to provide different ratios of certain ion pairs, based on the tails of the distributions of these ratios in our analysis of US Geological Survey data. Waters with high Ca:Mg (13.6 by mass) and low Ca:Mg (0.27) ratios (Table 2, set 5) were created by changing the salts added to LSW to achieve the target ratios without altering concentrations of any other ions in the water (Table 1). Similar approaches were used to create waters with high and low $Cl:SO_4$ ratios (4.2 and 0.072 by mass; Table 2, set 6) and high and low Na:K ratios (38 and 0.19 by mass; Table 2, set 7). Some of these waters required that LSW be initially diluted with deionized water to lower the concentrations of a target ion to its lowest value, followed by addition of salts to match the ALSW chemistry except for the ion pair being manipulated (Table 1). Where required, the CO_2-aided dissolution of $CaCO_3$ was used to restore alkalinity to waters based on diluted LSW.

Sets 8 and 9: Effects of manipulating alkalinity and pH. The effects of pH and alkalinity were evaluated in 2 ways. First, the alkalinity of ALSW was reduced to 10 mg $CaCO_3$/L and raised to 90 mg $CaCO_3$/L (resulting, respectively, in pHs of 7.3–7.5 and 8.1–8.3; Table 2, set 8). Alkalinity was raised simply by adding $NaHCO_3$, whereas it was lowered by diluting LSW and adding salts to restore all cations and Cl to their same values as in the high-alkalinity water, with SO_4 replacing the alkalinity (Table 1). Second, pH was reduced to approximately 6.8 and raised to approximately 8.5 (Table 2, set 9) without altering alkalinity or other ions by bubbling the ALSW used in test solution preparation with air containing 1% CO_2 and 0% CO_2, respectively, and enclosing test vessels in sealed chambers containing these same CO_2 concentrations [27].

Set 10: Effect of sodium on potassium toxicity. Based on the results of the tests contrasting MHRW and ALSW (set 3), we hypothesized that the toxicity of K salts was dependent on the Na concentration in the dilution water. To evaluate this hypothesis, the toxicity of KCl was tested in 6 different dilution waters with Na ranging from 1.6 mg/L to 300 mg/L (Table 2, set 10). Test waters were prepared by modifying the formulation of ALSW to have no added Na and Cl, then adding NaCl to achieve the desired levels of Na (Table 1).

Set 11: Effects of time-dependent calcium precipitation. Testing of the toxicity of $NaHCO_3$ and $MgCO_3$ resulted in oversaturation of $CaCO_3$ and possible loss of Ca from solution, which would affect toxicity given the established ameliorative effect of Ca. Although Ca was monitored in these tests, the sampling was too limited to precisely determine the Ca concentration to associate with the LC50. Therefore, an additional experiment was conducted to more thoroughly characterize the dependence of any Ca loss on time and test salt concentration. This was a 2 × 2 × 2 factorial experiment, the 3 factors being test salt ($NaHCO_3$, $MgCO_3$), dilution water (1× ALSW, 3× ALSW), and solution age (freshly prepared vs aged for 48 h before introducing test organisms; Table 2, set 11). Extra replicate test cups were prepared to allow sampling of Ca at multiple times and treatment concentrations. This experiment provided not only $NaHCO_3$ and $MgCO_3$ LC50s from tests that were monitored better but also data with which to better estimate Ca concentrations at the LC50 in other CO_3/HCO_3 salt tests.

Set 12: Toxicities of gluconate salts and mannitol. Charge balance requires including both cations and anions in any testing of ion toxicity, making it more difficult to infer the relative toxicities of the individual ions. In addition, results of other experiments in the present study led us to hypothesize that the toxicity of some salts might be related to their effect on the osmolarity of the test solution and thus on the osmotic gradient the organism experiences. To provide more information on the role of the individual cations, we tested the toxicity of the gluconate salts of Na, Ca, and Mg (Table 2, set 12). Gluconate is an organic ion that is not expected to be absorbed appreciably and, thus, should act only through its effect on osmotic potential or on charge gradients, providing an informative contrast to Cl, SO_4, and HCO_3/CO_3. This experiment also evaluated the toxicity of mannitol, a sugar alcohol also not expected to be appreciably absorbed, enabling us to manipulate the external osmolarity without adding any ions. NaCl was also included as a reference major ion salt.

Toxicity test procedures

Salts for all 12 combinations of the 4 major cations and 3 major anions; gluconate salts of Na, Ca, and Mg; and mannitol were obtained from Sigma-Aldrich or Fisher Scientific. All chemicals were American Chemical Society reagent grade or better, with the exception of $MgCO_3$ which was specified as meeting US Pharmacopeia requirements. The certificate of analysis for this salt was used to determine the ratio of $MgCO_3$ to total salt weight for computing nominal $MgCO_3$ concentrations. This certificate of analysis also specified the Ca content to be 0.73% of the Mg content, enough to appreciably affect the background Ca; this was used to adjust the nominal Ca background concentration for tests on the toxicity of $MgCO_3$.

Test organisms were <24-h-old *C. dubia* obtained from in-house cultures. Most test organisms were cultured in MHRW, but organisms for testing the effects of acclimation were also cultured in the dilution waters for the tests (for a least 2 wk prior to the tests); and some tests late in the present study were conducted with organisms cultured in ALSW after a switch of our culture to this water. The culture water for each toxicity test is indicated in Table 3. General culture procedures followed those described by the USEPA [24].

Static 48-h toxicity tests were conducted in 30-mL plastic cups (Berry Plastics) filled with 10 mL test solution and held in polystyrene boards with holes sized to the cups. These boards were floated in a temperature-controlled water bath with a glass sheet covering all test cups. Because preliminary studies indicated that the response curves for major ion salts were quite steep, we used closely spaced exposure concentrations, with each test concentration being approximately 80% of the next higher. Test solutions were prepared by combining the applicable salt and dilution water to achieve the highest test concentration (100%). From this, 2 additional solutions, 80% and 64%, were prepared by dilution of the first; then, each of these 3 solutions was serially diluted by 0.5× to produce a total of 9 to 12 exposure concentrations, plus a dilution water control. The highest salt concentration in each test varied according to its expected toxicity (based on a combination of preliminary testing, any preceding experiments, and literature values), but all tests used the same relative dilution spacing. Most experiments were structured to compare toxicity under 2 related conditions (e.g., high and low Ca:Mg); tests were assigned to experiments such that both conditions would be tested simultaneously for a particular salt, using the same preparation of dilution water and the same cohort of test organisms.

All salts except $CaCO_3$, $MgCO_3$, and $CaSO_4$ could be easily dissolved at concentrations high enough to cause mortality. For $CaCO_3$ and $MgCO_3$, the CO_2 procedure in the description of set 4 was used to dissolve the salts. After adjusting to pH 7.8, $MgCO_3$ solutions so prepared were stable for 48 h at lethal concentrations, thereby allowing for testing as for other salts. However, a 15 mM (1500 mg/L) $CaCO_3$ solution dissolved in ALSW using CO_2 showed substantial precipitation and settling once the pH was raised, with the total concentration being only 6.5 mM at test start and dropping to 2.4 mM (2.2 mM dissolved) after 24 h and to 1.2 mM after 48 h, with no mortality observed. For $CaSO_4$, consistent with the findings of Mount et al. [3], a saturated solution at 16.2 mM (2200 mg $CaSO_4$/L) was not acutely toxic to *C. dubia* in MHRW or ALSW. Based on these results, no further testing was conducted with $CaSO_4$ or $CaCO_3$.

Test cups were usually prepared in duplicate, but in a few tests with CO_3/HCO_3 salts more replicates were used to provide test solution for more monitoring of alkalinity or Ca than in other tests. Each cup received food in the form of 100 μL of a 50:50 mixture of yeast, cereal leaves, and trout chow [24] and algae (*Pseudokirchneriella subcapitata* at 3.5×10^7 cells/mL). Food was added based on previous work indicating that it had minimal effect on toxicity of major ions [3] and to avoid possible stress. Test temperature was $25 \pm 1\,^{\circ}C$ with fluorescent lighting on a 16:8-h light: dark photoperiod. Five *C. dubia* neonates were added to each cup, and survival was determined after 24 h and 48 h of exposure; death was defined as no visible movement after gentle prodding and at least 10 s of observation.

Exposure monitoring

Temperature was monitored daily in test cups from each simultaneous test and continuously in the surrounding water bath. Hardness and alkalinity of dilution waters in every test were measured by titration [28]. For tests with $MgCO_3$ and $NaHCO_3$, alkalinity was also measured in the highest test concentration. Conductivity was used to verify that dilution of the salt solutions was done properly and was measured (model 2052; Amber Science) in each treatment at the start of the exposure, in every treatment that had 100% mortality after 24 h, and in all remaining treatments at 48 h. The pH was measured (PH150 with A57198 probe; Beckman Coulter) at the start of each test in the highest concentration and control (in all concentrations for tests with CO_3/HCO_3 salts), in every concentration that had 100% mortality at 24 h, and in all remaining treatments at 48 h. Dissolved oxygen was measured (model 58; Yellow Springs Instruments) in the highest concentration and control at the start of the test, in the highest and lowest concentrations with 100% mortality at 24 h, and in 2 arbitrarily selected treatments at 48 h.

Because of the large number of exposure treatments (~1500), it was not practical to measure major ion concentrations in every treatment. Instead, the analytical sampling program was structured with 2 primary purposes: 1) to verify that the dilution waters and highest concentrations were prepared properly and 2) to verify the exposure concentrations near the LC50. Accordingly, analytical samples were collected at the start of exposure from each dilution water and from the highest concentration of each salt in each dilution water. In addition, analytical samples were collected from each exposure series after 24 h in the lowest concentration with 100% mortality and at 48 h from the concentration nearest the LC50. Beyond this routine sampling program, some tests included additional

Table 3. Results of tests on the acute toxicity of major ion salts, gluconate salts, and mannitol to *Ceriodaphnia dubia* in various tests waters[a]

Set #	Test Chemical	Culture Water	Test Water	LC50 Method	LC50 mg/L (≥95% CL)	LC50 mM (≥95% CL)	Na mM	K mM	Ca mM	Mg mM	Cl mM	SO4 mM	Alk meq/L	pH	LC50 µS/cm	Osmol mOsM
	NaCl	MHRW	0.33× MHRW	Midpoint	1860 (1740–1990)	31.8 (29.8–34.0)	32.2	0.02	0.116	0.16	31.8	0.28	0.38	7.40	3170	61.4
	NaCl	MHRW	MHRW	Probit	2060 (2020–2490)	35.3 (34.5–42.6)	36.5	0.05	0.348	0.50	35.4	0.84	1.14	7.90	3500	70.1
	Na_2SO_4	MHRW	0.33× MHRW	Midpoint	1860 (1730–2010)	13.1 (12.2–14.2)	26.6	0.02	0.116	0.16	0.02	13.4	0.38	7.60	2480	35.7
	Na_2SO_4	MHRW	MHRW	Probit	3060 (2810–3350)	21.5 (19.8–23.6)	44.2	0.05	0.348	0.50	0.05	22.4	1.14	8.00	3680	58.7
	NaCl	ALSW	ALSW	Midpoint	1680 (1560–1790)	28.7 (26.8–30.7)	28.9	0.04	0.364	0.17	28.9	0.16	0.86	7.95	2980	56.1
	NaCl	ALSW	0.33× ALSW	Probit	1560 (1420–1720)	26.7 (24.4–29.4)	26.8	0.01	0.121	0.06	26.8	0.05	0.29	7.55	2700	51.3
	NaCl	0.33× ALSW	0.33× ALSW	Probit	1770 (1580–1980)	30.2 (27.0–33.9)	30.3	0.01	0.121	0.06	30.3	0.05	0.29	7.55	3030	57.9
1	NaCl	0.33× ALSW	ALSW	Probit	1940 (1780–2110)	33.2 (30.5–36.2)	33.5	0.04	0.364	0.17	33.4	0.16	0.86	7.90	3180	64.5
	NaCl	MHRW	MHRW	Probit	1810 (1680–1950)	31.0 (28.8–33.4)	32.1	0.05	0.348	0.50	31.0	0.84	1.14	8.00	3310	62.0
	NaCl	0.33× MHRW	0.33× MHRW	Midpoint	1640 (1530–1760)	28.1 (26.2–30.1)	28.4	0.02	0.116	0.16	28.1	0.28	0.38	7.60	2990	54.4
	NaCl	0.33× MHRW	0.33× MHRW	Probit	1560 (1440–1710)	26.7 (24.6–29.2)	27.1	0.02	0.116	0.16	26.8	0.28	0.38	7.70	2770	51.9
	NaCl	MHRW	0.33× MHRW	Probit	1480 (1340–1640)	25.3 (23.0–28.1)	25.7	0.02	0.116	0.16	25.4	0.28	0.38	7.70	2690	49.3
	$MgCl_2$	0.33× ALSW	0.33× ALSW	Probit	529 (457–612)	5.56 (4.80–6.43)	0.09	0.01	0.121	5.61	11.2	0.05	0.29	7.45	1230	15.9
	$MgCl_2$	ALSW	0.33× ALSW	Probit	667 (556–810)	7.01 (5.84–8.51)	0.09	0.01	0.121	7.06	14.1	0.05	0.29	7.45	1510	19.7
	$MgCl_2$	MHRW	0.33× MHRW	Probit	305 (271–338)	3.20 (2.85–3.55)	0.38	0.02	0.116	3.37	6.42	0.28	0.38	7.55	813	10.2
	$MgCl_2$	0.33× MHRW	0.33× MHRW	Probit	312 (192–417)	3.28 (2.02–4.38)	0.38	0.02	0.116	3.44	6.57	0.28	0.38	7.60	835	10.3
	NaCl	0.125× MHRW	0.125× MHRW	Midpoint	907 (828–993)	15.5 (14.2–17.0)	15.7	0.01	0.044	0.06	15.5	0.11	0.14	7.45	1750	31.0
	NaCl	0.125× MHRW	MHRW	Midpoint	1810 (1640–1990)	30.9 (28.1–34.0)	32.0	0.05	0.348	0.50	31.0	0.84	1.14	8.05	3260	61.9
	NaCl	MHRW	0.125× MHRW	Midpoint	1010 (940–1080)	17.2 (16.1–18.4)	17.3	0.01	0.044	0.06	17.2	0.11	0.14	7.35	1860	34.1
	NaCl	MHRW	MHRW	Probit	1710 (1580–1850)	29.3 (27.1–31.7)	30.4	0.05	0.348	0.50	29.4	0.84	1.14	8.05	3040	58.9
	$MgCl_2$	0.125× MHRW	0.125× MHRW	Probit	69 (53–90)	0.72 (0.56–0.95)	0.14	0.01	0.044	0.79	1.46	0.11	0.14	7.25	229	3.3
	$MgCl_2$	MHRW	0.125× MHRW	Probit	116 (94–202)	1.22 (0.99–2.12)	0.14	0.01	0.044	1.28	2.44	0.11	0.14	7.25	351	8.8
	Na_2SO_4	0.125× MHRW	0.125× MHRW	Midpoint	671 (613–734)	4.72 (4.32–5.17)	9.59	0.01	0.044	0.06	0.01	4.83	0.14	7.40	1070	14.2
2	Na_2SO_4	MHRW	0.125× MHRW	Midpoint	916 (829–1012)	6.45 (5.84–7.12)	13.0	0.01	0.044	0.06	0.01	6.55	0.14	7.20	1360	18.8
	$MgSO_4$	0.125× MHRW	0.125× MHRW	Probit	160 (137–184)	1.33 (1.14–1.53)	0.14	0.01	0.044	1.39	0.01	1.43	0.14	7.05	630	3.6
	$MgSO_4$	MHRW	0.125× MHRW	Probit	291 (264–321)	2.42 (2.19–2.67)	0.14	0.01	0.044	2.48	0.01	2.52	0.14	7.20	978	5.2
	KCl	0.125× MHRW	0.125× MHRW	Probit	177 (159–197)	2.37 (2.13–2.64)	0.14	2.38	0.044	0.06	2.38	0.11	0.14	7.40	389	5.9
	KCl	MHRW	0.125× MHRW	Midpoint	224 (204–245)	3.0 (2.74–3.29)	0.14	3.01	0.044	0.06	3.01	0.11	0.14	7.40	474	7.1
	$NaHCO_3$	0.125× MHRW	0.125× MHRW	Midpoint	1070 (1000–1140)	12.7 (11.9–13.6)	12.9	0.01	0.044	0.06	0.01	0.11	12.9	9.20	1110	23.8
	$NaHCO_3$	MHRW	0.125× MHRW	Midpoint	1000 (920–1090)	11.9 (10.9–13.0)	12.0	0.01	0.044	0.06	0.01	0.11	12.0	9.15	1060	22.4
	$CaCl_2$	0.125× MHRW	0.125× MHRW	Midpoint	1670 (1570–1780)	15.1 (14.2–16.1)	0.14	0.01	15.1	0.06	30.2	0.11	0.14	6.90	2930	41.2
	$CaCl_2$	MHRW	0.125× MHRW	Probit	1660 (1620–1940)	15.0 (14.6–17.5)	0.14	0.01	15.0	0.06	30.0	0.11	0.14	6.95	2920	40.9
	KCl	MHRW	ALSW	Probit	351 (321–384)	4.71 (4.31–5.15)	0.28	4.75	0.364	0.17	4.92	0.16	0.86	7.95	781	11.1
	KCl	MHRW	MHRW	Probit	464 (395–568)	6.22 (5.30–7.62)	1.14	6.28	0.348	0.50	6.28	0.84	1.14	8.10	1140	15.7
	$CaCl_2$	MHRW	ALSW	Probit	1900 (1690–2170)	17.2 (15.2–19.5)	0.28	0.04	17.5	0.17	34.5	0.16	0.86	7.90	3220	46.9
	$CaCl_2$	MHRW	MHRW	Probit	1990 (1760–2260)	18.0 (15.9–20.4)	1.14	0.05	18.3	0.50	36.0	0.84	1.14	7.95	3250	50.3
	$MgCl_2$	MHRW	ALSW	Probit	861 (759–981)	9.04 (7.97–10.3)	0.28	0.04	0.364	9.21	18.3	0.16	0.86	7.85	1900	26.1
	$MgCl_2$	MHRW	MHRW	Probit	733 (615–895)	7.70 (6.46–9.40)	1.14	0.05	0.348	8.19	15.4	0.84	1.14	7.95	1730	24.2
	K_2SO_4	MHRW	ALSW	Midpoint	362 (339–386)	2.08 (1.95–2.22)	0.28	4.19	0.364	0.17	0.22	2.23	0.86	7.90	690	7.7
	K_2SO_4	MHRW	MHRW	Midpoint	606 (557–659)	3.48 (3.20–3.78)	1.14	7.01	0.348	0.50	0.05	4.32	1.14	8.05	1150	13.2
	Na_2SO_4	MHRW	ALSW	Midpoint	3410 (3150–3700)	24.0 (22.2–26.0)	48.3	0.04	0.364	0.17	0.22	24.18	0.86	7.90	4080	63.4
3	Na_2SO_4	MHRW	MHRW	Midpoint	3260 (3060–3460)	22.9 (21.5–24.4)	47.0	0.05	0.348	0.50	0.05	23.76	1.14	8.00	3970	62.2

(continued)

Table 3. (*Continued*)

Set #	Test Chemical	Culture Water	Test Water	LC50 Method	LC50 mg/L (≥95% CL)	LC50 mM (≥95% CL)	Na mM	K mM	Ca mM	Mg mM	Cl mM	SO4 mM	Alk meq/L	pH	LC50 µS/cm	Osmol mOsM
	$MgSO_4$	MHRW	ALSW	Probit	2040 (1880–2230)	16.9 (15.6–18.5)	0.28	0.04	0.364	17.1	0.22	17.10	0.86	7.85	2240	25.2
	$MgSO_4$	MHRW	MHRW	Probit	1990 (1830–2180)	16.6 (15.2–18.1)	1.14	0.05	0.348	17.1	0.05	17.4	1.14	8.00	2280	26.3
	$KHCO_3$	MHRW	ALSW	Probit	322 (306–340)	3.22 (3.06–3.40)	0.28	3.25	0.364	0.17	0.22	0.16	4.08	8.70	487	8.0
	$KHCO_3$	MHRW	MHRW	Probit	454 (407–507)	4.53 (4.07–5.06)	1.14	4.59	0.348	0.50	0.05	0.84	5.68	8.85	844	13.3
	$NaHCO_3$	MHRW	ALSW	Midpoint	1980 (1880–2090)	23.6 (22.3–24.9)	23.8	0.04	0.187[b]	0.17	0.22	0.16	24.4	9.35	1940	41.7
	$NaHCO_3$	MHRW	MHRW	Probit	1610 (1440–1700)	19.1 (17.2–20.3)	20.3	0.05	0.141[b]	0.50	0.05	0.84	20.3	9.25	1680	36.6
	NaCl	MHRW	ALSW	Probit	1910 (1730–2110)	32.7 (29.6–36.1)	33.0	0.04	0.364	0.17	32.9	0.16	0.86	8.00	3110	63.6
	NaCl	MHRW	MHRW	Midpoint	2000 (1830–2190)	34.3 (31.4–37.5)	35.4	0.05	0.348	0.50	34.4	0.84	1.14	8.10	3470	68.1
	$MgCO_3$	MHRW	ALSW	Midpoint	906 (859–956)	10.8 (10.2–11.3)	0.28	0.04	0.443	10.9	0.22	0.16	22.4	9.25	1160	23.2
	$MgCO_3$	MHRW	MHRW	Midpoint	892 (848–938)	10.6 (10.1–11.1)	1.14	0.05	0.426	11.1	0.05	0.84	22.3	9.25	1230	25.2
	$NaHCO_3$	MHRW	0.33× ALSW	Midpoint	1950 (1780–2130)	23.2 (21.2–25.4)	23.3	0.01	0.121	0.06	0.07	0.05	23.5	9.30	1800	40.3
	$NaHCO_3$	MHRW	3.0× ALSW	Probit	1710 (1560–1890)	20.4 (18.6–22.5)	21.2	0.12	0.231[b]	0.50	0.65	0.47	23.0	9.35	1800	39.1
	NaCl	MHRW	0.33× ALSW	Midpoint	1640 (1530–1760)	28.1 (26.2–30.0)	28.2	0.01	0.121	0.06	28.1	0.05	0.29	7.40	2700	53.9
	NaCl	MHRW	3.0× ALSW	Probit	1980 (1770–2160)	33.8 (30.2–36.9)	34.7	0.12	1.093	0.50	34.5	0.47	2.58	8.30	3490	69.1
	Na_2SO_4	MHRW	0.33× ALSW	Probit	1940 (1770–2100)	13.6 (12.5–14.8)	27.3	0.01	0.121	0.06	0.07	13.7	0.29	7.55	2480	36.5
	Na_2SO_4	MHRW	3.0× ALSW	Midpoint	3350 (3140–3570)	23.6 (22.1–25.2)	47.0	0.12	1.093	0.50	0.65	23.8	2.58	8.45	4090	65.4
	KCl	MHRW	0.33× ALSW	Midpoint	192 (180–205)	2.58 (2.41–2.75)	0.09	2.59	0.121	0.06	2.65	0.05	0.29	7.65	430	5.7
	KCl	MHRW	3.0× ALSW	Probit	481 (432–534)	6.45 (5.79–7.16)	0.85	6.57	1.093	0.50	7.10	0.47	2.58	8.45	1220	18.1
	$MgSO_4$	MHRW	0.33× ALSW	Midpoint	1590 (1440–1770)	13.2 (11.9–14.7)	0.09	0.01	0.121	13.3	0.07	13.3	0.29	7.45	1780	19.4
	$MgSO_4$	MHRW	3.0× ALSW	Midpoint	2860 (2620–3130)	23.8 (21.7–26.0)	0.85	0.12	1.093	24.3	0.65	24.2	2.58	8.35	2910	36.9
	$MgCl_2$	MHRW	0.33× ALSW	Probit	374 (344–409)	3.93 (3.61–4.30)	0.09	0.01	0.121	3.98	7.93	0.05	0.29	7.25	910	11.6
4	$MgCl_2$	MHRW	3.0× ALSW	Probit	1290 (1160–1490)	13.6 (12.2–15.7)	0.85	0.12	1.093	14.0	27.8	0.47	2.58	8.35	2760	40.8
	$CaCl_2$	ALSW	0.3× ALSW	Midpoint	1600 (1490–1710)	14.4 (13.4–15.4)	0.09	0.01	14.5	0.06	28.8	0.05	0.29	7.35	2870	38.7
	$CaCl_2$	ALSW	3.0× ALSW	Midpoint	2000 (1870–2130)	18.0 (16.9–19.2)	0.85	0.12	19.1	0.50	36.7	0.47	2.58	7.95	3510	52.4
	K_2SO_4	ALSW	0.3× ALSW	Probit	234 (215–254)	1.34 (1.23–1.46)	0.09	2.70	0.121	0.06	0.07	1.39	0.29	7.40	426	4.5
	K_2SO_4	ALSW	3.0× ALSW	Midpoint	518 (449–598)	2.97 (2.58–3.43)	0.85	6.06	1.093	0.50	0.65	3.44	2.58	8.50	1120	13.7
	$MgCO_3$	ALSW	0.3× ALSW	Midpoint	390 (365–417)	4.63 (4.33–4.95)	0.09	0.01	0.155	4.68	0.07	0.05	9.54	8.95	619	11.8
	$MgCO_3$	ALSW	3.0× ALSW	Midpoint	977 (916–1041)	11.6 (10.9–12.4)	0.85	0.12	0.318[b]	12.1	0.65	0.47	25.8	9.15	1280	28.9
	NaCl	ALSW	0.33× ALSW	Midpoint	1700 (1600–1800)	29.1 (27.4–30.9)	29.2	0.01	0.121	0.06	29.1	0.05	0.29	7.50	2960	55.7
	NaCl	ALSW	ALSW	Probit	1910 (1760–2070)	32.6 (30.1–35.4)	32.9	0.04	0.364	0.17	32.8	0.16	0.86	7.90	3320	63.5
	NaCl	ALSW	3.0× ALSW	Midpoint	2420 (2210–2660)	41.5 (37.8–45.5)	42.3	0.12	1.093	0.50	42.1	0.47	2.58	8.40	4110	83.1
	Na_2SO_4	ALSW	0.33× ALSW	Midpoint	1890 (1760–2020)	13.3 (12.4–14.2)	26.7	0.01	0.121	0.06	0.07	13.4	0.29	7.55	2480	35.7
	Na_2SO_4	ALSW	ALSW	Midpoint	3440 (3220–3670)	24.2 (22.6–25.8)	48.7	0.04	0.364	0.17	0.22	24.4	0.86	7.95	4000	63.8
	Na_2SO_4	ALSW	3.0× ALSW	Probit	3520 (3200–3880)	24.8 (22.5–27.3)	50.4	0.12	1.093	0.50	0.65	25.3	2.58	8.50	4280	68.5
	NaCl	MHRW	Low Ca:Mg	Midpoint	1580 (1420–1740)	27.0 (24.3–29.8)	27.2	0.04	0.075	0.46	27.2	0.15	0.86	7.90	2790	53.0
	NaCl	MHRW	HighCa:Mg	Midpoint	1920 (1790–2050)	32.8 (30.7–35.0)	33.1	0.04	0.475	0.06	33.0	0.15	0.86	7.90	3218	63.8
	$NaHCO_3$	MHRW	Low Ca:Mg	Midpoint	1540 (1440–1640)	18.3 (17.2–19.5)	18.6	0.04	0.075	0.46	0.22	0.15	19.2	9.25	1611	33.8
	$NaHCO_3$	MHRW	HighCa:Mg	Midpoint	2000 (1870–2140)	23.8 (22.2–25.5)	24.1	0.04	0.200[b]	0.06	0.22	0.15	24.6	9.30	1970	41.2
	Na_2SO_4	MHRW	Low Ca:Mg	Probit	1430 (1340–1560)	10.1 (9.41–11.0)	20.4	0.04	0.075	0.46	0.22	10.2	0.86	7.95	2027	28.6
5	Na_2SO_4	MHRW	HighCa:Mg	Midpoint	3140 (2940–3360)	22.1 (20.7–23.6)	44.5	0.04	0.475	0.06	0.22	22.3	0.86	8.00	3822	58.7
	KCl	MHRW	Low Ca:Mg	Midpoint	245 (229–263)	3.29 (3.07–3.53)	0.28	3.32	0.075	0.46	3.50	0.15	0.86	7.95	626	8.4
	KCl	MHRW	HighCa:Mg	Midpoint	281 (258–306)	3.77 (3.46–4.10)	0.28	3.81	0.475	0.06	3.99	0.15	0.86	7.95	663	9.3

(*continued*)

Table 3. (*Continued*)

Set #	Test Chemical	Culture Water	Test Water	LC50 Method	LC50 mg/L (≥95% CL)	LC50 mM (≥95% CL)	Na mM	K mM	Ca mM	Mg mM	Cl mM	SO4 mM	Alk meq/L	pH	LC50 µS/cm	Osmol mOsM
	$MgSO_4$	MHRW	Low Ca:Mg	Probit	935 (857–1020)	7.77 (7.12–8.52)	0.28	0.04	0.075	8.23	0.22	7.92	0.86	7.90	1292	13.3
	$MgSO_4$	MHRW	HighCa:Mg	Midpoint	2060 (1920–2200)	17.1 (15.9–18.3)	0.28	0.04	0.475	17.1	0.22	17.2	0.86	7.95	2232	25.4
	$MgCl_2$	MHRW	Low Ca:Mg	Probit	223 (203–245)	2.34 (2.13–2.57)	0.28	0.04	0.075	2.80	4.90	0.15	0.86	7.90	654	8.5
	$MgCl_2$	MHRW	HighCa:Mg	Probit	935 (865–1015)	9.82 (9.09–10.7)	0.28	0.04	0.475	9.88	19.9	0.15	0.86	7.90	2041	28.1
	Na_2SO_4	MHRW	Low Cl:SO4	Midpoint	3430 (3200–3670)	24.1 (22.5–25.8)	48.5	0.04	0.365	0.17	0.04	24.3	0.86	8.00	4001	63.5
	Na_2SO_4	MHRW	HighCl:SO4	Probit	2990 (2770–3250)	21.1 (19.5–22.9)	42.4	0.04	0.365	0.17	0.40	21.1	0.86	7.95	3632	56.2
	K_2SO_4	MHRW	Low Cl:SO4	Midpoint	377 (355–401)	2.16 (2.04–2.30)	0.28	4.37	0.365	0.17	0.04	2.38	0.86	8.00	710	7.8
	K_2SO_4	MHRW	HighCl:SO4	Midpoint	349 (322–379)	2.00 (1.85–2.17)	0.28	4.04	0.365	0.17	0.40	2.04	0.86	8.00	669	7.6
	$MgSO_4$	MHRW	Low Cl:SO4	Midpoint	1980 (1800–2170)	16.4 (15.0–18.0)	0.28	0.04	0.365	16.6	0.04	16.6	0.86	7.90	2172	24.4
6	$MgSO_4$	MHRW	HighCl:SO4	Probit	1860 (1670–2080)	15.5 (13.9–17.3)	0.28	0.04	0.365	15.6	0.40	15.5	0.86	7.90	2097	23.4
	$NaHCO_3$	MHRW	Low Cl:SO4	Probit	1990 (1830–2180)	23.7 (21.8–26.0)	24.0	0.04	0.190^b	0.17	0.04	0.22	24.6	9.35	1927	41.9
	$NaHCO_3$	MHRW	HighCl:SO4	Midpoint	2050 (1910–2200)	24.4 (22.8–26.1)	24.7	0.04	0.166^b	0.17	0.40	0.04	25.2	9.35	2003	42.2
	NaCl	MHRW	Low Cl:SO4	Probit	1660 (1500–1840)	28.4 (25.7–31.5)	28.7	0.04	0.365	0.17	28.4	0.22	0.86	7.95	2816	55.5
	NaCl	MHRW	HighCl:SO4	Midpoint	1700 (1600–1800)	29.1 (27.4–30.9)	29.4	0.04	0.365	0.17	29.5	0.04	0.86	7.90	2921	57.0
	$MgCO_3$	MHRW	Low Cl:SO4	Probit	948 (874–1030)	11.2 (10.4–12.2)	0.28	0.04	0.447	11.4	0.04	0.22	23.4	9.20	1149	24.7
	$MgCO_3$	MHRW	HighCl:SO4	Midpoint	894 (836–957)	10.6 (9.92–11.4)	0.28	0.04	0.442	10.8	0.40	0.04	22.1	9.20	1131	23.8
	NaCl	ALSW	Low K ALSW	Midpoint	1920 (1800–2040)	32.8 (30.9–35.0)	33.2	0.01	0.364	0.17	33.‘	0.16	0.86	7.85	3110	63.9
	NaCl	ALSW	High K ALSW	Probit	1980 (1830–2160)	34.0 (31.3–37.0)	34.0	0.26	0.364	0.17	34.1	0.16	0.86	7.85	3216	66.0
	Na_2SO_4	ALSW	Low K ALSW	Probit	2920 (2650–3230)	20.6 (18.7–22.8)	41.5	0.01	0.364	0.17	0.22	20.8	0.86	7.90	3351	54.9
	Na_2SO_4	ALSW	High K ALSW	Probit	3420 (3140–3730)	24.0 (22.1–26.3)	48.2	0.26	0.364	0.17	0.22	24.2	0.86	7.85	3858	63.5
	$NaHCO_3$	ALSW	Low K ALSW	Midpoint	2090 (1960–2220)	24.8 (23.3–26.5)	25.2	0.01	0.139^b	0.17	0.22	0.16	25.7	9.35	1867	43.8
7	$NaHCO_3$	ALSW	High K ALSW	Midpoint	2220 (2010–2450)	26.4 (23.9–29.1)	26.5	0.26	0.192^b	0.17	0.22	0.16	27.2	9.35	2007	46.2
	$CaCl_2$	ALSW	Low K ALSW	Probit	1870 (1690–2050)	16.9 (15.2–18.5)	0.33	0.01	17.2	0.17	34.0	0.16	0.86	7.90	3168	46.2
	$CaCl_2$	ALSW	High K ALSW	Probit	1710 (1550–1880)	15.4 (14.0–17.0)	0.08	0.26	15.8	0.17	31.1	0.16	0.86	7.85	2942	42.5
	$MgCl_2$	ALSW	Low K ALSW	Probit	970 (853–1103)	10.2 (8.96–11.6)	0.33	0.01	0.364	10.4	20.6	0.16	0.86	7.85	2022	29.1
	$MgCl_2$	ALSW	High K ALSW	Probit	912 (794–1050)	9.58 (8.34–11.0)	0.08	0.26	0.364	9.75	19.4	0.16	0.86	7.90	1968	27.5
	$MgSO_4$	ALSW	Low K ALSW	Probit	2030 (1790–2300)	16.8 (14.9–19.1)	0.33	0.01	0.364	17.0	0.22	17.0	0.86	7.95	2232	25.1
	$MgSO_4$	ALSW	High K ALSW	Probit	2240 (1980–2470)	18.6 (16.5–20.5)	0.08	0.26	0.364	18.8	0.22	18.8	0.86	8.00	2329	27.3
	KCl	MHRW	Low Alk ALSW	Midpoint	673 (632–718)	9.03 (8.48–9.63)	1.23	9.07	0.364	0.17	9.24	0.95	0.20	7.45	1433	20.2
	KCl	MHRW	High Alk ALSW	Probit	533 (487–584)	7.15 (6.53–7.83)	1.23	7.19	0.364	0.17	7.37	0.16	1.80	8.30	1188	17.6
	NaCl	MHRW	Low Alk ALSW	Probit	1860 (1690–2050)	31.8 (28.9–35.1)	33.1	0.04	0.364	0.17	32.1	0.95	0.20	7.40	3316	62.9
	NaCl	MHRW	High Alk ALSW	Probit	1970 (1790–2120)	33.8 (30.6–36.3)	35.0	0.04	0.364	0.17	34.0	0.16	1.80	8.25	3474	67.3
	$MgCl_2$	MHRW	Low Alk ALSW	Probit	899 (784–1008)	9.44 (8.23–10.6)	1.23	0.04	0.364	9.61	19.1	0.95	0.20	7.30	2076	27.9
8	$MgCl_2$	MHRW	High Alk ALSW	Midpoint	611 (571–654)	6.42 (6.00–6.87)	1.23	0.04	0.364	6.59	13.0	0.16	1.80	8.25	1542	21.0
	Na_2SO_4	MHRW	Low Alk ALSW	Probit	2960 (2700–3240)	20.8 (19.0–22.8)	42.9	0.04	0.364	0.17	0.22	21.8	0.20	7.45	3736	56.3
	Na_2SO_4	MHRW	High Alk ALSW	Probit	2830 (2560–3130)	19.9 (18.0–22.0)	41.0	0.04	0.364	0.17	0.22	20.1	1.80	8.25	3481	54.9
	$MgSO_4$	MHRW	Low Alk ALSW	Midpoint	2250 (2010–2510)	18.7 (16.7–20.8)	1.23	0.04	0.364	18.8	0.22	19.6	0.20	7.30	2475	28.2
	$MgSO_4$	MHRW	High Alk ALSW	Probit	2060 (1850–2450)	17.2 (15.4–20.3)	1.23	0.04	0.364	17.3	0.22	17.3	1.80	8.20	2263	27.1
	$MgCl_2$	MHRW	Low Alk ALSW	Probit	865 (754–985)	9.09 (7.92–10.4)	1.23	0.04	0.364	9.25	18.4	0.95	0.20	7.25	1986	27.0
	$MgCl_2$	MHRW	High Alk ALSW	Probit	623 (559–692)	6.54 (5.87–7.27)	1.23	0.04	0.364	6.71	13.3	0.16	1.80	8.10	1546	21.4
	NaCl	ALSW	ALSW	Probit	2080 (1890–2280)	35.5 (32.3–39.1)	35.8	0.04	0.364	0.17	35.8	0.16	0.86	7.85	3342	68.9
	NaCl	ALSW	Low pH ALSW	Midpoint	2160 (1980–2370)	37.0 (33.9–40.5)	37.3	0.04	0.364	0.17	37.2	0.16	0.86	6.75	3459	71.9
9	NaCl	ALSW	High pH ALSW	Probit	1880 (1730–2050)	32.2 (29.7–35.0)	32.5	0.04	0.364	0.17	32.4	0.16	0.86	8.20	3116	62.7
	$MgCl_2$	ALSW	ALSW	Probit	938 (841–1090)	9.85 (8.83–11.4)	0.28	0.04	0.364	10.0	19.9	0.16	0.86	7.85	2045	28.2

(*continued*)

Table 3. (*Continued*)

Set #	Test Chemical	Culture Water	Test Water	LC50 Method	LC50 mg/L (≥95% CL)	LC50 mM (≥95% CL)	Na mM	K mM	Ca mM	Mg mM	Cl mM	SO4 mM	Alk meq/L	pH	LC50 µS/cm	Osmol mOsM
	$MgCl_2$	ALSW	Low pH ALSW	Probit	1040 (894–1230)	10.9 (9.39–13.0)	0.28	0.04	0.364	11.0	22.0	0.16	0.86	6.75	2057	31.0
	$MgCl_2$	ALSW	High pH ALSW	Probit	1130 (1040–1280)	11.9 (10.9–13.4)	0.28	0.04	0.364	12.0	23.9	0.16	0.86	8.50	2257	33.2
10	KCl	MHRW	1.6 Na ALSW	Probit	153 (141–167)	2.05 (1.89–2.24)	0.07	2.09	0.364	0.17	2.09	0.16	0.86	7.95	422	5.6
	KCl	MHRW	3 Na ALSW	Probit	241 (206–268)	3.23 (2.76–3.59)	0.13	3.27	0.364	0.17	3.34	0.16	0.86	8.00	599	8.0
	KCl	MHRW	10 Na ALSW	Midpoint	393 (360–429)	5.27 (4.83–5.75)	0.43	5.31	0.364	0.17	5.68	0.16	0.86	7.95	872	12.5
	KCl	MHRW	30 Na ALSW	Midpoint	513 (469–560)	6.88 (6.29–7.51)	1.30	6.92	0.364	0.17	8.16	0.16	0.86	7.95	1193	17.3
	KCl	MHRW	100 Na ALSW	Midpoint	644 (602–690)	8.64 (8.08–9.26)	4.35	8.68	0.364	0.17	13.0	0.16	0.86	7.95	1656	26.4
	KCl	MHRW	300 Na ALSW	Probit	752 (641–1360)	10.1 (8.60–18.2)	13.0	10.13	0.364	0.17	23.1	0.16	0.86	7.90	2616	45.3
11	$NaHCO_3$	ALSW	ALSW Aged	Midpoint	1660 (1531–1790)	19.7 (18.2–21.3)	20.0	0.04	0.134[c]	0.17	0.22	0.16	20.6	9.35	1670	35.5
	$NaHCO_3$	ALSW	3.0× ALSW Aged	Midpoint	1670 (1530–1810)	19.8 (18.3–21.5)	20.7	0.12	0.126[c]	0.50	0.65	0.47	22.4	9.35	1793	38.2
	$NaHCO_3$	ALSW	ALSW Fresh	Probit	2150 (1990–2320)	25.6 (23.7–27.6)	25.9	0.04	0.207[c]	0.17	0.22	0.16	26.4	9.15	2087	47.2
	$NaHCO_3$	ALSW	3.0× ALSW Fresh	Midpoint	2240 (2080–2420)	26.7 (24.8–28.8)	27.5	0.12	0.139[c]	0.50	0.65	0.47	29.3	9.10	2284	51.7
	$MgCO_3$	ALSW	ALSW Aged	Probit	767 (699–843)	9.10 (8.29–10.0)	0.28	0.04	0.431	9.27	0.22	0.16	19.0	9.30	1043	20.9
	$MgCO_3$	ALSW	3.0× ALSW Aged	Probit	507 (467–551)	6.01 (5.54–6.54)	0.85	0.12	0.147[c]	6.52	0.65	0.47	14.6	9.30	879	18.0
	$MgCO_3$	ALSW	ALSW Fresh	Midpoint	792 (736–853)	9.39 (8.73–10.1)	0.28	0.04	0.433	9.56	0.22	0.16	19.6	9.15	1054	23.8
	$MgCO_3$	ALSW	3.0× ALSW Fresh	Probit	635 (529–752)	7.53 (6.27–8.92)	0.85	0.12	0.152[c]	8.04	0.65	0.47	17.6	9.00	941	18.4
12	NaCl	ALSW	ALSW	Midpoint	1950 (1840–2070)	33.4 (31.5–35.5)	33.7	0.04	0.364	0.17	33.6	0.16	0.86	7.85	3296	65.0
	Mannitol	ALSW	ALSW	Probit		73.7 (66.2–82.1)	0.28	0.04	0.364	0.17	0.22	0.16	0.86	7.75		75.7
	NaGluconate	ALSW	ALSW	Midpoint		39.2 (36.8–42.0)	39.5	0.04	0.364	0.17	0.22	0.16	0.86	7.85	2188	76.1
	CaGluconate	ALSW	ALSW	Probit		32.9 (29.9–36.2)	0.28	0.04	33.3	0.17	0.22	0.16	0.86	7.80	2074	84.6
	MgGluconate	ALSW	ALSW	Probit		17.1 (14.3–18.7)	0.28	0.04	0.364	17.3	0.22	0.16	0.86	7.80	1638	50.6

[a] See text for designations and formulations for test and culture waters; horizontal gaps delineate experiments and horizontal lines delineate experimental sets. For median lethal concentrations (LC50s), “Probit” denotes exposure-effects curve calculation by probit method; “Midpoint” denotes geometric mean of confidence limits; parentheses denote confidence limits; see text for methodology. Except where noted, LC50s and component concentrations are nominal, and expected to be within +/–10% based on analysis of subset of test solutions.

[b] Ca concentrations are an extrapolation of Ca measurements to median lethal concentration based on model for time dependence of Ca as a function of alkalinity; see Supplemental Data for more information.

[c] Ca concentrations are a weighted average of measurements and 24 and 48 hours that bracket the median lethal concentration.

ALSW = amended Lake Superior water; LC50 = median lethal concentration; MHRW = moderately hard reconstituted water.

sampling from intermediate treatments at the beginning and end of exposure to verify that exposure concentrations were stable over the course of the exposure. Every dilution water was analyzed for all 4 cations plus Cl and SO_4. Because cation analysis was more time-efficient than anion analysis, exposure concentrations of the salt tested were verified by analyzing for its cation. For tests of the toxicity of CO_3/HCO_3 salts, which caused oversaturation of $CaCO_3$ even at the low Ca concentrations in dilution water, Ca concentrations were also measured. In the Ca precipitation experiment with $NaHCO_3$ and $MgCO_3$ (set 11), extra test chambers were used to monitor Ca at 0 h, 24 h, and 48 h at the 25%, 50%, and 100% treatment concentrations, in addition to the cation samples normally taken.

For cation analysis, filtered (0.45-μm nylon syringe filter; Grainger) and unfiltered samples were collected in early experiments; but these analyses consistently showed no significant difference between the 2, so later cation samples were not filtered, except for tests with $NaHCO_3$ and $MgCO_3$ in which $CaCO_3$ precipitation was a concern. Samples for cation analysis were acidified by adding 0.2% (v/v) concentrated HNO_3 and held at room temperature; for tests with $NaHCO_3$ and $MgCO_3$, this amount of acid was increased by an amount calculated to neutralize the extra alkalinity. Cation measurements were made using an Agilent 240 FS flame atomic absorption spectrophotometer (Agilent Technologies), calibrated with a blank and a series of 5 standards, and verified with a quality control standard and an independent calibration standard at both the beginning and the end of the run.

Concentrations of Cl and SO_4 were determined on samples filtered into a polypropylene centrifuge tube, stored under refrigeration, and analyzed within 28 d. Quantifications were made using a Dionex DX600 Ion Chromatograph with an AS50 autosampler, an LC25 chromatography oven, an ED50 electrochemical detector, and a GP50 gradient pump (Thermo Fisher Scientific). A typical instrument run included a blank, a series of 8 standards, a blank spike, and a quality control standard analyzed at both the beginning and the end of the run. Thirteen percent of all ion analyses were run in duplicate; the average relative percentage difference was 1.6% (maximum 8%, standard deviation 1.6%).

Across all test solutions (i.e., samples other than controls) of the 10 major ion salts for which LC50s were determined, comparison of measured and nominal cation concentrations averaged 98.7% with a standard deviation of 4.9% ($n = 329$). Individual results ranged from 83% to 116% of nominal, and 95% fell between 90% and 110% of nominal. Conductivity measurements on all treatments confirmed that the intended gradient of exposure existed among samples not sampled for cation analysis. Based on these results, and the complexity of estimating concentrations in test solutions that were not directly measured, nominal exposure concentrations were used for calculating LC50 values.

Analysis of dilution waters yielded a similarly high average agreement between the measured cation concentrations and nominal values based on past reported measurements (99.3%, $n = 313$) but a higher standard deviation of 10.2%; 97% of values fell between 80% and 120% of nominal and 79% of samples fell between 90% and 110% of nominal. This higher variability is associated with the much lower ion concentrations in control waters, which can inflate percentage error values; in the more dilute waters, K in particular was at very low concentrations, sometimes lower than can be confidently quantified by the methods used (e.g., <0.5 mg/L). Wherever measured concentrations deviated more than 20% from nominal ($n = 8$), all data from that test were reviewed; and in each instance, the information suggested the large deviation was likely spurious (e.g., concentrations of all other ions measured in the same water were close to nominal). Accordingly, ion concentrations in dilution water were also assumed to be equal to nominal in the data analyses, except for Ca concentrations in tests on the toxicity of CO_3/HCO_3 salts, in which case Ca was based on measured concentrations. Because these Ca concentrations varied with time and added salt concentration, this involved interpolation of Ca measurements, which is further discussed in the Supplemental Data.

Data analysis

Concentration–response curves were extremely steep, so that even with the closely spaced exposure concentrations (0.8×), it was common for there to be only 1 (or no) exposure treatment with partial survival less than that attributable to background (control) mortality. For such tests, continuous concentration–effect models cannot be fit to provide point estimates for LC50s. Consequently, a tiered approach was used, based on the type of calculation the data would support. For each test, a background mortality range was defined as all treatments up to the highest concentration at which the fraction of mortality was no greater than at any lower concentration. The number of treatments with partial mortality (i.e., above this background mortality range and below the lowest treatment with complete mortality) was then determined and used to select the type of analysis.

For tests with at least 2 partial mortalities that increased with concentration, a tolerance distribution analysis was conducted, using a 3-parameter model that included a background survival parameter and assumed a log-normal distribution for the lethal concentrations. Parameters were estimated by maximum likelihood analysis using custom software written with Intel Professional Fortran Composer XE 2011. Of the 149 LC50s reported in the present study, 82 supported calculation of such "probit LC50s." The 95% confidence limits were calculated using the likelihood ratio method [29]. Statistical significance of LC50 differences between treatments within an experiment was assessed based on these confidence limits not overlapping, so that such differences have a significance level of at least 95%.

For tests with insufficient partial mortalities for this probit LC50 analysis, the same likelihood ratio method was used to calculate confidence limits for the LC50 (such confidence limits can be assigned even when a unique point estimate for the LC50 cannot be calculated). For these tests, we assigned the geometric mean of these confidence limits to be the point estimate for the LC50 ("midpoint LC50s"). For cases in which there were no partial mortalities, these confidence limits are the bracketing concentrations (the upper end of the background mortality range and the lowest treatment with complete mortality) and the LC50 is equivalent to linear interpolation of survival versus log concentration between these 2 concentrations. To test the performance of this methodology when tolerance distribution assumptions are met, it was applied to simulated data sets generated based on the range of observed parameters, which demonstrated the bias for LC50 estimation to be <1% and the confidence limits to equal or exceed 95%.

These LC50 calculations were based on the nominal concentration of added salt, without consideration of background ion concentrations, because different ion ratios in the dilution water and the added salt make it impossible to express total ion concentrations as an equivalent concentration of salt. Initial comparisons of how dilution water differences affect salt toxicity are also on the basis of added salt for the same reason.

Figure 1. Effects of culturing and testing organisms in different strengths of amended Lake Superior water and moderately hard reconstituted water on median lethal concentrations for selected major ion salts to *Ceriodaphnia dubia*. Error bars denote upper 95% confidence limits, and asterisks denote where confidence limits do not overlap. ALSW = amended Lake Superior water; LC50 = 48-h median lethal concentration; MHRW = moderately hard reconstituted water.

Background concentrations of the component ions of the test salt were <5% in all but 6 tests and never >10%; these had negligible impact on comparisons based on added salt except as noted in *Results and Discussion*. However, for further analyses of the aggregated data, exposure metrics included both background and added ions.

Although test preparation and chemical analysis were based on weight of the salts and the various ions, this is not a good basis for comparing the relative toxicity of the individual salts and ions because the toxic action of these ions should be related to their molarity, not their mass. As such, salt and ion concentrations were converted to molarity, which is the primary basis for toxicity comparisons of the added salts. In addition, evaluation of the dosimetry at these elevated ion concentrations should consider formation of complexes between the various cations and anions, which would affect their chemical reactivities and thus their toxicities. It should also consider that the high ionic strength of these solutions will reduce the reactivity of the ions, as represented in lower activity coefficients, and that exposure metrics thus would be best expressed as chemical activity rather than molarity. To this end, the ion composition at each LC50 was analyzed using the chemical speciation program Visual MINTEQ (Ver 3.0) to estimate chemical speciation and the chemical activity of each chemical species. The osmolarity of each LC50 solution was also calculated based on these MINTEQ activity estimates using the method described by Robinson and Stokes [30].

RESULTS AND DISCUSSION

For each test ($n = 149$), added-salt LC50 values with confidence limits are provided in Table 3, as both milligrams per liter and millimolarity, along with total ion concentrations and osmolarities at these LC50s. The pH associated with each LC50 at the end of exposure is also provided based on measurements in treatments bracketing the LC50. All reported tests had conductivity measurements that varied across treatments in a manner consistent with the intended exposure concentrations, and LC50s directly based on these conductivities are also provided in Table 3. Dissolved oxygen was always above 7.5 mg/L, and temperature was always within 1 °C of 25 °C. Measurements made at the beginning and end of experiments indicated that evapoconcentration during the experiment was low (<5%). Over the background mortality range, as defined in *Data analysis*, survival was ≥90% in all toxicity tests and averaged 98% overall.

Effects of acclimation to dilution water

Figure 1 compares LC50s for *C. dubia* cultured in dilute solutions to those from our standard culture waters. Culturing organisms in 1/3× ALSW or 1/3× MHRW had no significant effect on the toxicities of NaCl and $MgCl_2$, either in test waters at these dilutions or (for NaCl only) in 1× ALSW, the LC50 estimates differing by 20% or less. Culturing organisms in 1/8× MHRW also had no effect on the toxicities of NaCl, $NaHCO_3$, and $CaCl_2$, the LC50s differing by no more than 10%. However, for Na_2SO_4, KCl, $MgCl_2$, and $MgSO_4$, LC50s for 1/8× MHRW test water are 20% to 45% lower for organisms cultured in 1/8× MHRW than those cultured in 1× MHRW, these differences being statistically significant except for $MgCl_2$, for which the LC50 is uncertain because of highly variable responses in this dilute test water.

Thus, "acclimation" to 1/8× MHRW resulted in *C. dubia* being more, rather than less, sensitive to some salts when tested in this dilute solution. Although both we and Elphick et al. [19] established successful cultures in this water, based on our experience with *C. dubia*, such a dilute solution appears to be stressful, an indication of this being the greater variability of organism response to salt toxicity. It is not clear whether this should be considered an artificial stress created by manipulating organisms long adapted to laboratory waters with higher ion concentrations, or a natural consequence for a species not adapted to and not endemic to such dilute waters.

These acclimation experiments were prompted by the work of Elphick et al. [19], who acclimated cultures to dilutions waters (for 2 generations or more) and found that, relative to 1× MHRW, the 7-d NaCl LC50 for *C. dubia* decreased by 2.1-fold in 1/2× MHRW, 3.6-fold in 1/4× MHRW, and 8.4-fold in 1/8× MHRW. In contrast, for organisms both cultured and tested in the waters, we found only a 9% decrease in the NaCl LC50 between 1× MHRW and 1/3× MHRW and only a 1.9-fold decrease between MHRW and 1/8× MHRW (Figure 1). The lack of culture water effect on our LC50s for NaCl suggests that these differences between the 2 studies resulted from other factors, such as the longer duration in Elphick et al. [19] or a variability associated with culturing and/or testing *C. dubia* in such dilute waters. More recent communication with J. Elphick has indicated that they have observed variability in NaCl LC50s for *C. dubia* in very dilute test waters (D.R. Mount, personal communication).

Overall, we concluded that the absence of dilution water-specific culturing was not a substantial factor for most of our experiments but that data for the 1/8× MHRW waters should be interpreted with caution. The results presented hereafter will be only for organisms from our standard culture waters and will consider LC50s for the most dilute test waters to be more useful for qualitative insights into ion toxicity rather than establishing quantitative relationships. Any quantitative uncertainties of LC50s for such dilute waters are probably of limited practical concern because few unimpacted surface waters will likely experience elevations of a single salt to toxic levels while other ions remain at such low levels.

Our culture experiments only addressed more dilute waters than our standard culture water because discrepancies of our results from that of Elphick et al. [19] were noted for those waters. Although we did not evaluate effects of culturing at higher ion concentrations, the relationship of our NaCl LC50s to Ca concentration shows good agreement with that of Soucek et al. [18] and Elphick et al. [19], who did culture organisms in their different test waters. Also, Soucek and Kennedy [16] reported only small and statistically nonsignificant differences among Na_2SO_4 LC50s in MHRW for *C. dubia* cultured in MHRW versus MHRW with elevated Na_2SO_4 levels. This suggests that acclimation to higher ion concentrations may not be a significant factor, but more study would be needed to reach a definitive conclusion.

Salt toxicity in MHRW versus ALSW

Figure 2 compares LC50s for 10 major ion salts tested in both ALSW and MHRW. For NaCl, $MgCl_2$, $CaCl_2$, Na_2SO_4, $MgSO_4$, and $MgCO_3$, LC50 differences are <15% and not statistically significant. For $NaHCO_3$, the LC50 is 20% lower for MHRW and is statistically significant; however, this test involved significant precipitation of Ca, and this precipitation was more pronounced in the MHRW test (Table 3). Given previous studies on the effect of hardness on salt toxicity [16–21], such a Ca difference would contribute to the observed LC50 difference. The fact that MHRW provides a higher background Na concentration and alkalinity would also contribute to the difference in LC50s expressed in terms of added $NaHCO_3$.

Only for the 3 K salts are there clear, substantial differences between the 2 dilution waters. The LC50s are 32% to 67% higher for MHRW than for ALSW, and these differences were all statistically significant. Notable compositional differences between these waters (Table 1) include 4-fold higher Na in MHRW (because of the use of $NaHCO_3$ to add alkalinity), 3-fold higher Mg in MHRW (but similar Ca), 5-fold higher SO_4, and 3-fold lower Cl. The higher Na for MHRW is of particular interest because Na and K are linked physiologically through the central role of Na K adenosine triphosphatases in ion regulation and other key cellular processes [2]. Additional information regarding this issue is provided below (see *Effects of sodium on potassium toxicity*).

Comparing the LC50s in Figure 2 across salts also supports some inferences regarding the contributions of individual ions to toxicity. On a total molarity basis, the Na salts are significantly less toxic than salts of other cations with the same anion. On a total molarity basis NaCl is also significantly less toxic than the other Na salts. For Na_2SO_4, the greater toxicity could be caused by it containing 2, instead of 1, Na atoms, in addition to any anion effects. For $NaHCO_3$, the greater toxicity should be partly the result of lower Ca concentrations (Table 3) from the $CaCO_3$ precipitation induced by this salt. These differences among Na salts will be further addressed below (see *Effects of calcium on the toxicities of sodium salts*).

The K salts are much more toxic than the corresponding salts with other cations, especially Na (Figure 2). The ratio of the Na salt LC50 to the K salt LC50 ranges across the different anions from 4.2 to 6.6 for MHRW and from 5.8 to 9.1 for ALSW, with smaller ratios for MHRW resulting from the aforementioned dilution water effect on K toxicity. These large ratios indicate that K is the principal source of toxicity for these salts because the anion concentrations at the LC50s for K salts are in all cases a small fraction of those present at the LC50s for the Na salts. The LC50 on a molarity basis for K_2SO_4 is approximately half that of KCl, consistent with toxicity being related to the molarity of K rather than the molarity of the salt. Factors controlling K salt toxicity are explored further below (see *Effects of sodium on potassium toxicity*).

Figure 2. Median lethal concentrationsfor major ion salts to *Ceriodaphnia dubia* in moderately hard reconstituted water and amended Lake Superior water. Error bars denote upper 95% confidence limits, and asterisks denote where confidence limits do not overlap. ALSW = amended Lake Superior water; LC50 = 48-h median lethal concentration; MHRW = moderately hard reconstituted water.

Although less toxic than the K salts in these test waters, the Mg salts are also significantly more toxic than their corresponding Na salts (ratios of 1.4–4.4; Figure 2), suggesting that, like K, Mg is also more important to the toxicity of these salts than are the anions. On a total molarity basis, $MgSO_4$ was approximately 2-fold less toxic than $MgCl_2$. Given the same Mg stoichiometry in each salt, this suggests that the toxicity of Mg is differentially modified by the anions, perhaps by SO_4 complexing Mg and thus reducing its toxicity. Unlike for $NaHCO_3$, there was no Ca precipitation in the tests with $MgCO_3$ (Table 3; and see Supplemental Data for more information on Ca precipitation relationships), and $MgCO_3$ was only slightly less toxic than $MgCl_2$. The relationship of these toxicity differences among Mg salts to ion speciation and activity is discussed later (see *Effects of calcium on the toxicities of magnesium salts*).

For Ca, only the Cl salt was acutely toxic below its solubility. On a total molarity basis, $CaCl_2$ is more toxic (by 1.9 fold) than NaCl (Figure 2); however, because $CaCl_2$ has twice as many chlorides as NaCl, this result by itself provides no clear evidence regarding relative ion toxicities.

Salt toxicity in different strengths of ALSW

Figure 3 shows the effect of varying all ions in dilution water by comparing LC50s for 1/3×, 1×, and 3× ALSW. These comparisons were developed by combining data from simultaneous tests in 1/3× and 3× ALSW with the 1× ALSW data generated separately (Figure 2). Also, NaCl and Na_2SO_4 were evaluated in an additional experiment with all 3 waters tested simultaneously (marked “dup” in Figure 3); this was done to determine if combining data across experiments might influence conclusions. For the 3× ALSW water, ions in the dilution water equaled between 3% and 8% of the added salt LC50, so that any amelioration of toxicity in this water would be slightly underestimated when based just on the added salt.

For the K salts, there is a 2.3-fold to 2.5-fold increase in LC50s from 1/3× to 3× ALSW (Figure 3), with the LC50 for 1× ALSW being near the midpoint of these ranges. Because all ions varied proportionately between these dilution waters, the specific factors responsible for these differences are not directly identifiable. However, the decreased toxicity from 1× to 3× ALSW (entailing a 3-fold change in both Na and hardness) is similar to the change between ALSW and MHRW (Figure 2), between which Na differs 4-fold but hardness is just 1.5-fold different and Ca is virtually constant, suggesting that Na is more important than other cations to these toxicity differences.

For the Mg salts, there are substantial increases in the LC50s from 1/3× to 3× ALSW, totaling 3.4-fold, 2.5-fold, and 1.8-fold for $MgCl_2$, $MgCO_3$, and $MgSO_4$, respectively (Figure 3). For $MgCO_3$, the lack of difference between 1× and 3× ALSW is associated with substantial precipitation of $CaCO_3$ in 3× ALSW (Table 3). For $MgCl_2$ and $MgSO_4$, the LC50 increases between 1× and 3× ALSW are similar to the corresponding K salts; but because these Mg salts show no LC50 difference between ALSW and MHRW whereas the K salts do, the underlying reasons must be different. These effects of dilution water strength should reflect the effects of the anions on the speciation of Mg and Ca; whether such consideration of speciation accounts well for LC50 differences among dilution waters, and among the salts, is addressed later.

For the Na salts, LC50s show responses to ALSW strength that are different from each other and from the K and Mg salts (Figure 3). The LC50 for Na_2SO_4 increases by 1.8-fold from 1/3× to 3× ALSW in both replicate tests, similar to $MgSO_4$ and K_2SO_4; but the increase is all between 1/3× to 1× ALSW. The LC50 for NaCl increases much less from 1/3× to 3× ALSW (1.2-fold and 1.4-fold in the different replicates). For $NaHCO_3$, there is a nonsignificant but slight decrease of the LC50 from 1/3× to 3× ALSW. This is again associated with precipitation of Ca, this precipitation being more extensive in the higher ALSW strengths, leading to Ca concentrations actually being similar across these waters (Table 3) despite the original formulations being 9-fold different. Possible reasons for these LC50 differences across Na salts, including chemical speciation and the ameliorative effects of Ca, are further addressed below (see *Effects of calcium on the toxicities of sodium salts*).

For $CaCl_2$, there is an increase of only 1.2-fold in the LC50 from 1/3× to 3× ALSW of marginal statistical significance. This small dependence of Ca toxicity on ALSW strength, compared with the large dependence of Mg toxicity, results in the toxicity of $MgCl_2$ being very similar to $CaCl_2$ in 3× ALSW (a factor of 1.3 difference) and very different in 1/3× ALSW (a

Figure 3. Median lethal concentrations for selected major ion salts to *Ceriodaphnia dubia* in different strengths of amended Lake Superior water (ALSW). Error bars denote upper 95% confidence limits, and asterisks denote where confidence limits do not overlap for 0.33× and 3× ALSW. LC50 = 48-h median lethal concentration.

factor of 3.7 difference). To the extent that the toxicity of $MgCl_2$ (or another salt) depends on Ca, judging relative potencies is best done at high, but nontoxic, Ca concentrations because any adverse consequences of low Ca cannot, by definition, affect Ca toxicity. Thus, Ca and Mg should be considered to have similar intrinsic toxicities.

Effects of manipulating specific ion ratios

To evaluate the factors behind the responses in the 1/3× versus 3× ALSW and MHRW versus ALSW, 3 sets of experiments were conducted in which the Ca:Mg, Cl:SO_4, and Na:K ratios were manipulated whereas other ions remained constant. The LC50s for $MgCl_2$, Na_2SO_4, and $MgSO_4$ are at least 2-fold greater at high Ca:Mg than low (Figure 4). Because total hardness and all other ion concentrations were the same in high Ca:Mg and low Ca:Mg waters, these differences are presumably attributable to Ca, rather than overall hardness. These same salts have large LC50 differences in the 1/3× versus 3× ALSW comparisons (Figure 3), for which Ca also changed substantially (in concert with other ions), but small LC50 differences for the MHRW versus ALSW comparisons (Figure 2), for which Ca was essentially the same but other ions varied markedly. Overall, this suggests that Ca is the important factor in differences among these various dilution waters for these salts. The LC50s of NaCl and $NaHCO_3$ had smaller (20–30%) increases at high Ca:Mg (Figure 4), but the magnitude of these effects is also consistent with the results shown in Figures 2 and 3 and their associated Ca concentrations (Table 3). In contrast, although KCl has a higher (15%) LC50 at high Ca:Mg, this is not statistically significant and is much smaller than the LC50 differences for both the MHRW and ALSW comparison (Figure 2) and the 1/3× to 3× ALSW comparison (Figure 3) for this salt. This reinforces the earlier suggestion of the importance of Na to K salt toxicity (see *Salt toxicity in MHRW versus ALSW*).

When the Cl:SO_4 ratio was altered (Figure 4), no significant differences in LC50s ($<15\%$) exist for any of the tested salts (Table 2), indicating that these anions are not important characteristics of dilution water for major ion toxicity to *C. dubia*. When the Na:K ratio was altered (Table 3), there also are no significant effects on the LC50s of the tested salts. No K salts were included in Na:K ratio studies because the effect of Na on K salt toxicity was evaluated separately.

Effects of manipulating alkalinity and pH

Two sets of toxicity tests evaluated the effects of dilution water alkalinity and pH on salt toxicity (Figure 5). In the first set, alkalinity was decreased (by dilution, then restoring other ions) and increased (by $NaHCO_3$ addition) relative to 1× ALSW water, resulting in pHs of approximately 7.4 and 8.3 in the low-alkalinity and high-alkalinity treatments, respectively, compared with a typical pH of approximately 7.9 in ALSW. The effects of these combined changes in alkalinity and pH on LC50s are $<10\%$ and not statistically significant for NaCl, Na_2SO_4, and $MgSO_4$, but are larger and statistically significant for $MgCl_2$ and KCl. For $MgCl_2$, increasing the alkalinity decreased the LC50 by 1.4-fold. This test was repeated, yielding virtually identical results (Table 3). We initially speculated that complexation by the higher HCO_3/CO_3 decreased Ca activity and thus increased toxicity. However, to maintain a constant Na concentration in these tests, the low-alkalinity water had higher SO_4, which also complexes Ca. As a result, the calculated Ca activity in the high-alkalinity treatment is actually higher than that in the low-alkalinity treatment, so it would not explain the observed LC50 shift. We similarly have no explanation for the lower LC50 (20%) for KCl at high alkalinity. One area of needed future work is direct ion activity measurements to verify speciation model calculations before further exploring the cause of these effects.

In the second set of tests, pH was manipulated by CO_2 partial pressure, leaving alkalinity and all other ions constant as pH varied. For this experiment, pH had no effect on NaCl toxicity over the pH range 6.75 to 8.20 or on $MgCl_2$ toxicity over the pH range 6.75 to 8.50 (Figure 5). (The high pH treatment for the NaCl tests had lower pH than for the $MgCl_2$ tests because of an air leak in the chamber for the low CO_2 treatment, so that the range of tested pHs was less than intended.) This insensitivity of toxicity to pH manipulation suggests that something other than pH itself is responsible for the effects of altered alkalinity on $MgCl_2$ toxicity noted in the previous experiment. However, although these tests at different pHs and alkalinities indicate limited or no effect of these factors on the toxicity of SO_4 and Cl salts, they do not

Figure 4. Median lethal concentrations for selected major ion salts to *Ceriodaphnia dubia* in amended Lake Superior water modified to have different ratios of Ca to Mg and different ratios of Cl to SO_4. Error bars denote upper 95% confidence limits, and asterisks denote where confidence limits do not overlap. LC50 = 48-h median lethal concentration.

Figure 5. Median lethal concentrations for selected major ion salts to *Ceriodaphnia dubia* in amended Lake Superior water modified to have different alkalinities and pH or different pH with alkalinity unchanged. Error bars denote upper 95% confidence limits, and asterisks denote where confidence limits do not overlap. LC50 = 48-h median lethal concentration.

encompass the much higher pHs and buffering levels of the tests with HCO_3/CO_3 salts. Therefore, pH might still have a role in the relative toxicity of those salts.

Effects of sodium on potassium toxicity

To evaluate the suspected effect of Na on the toxicity of K salts, 6 dilution waters were prepared, each having the composition of ALSW except that the Na^+ concentrations were varied to be 0.070 mM (background in LSW), 0.13 mM, 0.43 mM, 1.3 mM, 4.3 mM, and 13 mM (1.62 mg/L, 3 mg/L, 10 mg/L, 30 mg/L, 100 mg/L, and 300 mg/L), added as NaCl so that the anion was the same as for the toxicant, KCl. The LC50 of KCl differs consistently and substantially across these dilution waters (Figure 6), from a low of 2.05 mM (153 mg/L) at the lowest Na to a high of 10.1 mM (752 mg /L) at the highest Na. The magnitudes of these changes are consistent with the behavior observed for KCl in the MHRW versus ALSW and 1/3× versus 3× ALSW experiments (Figures 2 and 3 and included in Figure 6).

Mount et al. [3] and our initial experiments (Figure 2) demonstrated that K is the most toxic of the major ions to *C. dubia* and, as a result, the effects of K seem to greatly exceed any effect of the different anions. This is demonstrated in Figure 6, which shows agreement of other K salts and dilution waters with the experiment regarding KCl toxicity across different NaCl levels. Note that by compiling data for tests conducted across a variety of dilution water compositions, the correlation of Na with other major ions that might exist in a single experiment is reduced, strengthening the case for Na being the primary factor influencing toxicity of K salts. There might be some lesser effects of other factors, as evidenced by slightly lower LC50s (expressed as K) for $KHCO_3$ than for KCl and K_2SO_4 (Figures 2 and 6). As noted previously, the carbonate tests entailed much higher pH and buffering for which effects are uncertain. However, any such effects appear to be of less consequence than the primary effect of Na on K toxicity.

Figure 6. Effect of Na on median lethal concentrations for K salts to *Ceriodaphnia dubia*. Error bars denote 95% confidence limits. LC50 = 48-h median lethal concentration.

One implication of the Na dependence of K toxicity demonstrated in the present study is that the high toxicity of K in typical test waters with low Na may be of limited field relevance because K would be unlikely to be present at toxic concentrations when Na is so low. For example, the K-dominated effluent evaluated by Jop and Askew [11] had approximately 1.5 mM Na, which would put the expected acute LC50 for K at 10 mM or higher (Figure 6). This is similar to the toxicity of Mg salts and only 2-fold to 3-fold more toxic than Na salts.

Effects of calcium on the toxicities of sodium salts

Aside from the effect of Na on the toxicity of K, Ca is the only other ion in the present study which exerted a substantial influence on the acute toxicity of major ion salts to *C. dubia*. Several studies have shown that hardness influences major ion toxicity to *C. dubia* [16–20], though the way in which hardness was manipulated differed across studies. Experiments by Soucek et al. [16–18] varied hardness by adding $CaCl_2 + MgCl_2$ (for NaCl toxicity tests) or $CaSO_4 + MgSO_4$ (for Na_2SO_4 toxicity tests) in fixed ratios and keeping the remaining ions constant, whereas Elphick et al. [19,20] varied all ions proportionately (as in the 1/8×, 1/3×, and 1× MHRW waters used in the present study). In the present experiments, the combination of manipulating all ions simultaneously in some tests and only specific ion pairs in others (at constant hardness) allowed us to conclude that the "hardness effect" on the toxicity of Na salts is primarily an effect of Ca, rather than total hardness or the other ions that covary with hardness. The same conclusion was reached by Davies and Hall [21], who manipulated both total hardness and the Ca:Mg ratio to show the dominance of Ca in determining toxicity of Na_2SO_4 to *D. magna*.

Figure 7 shows the relationship of LC50s to Ca for all our Na salt toxicity tests using organisms cultured in 1× ALSW or MHRW. Each panel considers different exposure metrics for how LC50 and Ca are expressed. As a comparative performance measure for these different metrics, residual standard deviations of the logLC50s (RSD_{log}) around the mean trend of logLC50 with Ca are provided. Mean data trends were calculated by least-square regression of logLC50 versus logCa using Sigmaplot with a model for an exponential rise to a maximum. This is not intended to provide a definitive model for the relationship of toxicity to Ca but rather only an empirical description of the mean data trend suitable for calculating the RSD_{log}.

In Figure 7A, LC50s are plotted as millimolarity of added salt and Ca is plotted as total concentration (measured Ca for $NaHCO_3$ tests, nominal for others). Although the 3 inorganic salts have a similar and substantial (2-fold to 3-fold) dependence on Ca, there is an apparent difference in the relative toxicities of the salts, with LC50s being ordered NaCl ≈ Na gluconate > $NaHCO_3$ > Na_2SO_4. The RSD_{log} is 0.110, for which ±2 SDs corresponds to nearly a 3-fold range in LC50 at a fixed Ca concentration, indicative of the substantial variability among the different salts.

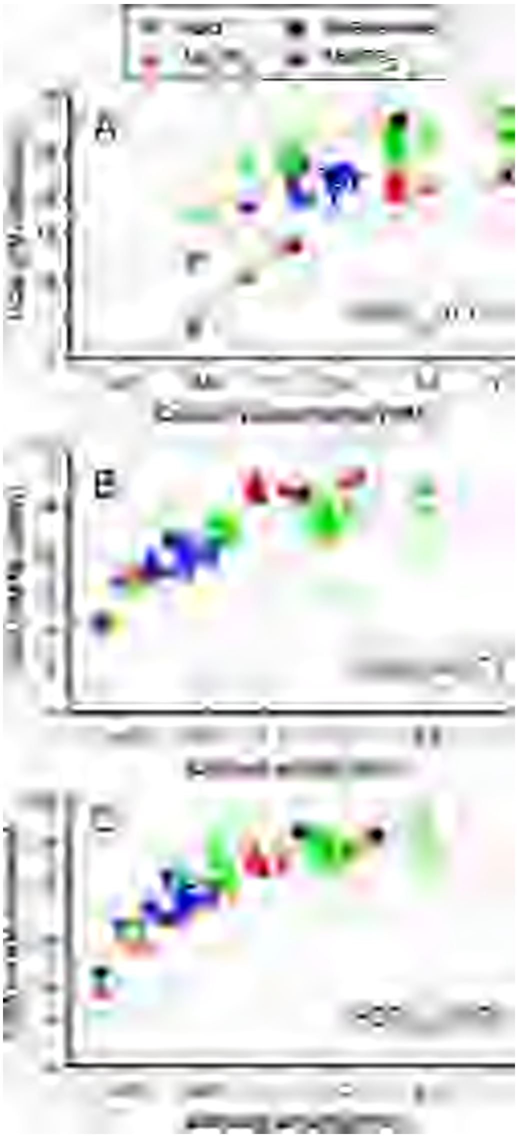

Figure 7. Effect of Ca on 48-h median lethal concentrations (LC50s) for Na salts to *Ceriodaphnia dubia* using different exposure metrics. (**A**) Metrics are LC50 as concentration of added salt and Ca concentration. (**B**) Metrics are Na and Ca activities. (**C**) The LC50 metric is changed to osmolarity. RSD_{log} denotes the residual standard deviation of log(LC50)s from their mean trend with Ca.

Because chemical reactions are governed by chemical activity rather than concentration, the relative toxicities of salts and their relationships to Ca should be based on chemical activities rather than total concentrations, as in Figure 7A. Activities will differ from total concentrations because of formation of chemical complexes as well as reduced activity coefficients associated with high ion concentrations (especially for the divalent Ca ion), and these factors will differ among the salts and dilution waters. Another issue for comparing toxicities across these salts is that, per mole of salt, Na_2SO_4 provides twice as many Na ions and 1.5 times as many total ions as do the other salts. To address these issues, in Figure 7B, Ca is plotted as Ca activity and the LC50s are based on the activity of Na. This results in much closer agreement among the salts, with the RSD_{log} reduced to 0.071 and the ±2 SD range for LC50 reduced to less than 2-fold; NaCl and $NaHCO_3$ now show similar toxicity because complexation of Ca by CO_3 and HCO_3 in the $NaHCO_3$ exposures causes Ca activity to be lower than that in NaCl exposures at the same total Ca concentration. Accounting for Na_2SO_4 having twice as many Na atoms as other Na salts has also made its LC50s more similar to the other salts; however, Na_2SO_4 now appears to be less, rather than more, toxic than NaCl, based on the repeated tests with Na_2SO_4 and NaCl in 1× ALSW and other waters with similar Ca concentration (i.e., the cluster of red triangles near 0.1 mM Ca activity and green circles near 0.2 mM Ca activity). This continued discrepancy between Na_2SO_4 and NaCl suggests that the anions play some role in determining toxicity beyond their effects on cation speciation.

A simple metric for expressing the aggregate effect of Na and anions is osmolarity, which reflects the combined, unweighted influence of all dissolved species. Figure 7C provides LC50s on the basis of calculated osmolarity, resulting in excellent agreement among all the tests of Na salt toxicity. The LC50s for the different salts now overlap within experimental variability; the RSD_{log} is reduced to 0.052 and the ±2 SD range to 1.6-fold. Further support for using osmolarity as an exposure metric comes from the test conducted with mannitol (black star in Figure 7C), whose LC50 is not significantly different from those of the Na salts even though its only expected effect would be on osmolarity. While this case for osmolarity is simply correlative and does not establish osmotic potential as the primary stressor, an exposure metric such as osmolarity that includes multiple ions in a nonspecific manner is indicated by these data.

Effects of calcium on magnesium toxicity

Figure 8 examines the relationship of LC50s to Ca for all our Mg salt toxicity tests using organisms cultured in 1× ALSW or MHRW. The panels address different exposure metrics parallel to those examined in Figure 7 for Na salts and include RSD_{log} as a performance measure for these different expressions of exposure.

In Figure 8A, LC50s are plotted as millimolarity of added salt and Ca is plotted as total concentration (measured Ca as appropriate for $MgCO_3$ tests, nominal for others). There is an even greater effect of Ca (~ 10-fold LC50 range over the 0.05–1.0 mM Ca range) for Mg salts than for Na salts (note scale change from Figure 7). On the basis of total added salt

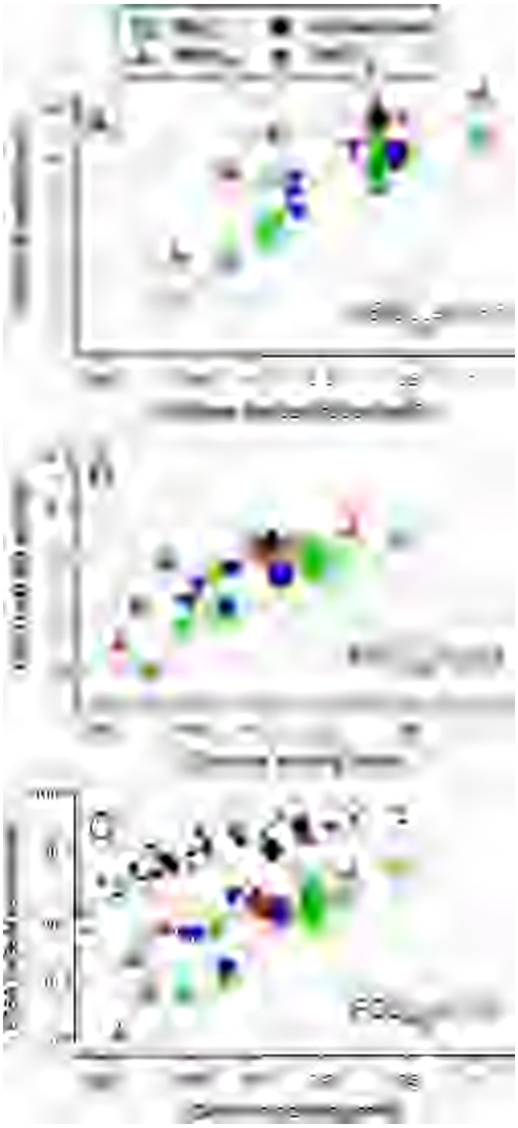

Figure 8. Effect of Ca on 48-h median lethal concentrations (LC50s) for Mg salts to *Ceriodaphnia dubia* using different exposure metrics. (**A**) Metrics are LC50 as concentration of added salt and Ca concentration. (**B**) Metrics are Mg and Ca activities. (**C**) The LC50 metric is changed to osmolarity, and black dots provide comparison to Na salt data from Figure 7C. RSD_{log} denotes the residual standard deviation of log(LC50)s from their mean trend with Ca.

concentrations, the salts have different apparent toxicities, with $MgSO_4$ and Mg gluconate being approximately 2-fold to 3-fold less toxic than $MgCl_2$ and $MgCO_3$. These differences make the RSD_{log} (0.167) even larger than for Na salts (Figure 7A), corresponding to a ±2 SD range of almost 5-fold at a fixed Ca concentration.

Expressing exposure on the basis of chemical activities of Mg and Ca (Figure 8B) reduces the residual variability and the differences between the salts because of the different degrees of complexation of both Ca and Mg by the different anions. The total range of the LC50s is also less because of a concentration dependence for complexation and activity coefficients. However, the closer agreement among the data is mainly for Ca activity >0.05 mM; at lower Ca activity, there remains considerable disparity between $MgSO_4$ and $MgCl_2$. As noted earlier (see *Effects of acclimation to dilution water*), these tests in very dilute test water raised some concerns about variable response and stresses associated with low ions, which might involve effects other than from Mg and Ca. Thus, although basing LC50s on Mg activity does provide a better metric than total Mg concentration, it does not account for all factors of significance. For all the data, the RSD_{log} is reduced to 0.123; but if only the data at Ca activity >0.05 mM are considered, the RSD_{log} is 0.102 and the ±2 SD range is then 2.5-fold, half of what is was for LC50s based on total added salt concentration.

Although osmolarity provides a possible unifying exposure metric for Na salts (Figure 7C), it is not a useful metric for Mg salt toxicity (Figure 8C). Using osmolarity to express Mg salt toxicity did not improve, but rather increased, the RSD_{log} of the data. More importantly, the osmolarities at the LC50 for Mg salts are 2-fold to 4-fold lower than those for Na salts at the same Ca activity. This indicates specific action of Mg and is incompatible with using a nonspecific, total ion metric such as osmolarity for the toxicity of Mg salts.

Our results indicate a strong effect of Ca on $MgSO_4$ and $MgCl_2$ toxicity, nearly a 10-fold change in LC50s from the Ca concentration in 1/8× MHRW (0.044 mM) to that in 1× MHRW (0.35 mM), with Figure 1 indicating even lower LC50s at 1/8× MHRW if organisms are cultured in this water. This is in contrast to results from van Dam et al. [31] for an Australian cladoceran (*Moinodaphnia macleayi*) collected from and cultured in very low ionic strength water (Ca <0.02 mM). In 6-d to 7-d reproductions tests, the $MgSO_4$ concentration reducing reproduction by 50% for *M. macleayi* was 2.6 mM in the culture water but only 2-fold higher (5.0 mM) when $CaSO_4$ was added to increase the Ca concentration by roughly 20-fold, to 0.34 mM. While there are several possible explanations for the difference in Ca effect for the 2 species, the van Dam et al. [31] study does reinforce concerns that species like *C. dubia* tested at very low ionic strength may not respond the same way as species (or strains) naturally adapted to low ionic strength water.

The toxicity of calcium

Because of solubility constraints, only limited testing of Ca salts could be conducted; and conclusions about any specific Ca toxicity could not be made based on simple comparisons of the toxicities of $CaCl_2$ and NaCl in our standard test waters. However, there are some indications that the toxicity of $CaCl_2$ is dominated by the cation, as are the toxicities of K and Mg salts. First, any comparison of the toxicity of $CaCl_2$ to NaCl should be made for NaCl tests in the dilution waters with the highest Ca. Otherwise, the comparison is confounded by the exacerbation of Na toxicity by low Ca. For the $CaCl_2$ tests (Table 3), osmolarities at the LC50s average 45 milliosmoles/L (range, 39–52), well below the average osmolarity of 76 milliosmoles/L (range, 69–83) for the NaCl tests in 3× ALSW. Unless the Cl from these 2 salts acts differently, this indicates some Ca-specific toxicity beyond the toxicity exerted by Na and the various anions (Figure 7). Second, in ALSW, the calculated Ca activity is 7.5 mM for $CaCl_2$ and 8.1 mM for Ca gluconate; such similarity would not be expected if these disparate anions were contributing to toxicity. However, the conclusion that there is some specific toxicity of Ca is tentative and will be further addressed in the next article in this series.

Relationship of toxicity to conductivity

Figure 9 replots the data from Figures 7 and 8 with LC50s based on conductivity. Given the results already presented for the toxicity of different salts, it is not surprising that these conductivity LC50s also show a strong dependence of toxicity on Ca and substantial variation across the different salts. The

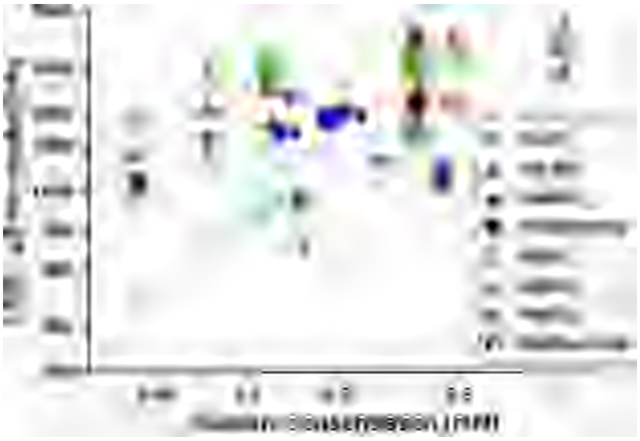

Figure 9. Median lethal concentration of Na and Mg salts expressed as conductivity. LC50 = 48-h median lethal concentration.

total range of conductivity LC50s is approximately 10-fold, with up to a 5-fold range among salts at the same Ca concentration. This indicates that assessment approaches using conductivity as an exposure metric should have a restricted scope of applicability regarding relative ion concentrations, as is the case for the conductivity benchmark of Cormier et al. [14,15].

SUMMARY AND IMPLICATIONS

In the present study, the key influences of background water chemistry on the acute toxicity of major ions salts to *C. dubia* were the effect of Na on toxicity of K salts and the effects of Ca on the toxicity of Na and Mg salts. Although the practical implications of the Na effect on K toxicity are limited because of the unlikelihood of K-dominated exposures, this result does mean that when K exposures are of concern [11] effects concentrations based on typical laboratory tests with low Na concentrations would overestimate risk. Regarding the Ca effects, although a hardness dependence of toxicity was already known for some Na and Mg salts [16–20] and Davies and Hall [21] implicated Ca as the specific factor for Na_2SO_4 toxicity, our results more thoroughly establish a role of Ca for a variety of salts. Predicting salt toxicity across waters will thus be more accurate if done on the basis of Ca rather than hardness, but the benefits of accuracy must be weighed against the availability of Ca versus hardness data. Uncertainties associated with using hardness as a surrogate for Ca can be addressed based on the regional variability of the Ca:Mg ratio, the resultant uncertainty on Ca, and the consequent uncertainty on toxicity estimated from Ca (e.g., Figures 7 and 8); in many cases, the resultant uncertainty should be limited.

An important tool in addressing the toxicity of ion mixtures has been the model of Mount et al. [3], and part of the purpose of the present study was to address some limitations of that model. The present results do indicate that not addressing the hardness (or Ca specifically) of the background water can introduce uncertainty into this model and raise questions regarding how joint toxicity across the different ions was addressed by Mount et al. [3]. However, any quantitative evaluation of that model relative to our results must wait for the alternative model to be presented in our subsequent article.

In addition to describing the dependence of major ion salt toxicities on background water chemistry, the results of the present study allowed some inferences about mechanisms and some conclusions about how salt toxicity dosimetry should be expressed. At least 3 separate mechanisms were apparent. First, a K-related mechanism is indicated by the high toxicities of K salts, the dependence of this toxicity on Na concentration, and its good correlation to K concentration. Second, a Mg-related mechanism is indicated based on the greater toxicity of Mg salts than Na salts and the correlation of Mg salt toxicity to Mg activity. Third, a mechanism related to multiple ions is indicated, based 1) on Na salts being less toxic than salts of other cations, 2) on anions affecting the toxicity of Na salts, 3) on the good correlation of this toxicity to osmolarity, and 4) on the agreement of toxicity from Na gluconate and mannitol with that of inorganic Na salts when expressed as osmolarity. Calcium salt toxicity also likely represents a mechanism different from that of Na salts because of the greater toxicity of $CaCl_2$ compared with Na salts tested at high but nontoxic Ca concentrations; however, this might be the same mechanism as Mg, which has similar toxicity to Ca when tested in waters with higher Ca background.

Regarding dosimetry, rigorously understanding and describing salt toxicity requires examining toxicity on the basis of chemical activities and consideration of various ion interactions. The cation-related mechanisms would be expected to be best related to cation activities, and the multiple ion mechanism associated with Na salts requires some measure, such as osmolarity, which addresses multiple chemical species. These conclusions regarding mechanisms and dosimetry are preliminary and will be further addressed in the subsequent articles mentioned in the *Introduction*, but it is evident that regulations based solely on a single ion may lack robustness from failing to address the relative toxicities and interactions of multiple ions.

One limitation of the present results is that they just concern the toxicities of single salts, with other ions being at low to moderate background concentrations reported for US waters. Most field situations would involve enrichment of more ions—to well above ambient levels, if not to toxic levels. For example, it would not be expected that Mg would be enriched to toxic levels while Ca stays at background levels, and the high toxicity of K salts depends on very low Na concentrations, which would be an unusual exposure scenario. The exposure metrics and relationships noted in the present study would be expected to apply to more complex mixtures (e.g., all ions contributing to osmolarity), but there is a need to better define the scope of applicability of these relationships. Their extension to more complex mixtures will be the subject of subsequent articles.

Although a complete model for the acute toxicity of major ions to *C. dubia* will be forthcoming, Figures 6 through 8 make it clear that this will be complicated, involving chemical speciation calculations, multiple toxicity mechanisms, and interactions among 7 toxicants. Any practical application will require simplifications, but we feel that the detail provided in the present study and in later articles will provide a starting point for determining what simplifications are possible and how they should be structured. Another application issue concerns the relevance of acute ion toxicity for *C. dubia* to the assessment of an entire aquatic community, especially in low-ion, lotic systems. This toxicity endpoint is the starting point for work that will address more endpoints and species. However, the relationships developed for *C. dubia* will be directly relevant to many aquatic systems and will also have value in developing and interpreting major ion toxicity data for other species.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3487.

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Disclaimer—The views expressed in the present study are those of the authors and do not necessarily reflect the views or policies of the US Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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

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COMMONWEALTH OF PENNSYLVANIA
DEPARTMENT OF ENVIRONMENTAL PROTECTION
BUREAU OF CLEAN WATER

TRIENNIAL REVIEW OF WATER QUALITY STANDARDS

RATIONALE FOR THE DEVELOPMENT OF AMBIENT WATER QUALITY CRITERIA FOR

CHLORIDE

PROTECTION OF AQUATIC LIFE

Introduction

Section 303(c)(1) of the Clean Water Act (33 U.S.C.A § 1313(c)(1)) requires that states periodically, but at least once every three years, review and revise as necessary their water quality standards. Water quality standards are instream water quality goals that are implemented by imposing specific regulatory requirements (such as treatment requirements and effluent limits) on individual sources of pollution. As part of the current review, the chloride criterion is being evaluated.

Chloride occurs naturally in the aquatic environment, especially in waters flowing through geologic formations of marine origin. The major anthropogenic sources of chloride include deicing salt for roads, urban and agricultural runoff, treated industrial waste, discharges from municipal wastewater plants and the drilling of oil and gas wells (EPA, 1988). Elevated levels of chloride are toxic to aquatic life in freshwater environments. A state-wide aquatic life criterion for chloride would provide an appropriate level of protection for all of Pennsylvania's waters.

Pennsylvania's existing chloride criterion was developed primarily for the protection of potable water supplies (PWS). It is not applied in all waters of this Commonwealth, but rather only at the point of water supply intake, pursuant to 25 Pa. Code § 96.3(d) (relating to water quality protection requirements). The current PWS criterion for chloride is included in Table 3 at 25 Pa. Code § 93.7 (relating to specific water quality criteria) and establishes a maximum level of 250 milligrams of chloride per liter of water, applicable only at the point of all existing or planned surface PWS withdrawals, unless otherwise specified by regulation.

The Pennsylvania Department of Environmental Protection has developed draft water quality criteria for chloride for the protection of aquatic life. The draft criterion is based on current science that shows that the water hardness and sulfate concentrations affect chloride toxicity to aquatic organisms. This relationship is incorporated into the newly developed equation used for calculating the acute and chronic numeric criteria for chloride in Pennsylvania waters. The Department is recommending that this chloride criterion be applied in all waters for the protection of aquatic life.

History

The U.S Environmental Protection Agency (EPA) published Ambient Water Quality Criteria (AWQC) for Chloride in February 1988, which summarized the published toxicity data that was available at that time for chlorides on freshwater plant and animal species. The acute and chronic effects of chlorides on aquatic animals were documented, along with the chronic effects of chloride on aquatic plants. The findings of 106 published scientific studies were considered in the development of the national aquatic life criteria for chloride. EPA developed the chloride criteria in 1988 for protection against adverse acute and chronic impacts on freshwater aquatic life based on the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses, NTIS PB85-227049 (Stephan, et al., 1985). EPA determined the four-day (chronic) and one-hour acute average concentrations based upon how quickly some aquatic species reacted to higher concentrations of pollutants. The Criteria Continuous Concentration (CCC) and Criteria Maximum Concentration (CMC) values should not be exceeded more than once every three years on the average (US EPA, 1988).

The 4-day average (CCC) criterion = 230 mg/l

The 1-hour average (CMC) criterion = 860 mg/l

In 2005, the state of Iowa with the help of EPA Region 5 began an investigation into updating the existing chloride AWQC. The revised WQS was promulgated by Iowa in 2009. EPA Office of Research and Development scientists served to link the relationship of chloride toxicity to aquatic organisms with water hardness and sulfate concentration. This relationship provided the basis for the revised WQS promulgated in Iowa.

The Department has reviewed the equation-based aquatic life criteria for chloride as developed by EPA and successfully implemented in Iowa. The researchers at the Great Lakes Environmental Center (GLEC) in Columbus, OH and the Illinois Natural History Survey (INHS) in Champaign, IL worked collaboratively under a contract with the EPA to determine the toxicity of chloride in freshwater invertebrate species. The research demonstrated a strong correlation between chloride toxicity and hardness and to a lesser extent with sulfate. The final results of this toxicity testing were published in the report “Acute Toxicity of Chloride to Select Freshwater Invertebrates” US EPA, October 28, 2008. Iowa Department of Natural Resources (IDNR) selected the appropriate acute and chronic criteria equations after considering input from many sources and two equations were promulgated by Iowa. Both the one-hour acute and ninety-six hour chronic criteria values should not be exceeded more than once every three years on the average (personal communication: Connie Dou, IDNR, November 2011).

Test Compound Determination

Chloride is one of the major anions commonly found in surface and wastewater. It is a constituent of naturally occurring minerals; it readily dissolves in water, and is important to living systems. As a solid, chloride is typically found as a salt bonded with a cation such as calcium, sodium, magnesium, or potassium. The salinity of a water body is measured by its total salt composition. Freshwater lakes are dominated by the cations: Ca^{2+}, Mg^{2+}, K^+ and Na^+ and the anions $HCO3^-$, SO_4^{2-} and Cl^-. (Wetzel, 1983) Data obtained from stream surveys of Pennsylvania waters confirmed this determination: the ionic composition is >40% $HCO3^-$/ Ca^{2+}, followed by SO_4^2, Mg^{2+}, Na^+, K^+ and Cl^-. Pennsylvania waters are calcium/bicarbonate dominant.

Chloride toxicity tests have been conducted through the addition of chloride salts such as sodium chloride (NaCl), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$) and potassium chloride (KCL). Results of tests with potassium and magnesium chloride suggest toxic effects observed can be due to the potassium and magnesium cation, rather than the chloride ion. It has been observed that the toxic effects of calcium chloride and sodium chloride are due to the chloride anion. In establishing the effect concentrations of the chloride ion, exposure to KCL and $MgCl_2$ salts are lower (more toxic) than the effect concentration of the exposures to $CaCl_2$ and NaCl salts (Canadian Council of Ministers of the Environment, 2011). Therefore the approximate order of chloride salt toxicity to freshwater organisms is KCL > $MgCL_2$ > $CaCl_2$ > NaCl (Mount et al 1997). Based on this information, chloride toxicity to freshwater organisms was evaluated using tests dosed with NaCl to ensure the effect concentrations were derived from tests where effects were based on the chloride anion, not the associated cations.

Other Modifying Factors

A long term study by Elphick et al. indicates that increased hardness may have an effect on the toxicity of chloride. GLEC and INHS (2008) also conducted some short-term exposure tests indicating that a hardness-chloride toxicity relationship exists for the water flea *Ceriodahpnia dubia*, the fingernail clam *Sphaerium simile*, the Oligochaete *Tubifex tubifex* and the aquatic snail *Gyraulus parvus*. (CCME, 2011)

EPA contracted with the GLEC and the INHS (2009) to perform toxicity testing for chloride. The results showed that chloride toxicity is heavily dependent on water hardness, and to a lesser degree, sulfate levels in water. PA Department staff has been monitoring sulfate and hardness levels at Water Quality Network (WQN) stations throughout PA. This data confirms that PA source waters have a varied amount of hardness and sulfate concentrations. As an example, the Aughwick Creek watershed is located in southcentral Pennsylvania. In this single watershed, the hardness values range from 10 mg/l in the small freestone streams to 250 mg/l in areas of limestone geology. There is a full range of hardness values between the 10 mg/l and 250 mg/l as the tributaries flowing through various geologies coalesce and mix. This is not an unusual situation as there are extensive limestone deposits in the Commonwealth. Pennsylvania has a legacy of abandoned coal mines that can discharge high levels of sulfate. Instream sulfate levels are elevated where there are concentrations of these abandoned discharges. Where there is a legacy of abandoned coal mines the sulfates often range between 100 to 500 mg/l and sometimes higher. In contrast, the streams in the less affected parts of the state have sulfate values less than 50 mg/l. Urban streams often have sulfate between 50 mg/l and 100 mg/l. The variation in the hardness and sulfate concentrations throughout the state confirms that it is appropriate to develop an equation based criterion that includes a modification for hardness and sulfate.

PA Site Specific Research/Literature Review

The Department contracted with the Stroud Water Research Center, in Avondale, Pennsylvania to perform chloride toxicity testing. The study was designed to provide the additional information needed to support the development of a chloride criterion that is protective across the range of aquatic habitats and species found in Pennsylvania waters. Benthic macroinvertebrates were used in this eco-toxicity study of chloride because they are an ecologically important group of aquatic organisms and are common components of the Pennsylvania bio-monitoring multi-metrics used in standard water quality assessment protocols (e.g., PA IBI; PADEP 2013)

The following mayfly species were included in the study: *Neocloeon triangulifer*, previously classified as *Centroptilum* (Jacobus and Wiersema, 2014), *Anafromtilum semirufum*, *Procloeon fragile*, *Ephemerella invaria*, *Maccaffertium modestum* and *Leptophlebia cupida*. All six species were evaluated for short term (acute) exposures to chloride. Chloride sensitivities were determined using sodium chloride because sodium is known to have little effect on the toxicity of chloride (Stroud 2015). Four species (*N. triangulifer*, *A. semirufum*, *P. fragile*, *M. modestum*) were subjected to a whole-life (chronic) toxicity test. The chronic test began with newly hatched larvae and ended when all larvae had emerged as adults (i.e., 20-48 days). The development time for a complete life cycle of *M. modestum* is 60-80 days. The experiment was ended before the adult emergence; therefore the chronic study of *M. modestum* was omitted.

The acute and chronic data obtained from the Stroud study was incorporated into the data set used to determine Pennsylvania-specific chloride criteria. It has been shown that some aquatic organisms show significantly more sensitivity when tested in reconstituted laboratory water compared to natural waters (CCME, 2011). The Stroud study was conducted in water from three source water streams: Spruce Run, a soft water stream (hardness 6 mg/L) in Union County, PA; House Run, a moderately hard water stream (hardness 94 mg/L) in Greene County, PA and Cedar Run, a hard water (hardness 212 mg/L) stream in Union County, PA. The reference stream was White Clay Creek which is a moderately hard water stream (hardness 89 mg/L) located at the Stroud Water Research Center in Chester County, PA, where all the study species were originally collected. (Stroud, 2015)

Other toxics data sources used:

Toxics data that was compiled by Charles Stephan, November 06, 2007 – This document contains acceptable acute and chronic data obtained from several significant studies (Birge et al., 1985; Spehar 1987; Cowgill and Milazza 1990; and Wisloh 2007). It also contains a list of the studies reviewed previous to 2007 that were not approved and the reasons for the disapproval.

Data from Canadian Council of Minister of the Environment, 2011 – This document contained additional studies (Harmon et al 2003; Collins & Russell 2009; Gillis 2011, GLEC & INHS 2008; Elphick et al 2011; Wang and Ingersoll 2010; US EPA 2010, Valenti et al 2007; Bringolf et al 2007) In particular, the Valenti et al, Bringolf et al and Gillis studies contained valuable data on sensitive and endangered mussels. This document also contains studies that have relevant chronic toxicity data.

US EPA, 2015 – EPA's data set contained additional peer reviewed studies. (GLEC and INHS 2010; Wang et al 2013; Garner and Royer 2010; Soucek 2012, 2013; Environ 2009; Sanzo and Hecnar 2006; Garibay and Hall 2004).

In addition, PA DEP staff evaluated Maryland's Freshwater Chloride Development Methodology and reviewed the data used in their chloride criteria development. (MDE, 2013)

Acute criteria determination

Acute values were used from all acceptable data contained in the reports listed above. This resulted in 219 acute toxicity results for aquatic species (51 genera). The LC50's are in mg/L for all acceptable data, including Stroud mayfly data. The four genera most sensitive to acute testing were *Epioblasma* (freshwater mussel); *Sphaerium* (fingernail clam); *Neocloeon* (mayfly); and *Lampsilis* (freshwater mussel). The genus mean acute value (GMAV) for the most sensitive organism (*Epioblasma*) was 698 mg/L. This value was lower than the calculated final acute value (FAV) of 874.8 mg/L. In order to protect for this sensitive freshwater mussel, the species mean acute value (SMAV) for the *Epioblasma* (698 mg/L) was used as the FAV. The final acute value is 349 mg/L, which will be incorporated into the hardness/sulfate modifying equation to determine the final acute chloride criterion.

Acute Data

Genus	GMAV (mg/L)
Epioblasma	698
Sphaerium	785
Neocloeon	959
Lampsilis	991
Anafroptilum	1090
Ambystoma	1178
Ceriodaphnia	1197
Elliptio	1437
Procloeon	1449
Megalonaisas	1517
Lasmigona	1577
Margaritifera	1577
Brachionus	1645
Daphnia	1765
Isonychia	1880
Musculium	1930
Villosa	2215
Gyraulus	2430
Diaptomus	2571
Physa	2667
Rana	2680
Pseudacris	2882
Lirceus	2950
Macaffertium	3052
Planorbella	3731
Ephemerella	3759
Limnodrilus	3761
Bufo	3926
Caecidotea	4049
Lumbriculus	4254
Nephelopsis	4310
Erpobdella	4550
Ameriurus	4849
Pimephales	5010
Tubifex	5311
Chironomus	5371
Leptophlebia	5473
Lithobates	5846
Aciperser	5903
Cyprinella	5956
Lepidostoma	6000
Lepomis	6634
Carassius	6959
Gambusia	7786
Oncorhynchus	8379
Libellulidae	9671
Fundulus	9706
Gasterosteus	10200
Cambarus	10557
Anguilla	11929
Agria	14255

Rank	Genus/ Species	GMAV mg/L
4	*Lampsilis (freshwater mussel)*	991
3	*Neocloeon (mayfly)*	959
2	*Sphaerium (fingernail clam)*	785
1	*Epioblasma (freshwater mussel)*	698

Chronic criteria determination

The chronic toxicity data set included 10 aquatic species. *Pimephales promelas*, *Oncorhynchus mykiss*, *Daphnia ambigua*, *Daphnia magna*, *Daphnia pulex*, *Ceriodaphnia dubia*, *Lumbriculus variegatus*, *Neocloeon triangulifer*, *Anafroptilum semirufum* and *Procloeon fragile*. The final calculated acute/chronic ratio (FACR) from the acceptable data is 6.2. The final chronic value is 112.7 mg/L (113 mg/L), which will be incorporated into the hardness/sulfate modifying equation to determine the final chronic chloride criterion.

Species	SCV mg/L	SAV (mg/L)	GM ACR	Source
Pimephales promelas	433.1	6570	10.2	Birge et al 1985
	598	4079		Elphick et al. 2011
Oncorhynchus mykiss	922.7	8379	9.1	Stephan 2007
Daphnia ambigua	259	1213	5.4	Harmon et al 2003
Daphnia magna	2382	4731		Stephan 2007
	421	3630		Elphick et al. 2011
Daphnia pulex	372	1470		Stephan 2007
Ceriodaphnia dubia	925	1395	2.6	Stephan 2007
	425	1677		Stephan 2007
	454	1068		CCME, 2011
Lumbriculus variegatus	825	3100	3.8	Elphick et al. 2011
Neocloeon triangulifer	109	704	8.4	Stroud, 2015
	175	2141		Stroud, 2015
	188	1420		Stroud, 2015
Anafroptilum semirufum	114	107	8.3	Stroud, 2015
	279	1827		Stroud, 2015
	128	1336		Stroud, 2015
Procloeon fragile	168	472	6.1	Stroud, 2015
	332	2110		Stroud, 2015
	245	1765		Stroud, 2015
		Final ACR	**6.2**	

Chronic Data Ranked

Rank	Species	SMCV mg/L
4	*Daphnia ambigua* (water flea)	259
3	*Procloeon fragile* (mayfly)	248
2	*Anafroptilum semirufum* (mayfly)	174
1	*Neocloeon triangulifer* (mayfly)	157

FAV	874.8
FAV for lowest GMAV	698
FACR	6.2
CMC based on FAV for lowest GMAV	349
CCC	112.7

Chloride Criteria Development

The hardness and sulfate values used to derive the appropriate chloride criteria shall be determined by instream measurements, statewide water quality network (WQN) or other values as approved by the Department. The proposed chloride criterion is calculated using the following equations that incorporate the hardness and sulfate modifiers based on the GLEC studies:

Acute Chloride Criterion Equation – One hour average concentration should not exceed

$$\text{Acute Criterion (mg/L)} = \mathbf{349}(\text{Hardness})^{0.2058}(\text{Sulfate})^{-0.0745}$$

Chronic Chloride Criterion Equation – 4 day average concentration should not exceed

$$\text{Chronic Criterion (mg/L)} = \mathbf{113}(\text{Hardness})^{0.2058}(\text{Sulfate})^{-0.0745}$$

Recommendation

On April 17, 2012 the EQB adopted a proposed rulemaking for the promulgation of an aquatic life criterion for chloride. Based on the comments received during the public comment period, which ended August 8, 2012, the Department, in this new proposed rulemaking has re-evaluated and incorporated Pennsylvania-specific data into the determination of the chloride criterion. The Department has developed a state-specific equation-based aquatic life criterion for chloride. It incorporates additional state-specific aquatic toxicity data, as related to the ion composition of our waterbodies, and the necessary adjustment for the effects of hardness and sulfate on the toxicity of chloride.

The Department recommends adopting the Pennsylvania-specific equation-based aquatic life criterion for chloride.

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FULL-LIFE CHRONIC TOXICITY OF SODIUM SALTS TO THE MAYFLY *NEOCLOEON TRIANGULIFER* IN TESTS WITH LABORATORY CULTURED FOOD

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Abstract: Although insects occur in nearly all freshwater ecosystems, few sensitive insect models exist for use in determining the toxicity of contaminants. The objectives of the present study were to adapt previously developed culturing and toxicity testing methods for the mayfly *Neocloeon triangulifer* (Ephemeroptera: Baetidae), and to further develop a method for chronic toxicity tests spanning organism ages of less than 24 h post hatch to adult emergence, using a laboratory cultured diatom diet. The authors conducted 96-h fed acute tests and full-life chronic toxicity tests with sodium chloride, sodium nitrate, and sodium sulfate. The authors generated 96-h median lethal concentrations (LC50s) of 1062 mg Cl/L (mean of 3 tests), 179 mg N-NO_3/L, and 1227 mg SO_4/L. Acute to chronic ratios ranged from 2.1 to 6.4 for chloride, 2.5 to 5.1 for nitrate, and 2.3 to 8.5 for sulfate. The endpoints related to survival and development time were consistently the most sensitive in the tests. The chronic values generated for chloride were in the same range as those generated by others using natural foods. Furthermore, our weight-versus-fecundity plots were similar to those previously published using the food culturing method on which the present authors' method was based, indicating good potential for standardization. The authors believe that the continued use of this sensitive mayfly species in laboratory studies will help to close the gap in understanding between standard laboratory toxicity test results and field-based observations of community impairment. *Environ Toxicol Chem* 2015;34:2126–2137. © 2015 SETAC

Keywords: Mayfly Toxicity testing Chronic toxicity Major ions

INTRODUCTION

Despite the fact that insects occur in nearly all freshwater ecosystems and are often the dominant class of macroinvertebrate in those systems [1], few insect models exist for use in determining the toxicity of contaminants. Among the most commonly used insect species in toxicity testing are the midges *Chironomus riparius* and *Chironomus dilutus*, which can be cultured in the laboratory but tend to be among the more insensitive invertebrates. Efforts have been made to assess the potential for use of abundant, wild-caught mayflies (Ephemeroptera) for standardized toxicity test development [2,3], but knowledge of exposure history, health, and age of test organisms before testing provides for better interlaboratory test result comparisons [4].

A major step toward the development of a sensitive insect model organism for toxicity testing was taken when Sweeney and Vannote [5] characterized the effects of several variables on life history characteristics of the parthenogenetic mayfly *Neocloeon triangulifer* McDunnough (originally described as *Cloeon triangulifer* [6], later transferred to *Centroptilum* [7], and most recently assigned to *Neocloeon* [8]). The same research group (Stroud Water Research Center) later developed a method of culturing this organism in the laboratory and using it in toxicity tests [9]. The advantage of *N. triangulifer* is that because it is parthenogenetic, it does not require a large amount of space for mating to take place; female clones can emerge into small containers (e.g., 300-mL beakers) and then deposit viable eggs. Furthermore, parthenogenic organisms in general are desirable for use in toxicity testing because being clonal eliminates genetic variability as a confounding factor. The Stroud Water Research Center group uses naturally colonized periphyton plates to feed the organisms, and this approach has now been adapted by others to conduct toxicological investigations with various contaminants [10–13].

Because food quality and quantity appear to influence the response of *N. triangulifer* to contaminants [10,11,13], developing a cultured food consisting of 1 or more diatom species is necessary to further allow standardization and more widespread use of this organism in toxicity testing. Great progress has been made toward this end by Weaver et al. [14], who developed a method of culturing 3 different diatom species (*Mayamea atomus, Nitzschia* cf. *pusilla,* and *Achnanthidium minutissimum*) in the laboratory, and then using stocks of monocultures of the species to colonize microscope slides, which are then offered to nymphs as biofilms. This approach was successfully used to culture 13 successive generations of the organisms, and both acute and short-term chronic (i.e., less than full-life) toxicity data have been generated using organisms cultured with this food [15].

Previously reported chronic toxicity tests with this species (or genus) have been conducted using wild-caught individuals [16], using cultured individuals with naturally colonized periphyton plates ([9–13,17,18]; J. Jackson, Stroud Water Research Center, Avondale, PA, USA, unpublished data), or for less than a full life cycle [15,19]. The objectives of the present study were to adapt the previously developed diatom culturing and toxicity testing methods for *N. triangulifer* [14,15] and to further develop a method for chronic toxicity tests spanning organism ages of less than 24 h post hatch to adult emergence, with the goal of producing data that are suitable for use in the development of chronic water quality criteria, as prescribed by Stephan et al. [20] (i.e., similar in scope to the daphnid and mysid life-cycle tests). We chose 3 sodium salts that are contaminants of interest in the Great Lakes region: sodium chloride, sodium nitrate, and sodium sulfate (E. Hammer, United States Environmental Protection Agency [USEPA],

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All Supplemental Data may be found in the online version of this article.

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Washington, DC, USA, personal communication), and conducted 96-h fed acute tests and full-life chronic toxicity tests so that acute-to-chronic ratios could be calculated.

METHODS

Culturing of test organisms and food

Mayfly and diatom biofilm culturing methods were based on those developed by Weaver et al. [14], with several modifications. As recommended [14], we initially used 3 species of diatom for feeding of mayflies, but we stopped using *Achnanthidium* sp. because it was more difficult to culture than the other diatoms, and culture results indicated that it was not necessary (DJ Soucek, personal observation). Diatoms used to feed mayflies included *Mayamea* sp. and *Nitzschia* sp. Both diatoms were obtained from Carolina Biological Supply, sold as *Navicula* sp. and *Synedra* sp., respectively. We had the genus-level identities taxonomically confirmed by an expert (S. Decelles) at USEPA-ORD, Cincinnati, Ohio, USA.

Mixed diatom stocks

To culture diatoms, we autoclaved (30 min at 121 °C, liquid cycle) a 4-L flask containing 4 L filtered (Whatman 934-AH) dechlorinated tap water and a 2-inch-long, Teflon-coated stir bar. After allowing it to cool, sterile technique was used to add 1.3 mL Kent Proculture Professional F/2 Algal culture formula A, 1.3 mL Kent Proculture Professional F/2 Algal culture formula B, 150 mg sodium metasilicate ($Na_2SiO_3 \cdot 9H_2O$), and 200 mL fresh diatom stock solution (just removed from stir-plate). Both diatom species were present in combination in stock cultures. We had initially kept the species stocks separate, but combining the species in stocks did not appear to impact the performance of the mayflies, so we adopted the combined stock approach. The flasks were placed on stir-plates with moderate to fast stirring (a large vortex was visible) in an environmental chamber set for a 16:8-h light:dark photoperiod and 25 °C. Diatom stocks were allowed to grow for 5 d, then 200 mL stock was used to seed the next flask and cages for mixed diatom slides (see below *Mixed-diatom slides*). Stocks were not refrigerated before seeding subsequent flasks or mixed-diatom slide cages.

Mixed-diatom slides

To culture mixed-diatom slides, 15 fully frosted microscope slides (catalogue no. 12-544-5CY, Fisherscience) were placed in a single layer (with frosted side facing up) on the bottom of a 7.2-L (189 mm × 297 mm × 128 mm) autoclavable polysulfone mouse cage (#PC7115HT, Ace Caging) filled with 2.5 L filtered (Whatman 934-AH) dechlorinated tap water. The container with the slides was autoclaved (30 min at 121 °C, liquid cycle) and allowed to cool. Sterile technique was used to add 1.3 mL Kent Proculture Professional F/2 Algal culture formula A, 1.3 mL Kent Proculture Professional F/2 Algal culture formula B, 150 mg sodium metasilicate ($Na_2SiO_3 \cdot 9H_2O$; dissolved in a small amount of deionized water before addition), and 200 mL fresh (never refrigerated) mixed-diatom stock. The container with slides was covered with clear plastic wrap and placed in an environmental chamber set for a 16:8-h light:dark photoperiod and 25 °C. Slides tended to have the most robust biofilms if used within approximately 6 d to 10 d after inoculation, but slides as old as 1 mo were used with success as long as most of the material appeared to be distinct round or spindle-shaped diatom cells when examined under a compound microscope. Poor-quality slides would have very few distinct cells on microscopic examination and would be mostly masses of amorphous material. If, after approximately 5 d, the biofilms appeared to be thin, we mildly aerated cages containing slides rather than adding more nutrients. We made the observation that color of the biofilm slides, as apparent to the naked eye, was not necessarily predictive of good mayfly performance. Therefore, before feeding to mayflies in toxicity tests or cultures, diatom biofilms were examined under a compound microscope to ensure that most of the biofilm material was distinct diatom cells, rather than being amorphous material.

Mayfly nymph rearing method

Mayflies (*Neocloeon triangulifer*; Stroud Water Research Center Clone #WCC-2) were reared in an environmental chamber at 25 °C, and a 16:8-h light:dark photoperiod. Culture water was a reconstituted water (hereafter referred to as Duluth 100) with a nominal hardness of 100 mg/L as $CaCO_3$, prepared according to a formula developed at the USEPA laboratory in Duluth, Minnesota, USA. To make this water, the following salt concentrations were added to deionized water from a Barnstead "E-pure" filtration system: $KHCO_3$, 10 mg/L; $NaHCO_3$, 125 mg/L; $MgSO_4$, 38 mg/L; $CaSO_4$, 40 mg/L; $CaCl_2$, 43 mg/L; NaBr, 0.05 mg/L. This water recipe was designed with the goal of better mimicking chemistry of "typical" North American freshwaters relative to other commonly used reconstituted waters (D. Mount, USEPA, Washington, DC, USA, personal communication). When eggs hatched, approximately 250 mL culture water was added to a 300-mL "I-chem" jar. All water was filtered using Whatman #934-AH glass microfiber filters. One mixed diatom slide was added to the jar. Newly hatched mayfly larvae (100–1000s) were then added to the jar, the lid was loosely placed, and the jar was covered with aluminum foil to block direct overhead lighting. When mayflies were 4 d to 8 d old (usually 6 d or 7 d), 40 individuals were placed in a 1-L beaker containing 400 mL Duluth 100 reconstituted water, and fed as described for the I-chem jar. The diatom slide was placed in the beaker before adding mayflies to avoid injury. Again, the container was covered with aluminum foil to block direct overhead lighting. When mayflies were 11 d to 12 d old, 20 individuals were transferred to a 19-cm × 24-cm × 6.5-cm Pyrex casserole dish containing 1.5 L Duluth 100 water and 5 mixed diatom slides. Slides were replaced when diatom biofilms were depleted, and water was changed twice per week or more if water appeared to be littered with loose diatoms and waste products. The container was covered loosely with foil. Using this method, aeration was not necessary at any point during mayfly culturing.

When pre-emergent nymph stages (determined by presence of black wing pads) appeared (days 20–23), they were placed in a 300-mL I-chem jar containing culture water and a mixed diatom slide. A screened cover was placed on the jar to allow for emergence of sub-imagoes and molting to imago stage (within 24 h after pre-emergent nymph stage). To induce the imago to release its eggs, we held it by the wings with forceps and touched its abdomen to culture water held in a small petri dish. This procedure was conducted with the aid of a dissecting microscope. Eggs were then pipetted into a scintillation vial; when possible, eggs of 3 females were combined in each vial. Eggs were either allowed to hatch or placed in an environmental chamber at 10 °C for later use. We observed a predictable relationship between the number of days eggs were held at 10 °C and the number of days to hatch on transfer of eggs to 25 °C (Figure 1). In some cases, eggs hatched over multiple days. For the purposes of generating a predictive equation for time to egg hatch, we used half-day intervals; for

Figure 1. Predictive relationship between the number of days *Neocloeon triangulifer* eggs are held at 10 °C and number of days to hatch on moving eggs to 25 °C.

example, if approximately equal numbers of eggs hatched on days 2 and 3 after placement in 25 °C, we used 2.5 d in the regression model. The predictive model is as follows: # days to hatch on moving eggs to 25 °C = 0.0009 (# days at 10 °C)^2 – 0.1385(# days at 10 °C) + 6.809.

Test chemicals and dilution water

The nitrate, chloride, and sulfate sources for acute and chronic toxicity tests were reagent-grade sodium salts ($NaNO_3$ CAS # 7631-99-4; NaCl CAS # 7647-14-5; Na_2SO_4 CAS # 777-82-6). All acute and chronic tests were conducted in Duluth 100 hard water. As noted in the section *Mayfly nymph rearing method*, this was also the mayfly culture water, and eggs were stored in this water, so no acclimation was required.

Starvation test

To confirm previous observations that young mayflies are unable to survive extended periods without food [15], we conducted a starvation experiment in which we placed 1 <24-h-old mayfly into each of twenty 30-mL beakers containing 20 mL Duluth 100 water at 25 °C. We added no food to the beakers and observed mortality over the next 48 h. All organisms were alive at 24 h, but by the next day (48 h), survival was down to 22% (Figure 2). Based on this finding, all acute toxicity tests were fed a scraping of mixed diatom biofilm (described in *Acute test procedures*). Because all tests were conducted with sodium salts, food was not expected to impact availability of the contaminants, and analytical chemistry confirmed this (described in *Acute test procedures*).

Figure 2. Percentage of survival of *Neocloeon triangulifer* larvae over time with no food. Organisms were less than 24 h old at the start of the test.

Table 1. Test conditions for acute toxicity tests with *Neocloeon triangulifer*

Condition	Value
1. Temperature (°C)	25 ± 1
2. Photoperiod (light:dark)	16:8
3. Test chamber size	30 mL
4. Test solution volume	20 mL
5. Age of organisms	<24 h
6. Dilution water	Duluth 100
7. Substrate	None
8. No. of organisms per chamber	5
9. No. of chambers/treatment	4
10. Food	Scraping of live diatom biofilm
11. Aeration	None
12. Test type	Static
13. Renewal frequency	None
14. Test duration	96 h
15. Control survival	≥90%
15. Endpoint	Survival

Acute test procedures

Static, nonrenewal, acute toxicity tests were conducted according to guidelines detailed in ASTM International E729-96 [4]. Treatments comprised a 50% dilution series. Five concentrations were tested in addition to controls. Further general details on test conditions are provided in Table 1. Organisms were less than 24 h old at the beginning of the test. Test chambers were fed by grasping a mixed diatom slide (cultured as described previously in *Mixed-diatom slides*) with a forceps, and scraping off an approximately 5-mm × 10-mm × 25-mm area of biofilm with another clean microscope slide, and releasing the biofilm into the test chamber. Chambers were fed on day 0 only, because 1 biofilm scraping was more than enough for the 96-h test duration. Mortality was assessed daily, using a dissecting microscope. Individuals were considered dead if they did not respond to gentle prodding with a blunt instrument. All median lethal concentration (LC50) values were calculated by using the trimmed Spearman-Karber method [21].

Standard water chemistry parameters were measured at both the beginning and the end of each exposure period, including temperature, pH, conductivity, and dissolved oxygen. Alkalinity and hardness were measured at the beginning of the test only. The pH measurements were made by using an Accumet (Fisher Scientific) model AB15 pH meter equipped with an Accumet gel-filled combination electrode (accuracy $< \pm 0.05$ pH at 25 °C). Dissolved oxygen was measured using an air-calibrated Yellow Springs Instruments (RDP) model 55 meter. Conductivity measurements were made using a Mettler Toledo (Fisher Scientific) model MC226 conductivity/total dissolved solids meter. Alkalinity and hardness were measured by titration [22]. Water samples from each treatment were collected at the beginning and end of acute tests and submitted to the Illinois State Water Survey analytical laboratory for measurement of nitrate, chloride, and sulfate concentrations as appropriate, using ion chromatography. Conductivity varied with salt concentration in all 3 tests. For the NaCl tests, mean (±standard deviation [SD]) temperature, pH, dissolved oxygen, alkalinity, and hardness were 25.0 ± 0.2 °C, 8.3 ± 0.1, 7.5 ± 0.3 mg/L, 84 ± 2 mg/L as $CaCO_3$, and 93 ± 1 mg/L as $CaCO_3$, respectively. For the $NaNO_3$ test, the values were 24.7 ± 0.3 °C, 8.3 ± 0.1, 7.8 ± 0.1 mg/L, 83 ± 1 mg/L as $CaCO_3$, and 99 ± 1 mg/L as $CaCO_3$, respectively. For the Na_2SO_4 test, the values were 24.8 ± 0.2 °C, 8.3 ± 0.1, 7.6 ± 0.2 mg/L, 84 ± 2 mg/L as $CaCO_3$, and 99 ± 1 mg/L as $CaCO_3$, respectively. For the NaCl test, measured chloride concentrations averaged 103%

of nominal (range, 100–106%); for the $NaNO_3$ test, measured $N\text{-}NO_3$ averaged 105% of nominal (range, 103–107%); for the Na_2SO_4 test, measured sulfate averaged 107% of nominal (range, 101–110%). All reported LC50 values are based on measured concentrations.

Chronic toxicity testing procedures

For chronic toxicity tests, 6 treatments, including a control (dilution water), were tested. Nominal chloride concentrations for the treatments were as follows: 37 mg Cl^-/L (control), 70 mg Cl^-/L, 112 mg Cl^-/L, 196 mg Cl^-/L, 364 mg Cl^-/L, and 700 mg Cl^-/L. Nominal $N\text{-}NO_3$ concentrations were as follows: 0 mg $N\text{-}NO_3$/L (control), 12.5 mg $N\text{-}NO_3$/L, 25 mg $N\text{-}NO_3$/L, 50 mg $N\text{-}NO_3$/L, 100 mg $N\text{-}NO_3$/L, and 200 mg $N\text{-}NO_3$/L. Nominal SO_4^{2-} concentrations were as follows: 59 mg SO_4^{2-}/L (control), 136 mg SO_4^{2-}/L, 214 mg SO_4^{2-}/L, 369 mg SO_4^{2-}/L, 679 mg SO_4^{2-}/L, and 1300 mg SO_4^{2-}/L.

Test conditions are summarized in Table 2. In an attempt to minimize the mass of diatoms required for each test chamber, we initially used the model of the ASTM International *Ceriodaphnia dubia* chronic method [23], in which 1 organism is added per test chamber, and each treatment has 10 replicates. The NaCl and $NaNO_3$ chronic tests were conducted in this manner, but for the Na_2SO_4 chronic test, we used 2 organisms per test chamber (a total of 20 organisms per treatment) to decrease the chance of random control mortalities causing test failure. Because having 2 individuals per chamber did not require substantially higher numbers of diatom slides through the course of the test, we recommend using this organism loading rate for further tests.

Before the start of a test, a vial containing eggs from 3 females was moved from the 10 °C environmental chamber to the 25 °C chamber to encourage hatching of the eggs. The test began when sufficient numbers hatched to conduct the test; organisms were less than 24 h old at the start. Tests were conducted at 25 ± 1 °C and a 16:8-h light:dark photoperiod. All test chambers were covered with plastic wrap to minimize evaporation and with aluminum foil to eliminate direct overhead light. Light intensity in test chambers was approximately 110 lux to 160 lux. The test chambers at the beginning of the test were 30-mL glass beakers with 20 mL test water (or control). No substrate was used, but a scraping from a mixed diatom biofilm slide was added to each beaker for food as described previously in *Acute test procedures* for acute testing. Chambers were fed on day 0 and day 5, then on every water change day thereafter. Beginning on day 17 and until the end of the tests, biofilm scrapings were added to test chambers daily. Beakers were never allowed to be empty of food before the next feeding. We used scrapings of live biofilms for food, because numerous previous experiments using concentrated, refrigerated mixed diatom suspension made from concentrated stocks of freshwater diatoms, as recommended by Struewing et al. [15], resulted in lack of development of nymphs (DJ Soucek, personal observation). Water was not renewed for the first 4 d of the test, because the small size of the test organisms at that time made handling a potential source of error. Beginning on day 5 and every Monday, Wednesday, and Friday thereafter, complete water changes were conducted, with organisms being transferred to new beakers with freshly made water. Organisms were held in 30-mL beakers until day 14, when they were moved to 150-mL glass beakers with 100 mL test water. The 150-mL beakers were used until just before emergence.

Table 2. Test conditions for chronic toxicity tests with *Neocloeon triangulifer*

Condition	Value
1. Temperature (°C)	25 ± 1
2. Photoperiod (light: dark)	16:8
3. Test chamber size/solution volume	Day 0–14: 30 mL/20 mL; day 14 pre-emergent nymph stage: 150 mL/100 mL; emergence chamber: 300 mL/125 mL
4. Age of organisms at start of test	<24 h
5. Dilution water	Duluth 100
6. Substrate	None
7. No. of organisms per chamber	2 (2 of our tests had 1 per chamber)
8. No. of chambers/treatment	10
9. Food	Scrapings of live diatom biofilms from slides Day 0–16 on water change days; thereafter, daily
10. Aeration	None
11. Test type	Static/renewal
12. Renewal frequency	Days 0–4: none; day 5—end of test: MWF
13. Control survival[a]	≥80%
14. Endpoints	%Survival to pre-emergent nymph, % pre-emergent nymph when controls finished, no. of days to pre-emergent nymph, % emergence, pre-egg laying wet weight of adult, no. of eggs/original female

[a]Control survival was evaluated as no. of organisms surviving to pre-emergent nymph stage.
MWF = Monday, Wednesday, Friday.

Mayflies began to reach the pre-emergent nymph stage on day 20 to day 22, depending on the test. Slower developing individuals took as many as 36 d to reach this stage, but most had done so by day 25 in all 3 tests. When pre-emergent nymph stages were observed, they were transferred to 300-mL glass beakers with 125 mL appropriate test or control water and food. In early experiments to test this method, we added a clean microscope slide placed at a slant in the beaker to provide a surface for organisms to walk up and exit the water surface, but we later learned that this was not necessary. The larger beakers were used to provide more space for the flying sub-imagoes/imagoes, and they were covered individually with plastic wrap to prevent escape of the organisms. Sub-imagoes and imagoes were able to rest by clinging to the beaker wall or plastic wrap cover. For the NaCl and $NaNO_3$ tests, each organism had its own emergence chamber. In the case of the Na_2SO_4 chronic, which had 2 organisms per chamber at the beginning of the test, if both individuals reached pre-emergent nymph stage on the same day, they were placed in the same emergence chamber. Otherwise, each individual had its own emergence chamber. The pre-emergent nymph-staged nymphs were observed in the morning, and by the next morning, if successful, they had molted through the sub-imago stage to the imago stage. Imagoes then were grasped by the wings with a forceps, and weighed live to the nearest 0.001 mg by using a Cahn C-35 microbalance. Then, holding them again by the wings using a forceps, we held the ventral surface of their abdomen to Duluth 100 water held in a thick depression slide (85 mm × 14 mm × 33 mm), allowing the adult to release its eggs. When this procedure is performed under a dissecting scope, a faint yellowish coloration formed by the eggs is visible in the side of the abdomen. When all of the eggs are released, this coloration is no longer visible. In addition, we watched through the scope as the eggs were released and continued to hold the abdomen to the water for some time after eggs stopped appearing, to ensure that all eggs were released. With the eggs in the depression slide, we used a dissection probe to break the surface tension, allowing the eggs to fall to the bottom of the depression. The concave nature of the depression caused eggs to gather in the bottom. We then used the probe to spread the eggs into a monolayer, and photographed the monolayer using an Olympus Q-color 3 camera mounted on a dissecting scope and QCapture Ver 2.7.3 software. The images were then printed to hard copy, and eggs were counted manually.

Standard water chemistry parameters were measured throughout the exposure period, including temperature, pH, conductivity, dissolved oxygen, alkalinity, and hardness, as detailed previously in *Acute test procedures* for acute toxicity tests. Water samples from each treatment were submitted to the Illinois State Water Survey analytical laboratory for confirmation of chloride, sulfate, or nitrate concentrations by using ion chromatography. With the exception of conductivity, which varied with salt concentration in all 3 tests, measured water quality parameters varied little in the chronic toxicity tests. For the NaCl test, mean (±SD) temperature, pH, dissolved oxygen, alkalinity, and hardness were $25.2 \pm 0.5\,^{\circ}$C, 8.3 ± 0.1 mg/L, 7.4 ± 0.4 (lowest value = 6.8) mg/L, 86 ± 4 mg/L as $CaCO_3$, and 94 ± 3 mg/L as $CaCO_3$, respectively. For the $NaNO_3$ test, the values were $25.2 \pm 0.3\,^{\circ}$C, 8.3 ± 0.1 mg/L, 7.5 ± 0.5 (lowest value = 6.6) mg/L, 85 ± 5 mg/L as $CaCO_3$, and 95 ± 7 mg/L as $CaCO_3$, respectively. For the Na_2SO_4 test, the values were $25.0 \pm 0.3\,^{\circ}$C, 8.4 ± 0.1 mg/L, 7.3 ± 0.4 (lowest value = 6.1) mg/L, 83 ± 3 mg/L as $CaCO_3$, and 95 ± 4 mg/L as $CaCO_3$, respectively. For the NaCl test, measured chloride concentrations averaged 100% of nominal (range, 92–109%); for the $NaNO_3$ test, measured $N\text{-}NO_3$ averaged 102% of nominal (range, 96–107%); for the Na_2SO_4 test, measured sulfate averaged 97% of nominal (range, 90–106%).

Endpoints measured included percentage of survival to pre-emergent nymph stage; percentage of pre-emergent nymph when controls finished (% pre-emergent nymph when controls finished, calculated as the number of individuals in a treatment that had successfully reached pre-emergent nymph stage by the day the last individual in the control reached pre-emergent nymph stage); mean number of days until pre-emergent nymph stage; percentage of emergence (calculated as the number successfully emerging to imago stage divided by the number of individuals at the start of the test), mean pre-egg-laying wet (live) weight of imago; number of eggs per female (i.e., number of eggs per emerging female, a measure of mean individual fecundity); number of eggs per original female (i.e., overall number of eggs produced in a treatment divided by the number of individuals at the start of the test). The latter endpoint is analogous to population growth rate, which would include development time as well, but because the results of the 2 calculations were highly correlated, we only included number of eggs per original female in the present study. Another endpoint reported by the Stroud Water Research Center (J. Jackson, Stroud Water Research Center, Avondale, PA, USA, unpublished data) was instantaneous growth rate, which incorporates adult and hatchling mass and development time. This endpoint was not responsive to any of the salts tested in the present study, so for the sake of brevity, we have not included it. Percentage of survival was analyzed statistically by using Fisher's exact test, and means for the remaining endpoints except for percentage of emergence and number of eggs per original female were compared by using analysis of variance with post hoc pairwise comparisons conducted using Tukey's honest significant difference. Dunnett's test was not used for post hoc tests for sublethal endpoints, because the treatments did not have equal replication because of mortality. The maximum allowable toxicant concentration was calculated as the geometric mean of the no observable effect concentration (NOEC: the highest concentration at which means were not significantly different from the control) and the least observable effect concentration: the lowest concentration at which means were significantly different from that of the control). When possible, 20% effective concentrations (EC20s) were calculated by using TRAP software (Ver 1.21A; R.J. Erickson, USEPA, MED, Duluth, MN, USA).

RESULTS

Acute toxicity tests

In the five 96-h, fed, acute toxicity tests conducted with *Neocloeon triangulifer* (3 with sodium chloride and 1 each with sodium sulfate and sodium nitrate), control survival was 100% in all cases except for the sodium sulfate test, which had 95% control survival. For the sodium chloride tests, individual 96-h LC50s (95% confidence intervals) were 1140 (994–1309) mg Cl/L, 910 (719–1153) mg Cl/L, and 1153 (96–1374) mg Cl/L. The geometric mean of these 3 tests (1062 mg Cl/L) was used to calculate the acute-to-chronic ratios for chloride. The 96-h LC50 for the nitrate test was 179 (165–205) mg $N\text{-}NO_3$/L, and the value for the sulfate test was 1227 (1073–1404) mg SO_4/L.

Chronic toxicity tests

Chloride test. Survival to pre-emergent nymph stage was high for the controls and the 3 lowest-exposure treatments (Table 3). In the 194-mg/L treatment, 1 individual was killed because of technician error, so survival was 8 of 9 individuals/replicates. The 362-mg/L and 701-mg/L treatments had significantly lower survival to pre-emergent nymph stage than the other treatments. All of the organisms in the 701-mg/L treatment were dead by day 4, and all mortality in the 362-mg/L treatment occurred on day 7 or before (Figure 3A). Percentage of pre-emergent nymph when controls finished was slightly more sensitive than the percentage of survival to pre-emergent nymph stage (20% vs 60%, respectively, in the 362 mg Cl^-/L treatment), but calculated effects levels were similar in the 2 endpoints. Controls reached the pre-emergent nymph stage by day 23 on average, as did the 70-mg/L, 112-mg/L, and 194-mg/L treatments. Organisms that survived in the 362-mg/L treatment had significantly delayed (by ~3 d) development to pre-emergent nymph stage. The percentage of emergence was relatively high in the controls, but although the highest concentration had no emergence, no dose–response relationship was observed for this endpoint. The measured maximum allowable toxicant concentration and EC20 (95% confidence interval) for percentage of pre-emergent nymph when controls finished were 265 mg Cl^-/L and 165 mg Cl^-/L (119–230 mg Cl^-/L), respectively, and for percentage of survival to pre-emergent nymph stage were 265 mg Cl^-/L and 190 mg Cl^-/L (129–280 mg Cl^-/L), respectively. We could not calculate an EC20 for number of days to pre-emergent nymph stage (as 1/mean number of days) or percentage of emergence because of insufficient slope, but the maximum allowable toxicant concentrations were 265 mg Cl^-/L and 504 mg Cl^-/L, respectively.

The sublethal endpoints of pre-egg-laying weight, number of eggs per female, and number of eggs per original female were not as sensitive as the survival-related endpoints. In fact, mean weights and number of eggs for the surviving individuals in the 362-mg Cl^-/L treatment were nominally higher than those for

Table 3. Chronic chloride (as NaCl) toxicity data for the mayfly *Neocloeon triangulifer*[a]

$[Cl^-]$[b] (mg/L)	Conductivity[c] (μmhos/cm)	% pre-emergent nymph WCF[d]	% survival to pre-emergent nymph stage	No. of days to pre-emergent nymph	% emergence	Pre-egg laying weight (mg)	No. of eggs per female	No. of eggs per original female
27	371 ± 11	100 A	100 A	23.3 ± 1.1 A	70 A	3.131 ± 0.311 A	1446 ± 146 A	964
70	516 ± 15	100 A	100 A	23.1 ± 0.7 A	50 A	2.949 ± 0.290 A	1302 ± 216 A	651
112	658 ± 15	90 A	90 A	23.1 ± 0.6 A	40 A	3.075 ± 0.293 A	1326 ± 107 A	531
194	939 ± 11	89 A	89 A	23.0 ± 0.5 A	78 A	2.996 ± 0.145 A	1206 ± 163 A	938
362	1479 ± 33	20 B	60 B	26.5 ± 2.0 B	60 A	3.246 ± 0.179 A	1497 ± 113 A	898
701	2550 ($n = 1$)	0 B	0 B	NA	0 B	NA	NA	0
MATC (mg Cl/L)		265	265	265	504	NC	NC	NC
EC20 (mg Cl/L)		165	190	NC	NC	NC	NC	NC
EC20 95% CI		119–230	129–280	NC	NC	NC	NC	NC
ACR[e]		6.4	5.6	4.0	2.1	NC	NC	NC

[a] Within endpoint columns, means followed by different capital letters are significantly different ($p < 0.05$).

[b] Mean measured Cl^- concentrations are shown.

[c] Conductivity values shown are means (±Standard deviation) of all measurements for the duration of the test.

[d] WCF = when controls finished (i.e., on the day of the appearance of the last pre-emergent nymph stage in the control).

[e] Calculated using the geometric mean of three 96-h LC50 (1062 mg Cl/L) divided by EC20 when available, otherwise by maximum acceptable toxicant concentration.

MATC = maximum acceptable toxicant concentration; WCF = when controls finished; NA = not applicable; NC = not calculated (not statistically possible); EC20 = 20% effect concentration; CI = confidence interval; ACR = acute to chronic ratio.

any other treatment (Table 3). Neither maximum allowable toxicant concentrations nor EC20s could be calculated for any of these 3 endpoints. However, variability for these endpoints was relatively low, with coefficients of variation ranging from a minimum of 5 to a maximum of 17, suggesting that they may be useful endpoints for other contaminants with different modes of action.

With EC20s or maximum allowable toxicant concentrations ranging from 165 mg Cl^-/L to 504 mg Cl^-/L, and a mean 96-h LC50 of 1062 mg Cl^-/L, the acute to chronic ratios for chloride ranged from 2.1 to 6.4 (Table 3).

Nitrate test

Percentage of survival to pre-emergent nymph stage was high for the controls and up to 51 mg N-NO_3/L (Table 4). None of the mayflies in the 101-mg/L and 201-mg/L treatments survived to pre-emergent nymph stage. All of the organisms in the highest treatment were dead by day 16. In contrast to the chloride chronic test in which all mortality in the second highest exposure concentration occurred on day 7 or before, in the nitrate test, all mortality in the 101-mg/L treatment occurred on or after day 20, when organisms in other treatments were reaching the pre-emergent nymph stage (Figure 3B). Percentage of pre-emergent nymph when controls finished was more sensitive than percentage of survival to pre-emergent nymph stage, with a maximum allowable toxicant concentration of 36 mg/L compared with 72 mg/L for the latter. The EC20s could not be calculated for either of the survival endpoints because of inadequate partial effects. Controls reached the pre-emergent nymph stage by day 21 on average, and the 12.8 mg/L and 26 mg/L treatments had similar means for number of days to pre-emergent nymph stage (Table 4). Organisms in the 51-mg/L treatment reached the pre-emergent nymph stage on day 22 on average, and this mean was significantly different from that of the control. Although none of the organisms in the 101-mg N-NO_3/L treatment reached the pre-emergent nymph stage, 1 individual in this treatment was alive until day 31. Percentage of emergence was 60% in the controls but even higher in the 12.8-mg/L and 26-mg/L treatments.

As was true of the chloride chronic test, the sublethal endpoints of pre-egg-laying weight and number of eggs per

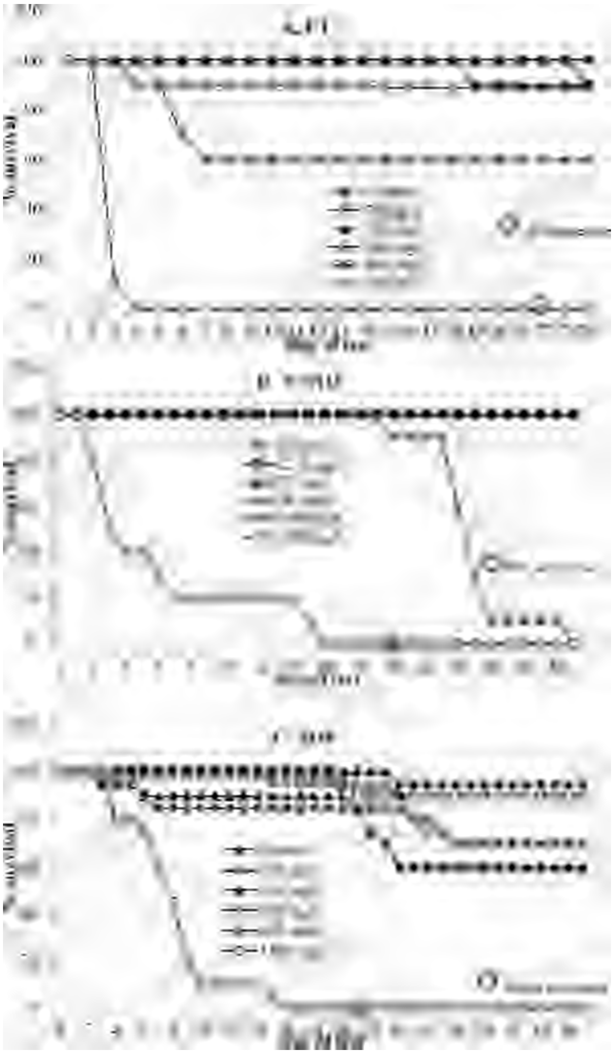

Figure 3. Survival of *Neocloeon triangulifer* larvae to pre-emergent nymph stage at different (**A**) sodium chloride, (**B**) sodium nitrate, and (**C**) sodium sulfate concentrations. Nominal treatment concentrations are shown in legend. Measured values are presented in Tables 4 and 5, respectively.

Table 4. Chronic nitrate (as $NaNO_3$) toxicity data for the mayfly *Neocloeon triangulifer*[a]

$[N\text{-}NO_3^-]$[b] (mg/L)	Conductivity[c] (µmhos/cm)	% pre-emergent nymph WCF[d]	% survival to pre-emergent nymph stage	No. of days to pre-emergent nymph	% emergence	Pre-egg laying weight (mg)	No. of eggs per female	No. of eggs per original female
0.03	374 ± 23	100 A	100 A	21.3 ± 0.6 A	60 A	3.030 ± 0.278 A	1395 ± 244 A	837
12.8	481 ± 17	100 A	100 A	20.7 ± 0.6 A	90 A	3.263 ± 0.179 A	1647 ± 117 A	1483
26	593 ± 22	100 A	100 A	20.4 ± 0.6 A	66 A	3.232 ± 0.103 A	1500 ± 202 A	1000
51	804 ± 24	60 B	100 A	22.4 ± 1.1 B	40 A	3.283 ± 0.239 A	1614 ± 144 A	646
101	1209 ± 30	0 B	0 B	NA	0 B	NA	NA	0
201	2042 ± 73	0 B	0 B	NA	0 B	NA	NA	0
MATC (mg $N\text{-}NO_3$/L)		36	72	36	72	NC	NC	NC
EC20 (mg $N\text{-}NO_3$/L)		NC	NC	NC	39	NC	NC	35
EC20 95% CI		NC	NC	NC	9.2–69	NC	NC	14–85
ACR[e]		5.0	2.5	5.0	4.6	NC	NC	5.1

[a] Within endpoint columns, means followed by different capital letters are significantly different ($p < 0.05$).

[b] Mean measured $N\text{-}NO_3^-$ concentrations are shown.

[c] Conductivity values shown are means (±Standard deviation) of all measurements for the duration of the test.

[d] WCF = when controls finished (i.e., on the day of the appearance of the last pre-emergent nymph stage in the control).

[e] Calculated using 96-h LC50 (179 mg $N\text{-}NO_3$/L) divided by EC20 when available, otherwise by maximum acceptable toxicant concentration.

MATC = maximum acceptable toxicant concentration; NA = not applicable; NC = not calculated (not statistically possible); EC20 = 20% effect concentration; CI = confidence interval; ACR = acute to chronic ratio.

female were not as sensitive as survival or number of days to pre-emergent nymph stage (Table 4). In fact, mean weights and number of eggs for the surviving individuals in the nitrate-exposed treatments were nominally higher than those in the control. We were, however, able to calculate an EC20 for number of eggs per original female of 35 mg $N\text{-}NO_3$/L, which was the lowest chronic value obtained. Variability for pre-egg-laying weight and number of eggs per female were relatively low, with coefficients of variation ranging from 3 to 17.

With EC20s or maximum allowable toxicant concentrations ranging from 35 mg $N\text{-}NO_3$/L to 72 mg $N\text{-}NO_3$/L, and a 96-h LC50 of 179 mg $N\text{-}NO_3$/L, the acute-to-chronic ratios ranged from 2.5 to 5.1 for nitrate (Table 4).

Sulfate test

Percentage of survival to pre-emergent nymph stage was high for the controls and the 129 mg SO_4/L treatment, but was significantly reduced at 209 mg/L and above (Table 5). None of the mayflies in the 1277-mg/L treatments survived to pre-emergent nymph stage. As was the case for the nitrate test, all of the organisms in the highest treatment were dead by day 16 (Figure 3C). For the other treatments that had 60% to 70% survival to pre-emergent nymph stage, most of the mortality occurred after day 21, similar to what was observed in the nitrate test. Percentage of pre-emergent nymph when controls finished was again more sensitive than percentage of survival to pre-emergent nymph stage. Both endpoints had the same maximum allowable toxicant concentration (164 mg/L), but the EC20 for percentage of pre-emergent nymph when controls finished (170 mg/L) was substantially lower than that of the percentage of survival to pre-emergent nymph stage (289 mg/L). Controls reached the pre-emergent nymph stage by day 23 on average, and 2 treatments had significant delays in development, with the 359-mg/L treatment averaging 26 d and the 661-mg/L treatment averaging 32 d (Table 5). None of the organisms in the 1277-mg/L treatment reached the pre-emergent nymph stage. Percentage of emergence was 70% in the controls and roughly decreased with increasing sulfate dose, resulting in an EC20 of 145 mg/L (Table 5).

Again, the sublethal endpoints of pre-egg-laying weight and number of eggs were not as sensitive as survival or number of

Table 5. Chronic sulfate (as Na_2SO_4) toxicity data for the mayfly *Neocloeon triangulifer*[a]

$[SO_4^{2-}]$[b] (mg/L)	Conductivity[c] (µmhos/cm)	% pre-emergent nymph WCF[d]	% survival to pre-emergent nymph stage	No. of days to pre-emergent nymph	% emergence	Pre-egg laying weight (mg)	No. of eggs per female	No. of eggs per original female
57	371 ± 12	95 A	95 A	23.0 ± 1.0 A	70 A	2.550 ± 0.282 A	1124 ± 148 A	749
129	540 ± 10	85 A	90 A	23.5 ± 1.3 A	45 A	2.428 ± 0.410 A	1060 ± 220 A	446
209	715 ± 9	60 B	60 B	23.4 ± 1.1 A	55 A	2.624 ± 0.383 A	1177 ± 209 A	648
359	1057 ± 11	40 B	70 B	26.1 ± 2.1 B	30 B	2.629 ± 0.229 A	1141 ± 130 A	342
661	1719 ± 17	0 B	70 B	32.2 ± 1.7 B	30 B	3.219 ± 0.367 B	1354 ± 150 A	406
1277	3003 ± 39	0 B	0 B	NA	0 B	NA	NA	0
MATC (mg SO_4/L)		164	164	274	274	NC	NC	NC
EC20 (mg SO_4/L)		170	289	528	145	NC	NC	281
EC20 95% CI		128–226	139–437	492–566	69–305	NC	NC	70–1138
ACR[e]		7.2	4.2	2.3	8.5	NC	NC	4.4

[a] Within endpoint columns, means followed by different capital letters are significantly different ($p < 0.05$).

[b] Mean measured SO_4^{2-} concentrations are shown.

[c] Conductivity values shown are means (±Standard deviation) of all measurements for the duration of the test.

[d] WCF = when controls finished (i.e., on the day of the appearance of the last pre-emergent nymph stage in the control).

[e] Calculated using 96-h LC50 (1227 mg SO_4/L) divided by EC20 when available, otherwise by MATC.

MATC = maximum acceptable toxicant concentration; NA = not applicable; NC = not calculated (not statistically possible or valid); EC20 = 20% effect concentration; CI = confidence interval; ACR = acute to chronic ratio.

days to pre-emergent nymph stage (Table 5), but we were able to calculate an EC20 for number of eggs per original female of 281 mg SO_4/L. Mean weights for the surviving individuals in the 661-mg/L treatment were significantly higher than those in the control, likely because of the longer development time. Again, variability for pre-egg-laying weight and number of eggs per female were relatively low, with coefficients of variation ranging from 9 to 21.

With EC20s ranging from 145 SO_4/L to 528 mg SO_4/L, and a 96-h LC50 of 1227 mg SO_4/L, the acute-to-chronic ratios ranged from 2.3 to 8.5 for sulfate (Table 5).

Relative sensitivity of endpoints

We were able to generate at least 4 acute-to-chronic ratios for each of the 3 chronic tests conducted (Table 6). To assess the relative sensitivity of the various endpoints over the 3 tests, we calculated relative acute-to-chronic ratios by dividing each individual acute-to-chronic ratio by the highest acute-to-chronic ratio for each particular test, thereby creating a ranking with a maximum of 1.00 (highest acute-to-chronic ratio) and a minimum of 0 (no acute-to-chronic ratio calculated). By taking the average relative acute-to-chronic ratio for each endpoint, we determined that percentage of pre-emergent nymph when controls finished was the most sensitive endpoint across the 3 tests, with an average relative acute-to-chronic ratio of 0.94, and pre-egg-laying weight and number of eggs per female were the least sensitive, with no acute-to-chronic ratios calculated (Table 6).

DISCUSSION

Our mean 96-h chloride LC50 of 1062 mg Cl/L at 25 °C and a hardness of 93 mg/L was higher than the 48-h LC50 of 399 mg Cl/L (calculated from the reported value for NaCl) reported by Struewing et al. [15] at the same temperature and a similar hardness. However, in the same paper, Struewing et al. [15] reported a 14-d LC50 of 505 mg Cl/L, which, being higher than their 48-h LC50, suggests that perhaps their reported acute tests were an overestimate of sensitivity. Based on results of separate experiments conducted in our laboratory (see Supplemental Data, Table S1), possibly this discrepancy is attributable to the feeding method used by the respective laboratories. The Struewing [15] study fed acute tests using a concentrated mixed diatom suspension, whereas the present study used live diatom biofilm scrapings as food. We conducted acute toxicity tests with *N. triangulifer* using both food types (refrigerated mixed diatom suspension and live biofilm scrapings) but otherwise with identical methods, and obtained substantially different LC50 values (~2-fold difference with non-overlapping confidence intervals) in the chloride salt tests (Supplemental Data, Table S1). Interestingly, although a similar difference was observed in tests with potassium chloride, the same comparisons with sulfate and nitrate salts resulted in LC50s that were quite similar between food types (Supplemental Data, Table S1), so this appears to be a phenomenon specific to chloride. At this time we are unable to speculate as to the mechanism for this difference. Our acute chloride toxicity results lie between those previously mentioned [15] and the findings of the Stroud Water Research Center (J. Jackson, Stroud Water Research Center, Avondale, PA, USA, unpublished data), who generated 48-h LC50s in tests conducted at 20 °C of 1423 mg Cl/L at a hardness of 22 to 42 (Dyberry Creek dilution water), and 2459 mg Cl/L at a hardness of approximately 100 (White Clay Creek dilution water), using naturally colonized live algal biofilm plates as food. With longer duration and a higher test temperature, believing that the Stroud Water Research Center results would be similar to ours is reasonable. All of these described tests were conducted with first instar larvae.

Our full-life chronic toxicity values for chloride, EC20s of 165 mg Cl/L to 190 mg Cl/L, and maximum allowable toxicant concentrations of 265 Cl/L to 504 mg Cl/L are similar to 14-d 25% inhibitory concentrations (IC25s) reported by Struewing et al. [15] in water with a similar hardness (~90 mg/L). Their values ranged from 139 Cl/L to 224 mg Cl/L (calculated from reported NaCl IC25s). Their acute-to-chronic ratios ranged from 0.79 to 2.9, whereas ours ranged from 2.1 to 6.4, a function of our higher LC50. Although they found weight and body length to be sensitive endpoints, our survival and development time data were more sensitive to chloride. A potential explanation for this different finding is that our weights were for egg-bearing adults that successfully emerged, whereas Struewing et al. [15] measured weight on approximately 14-d-old nymphs. Because we observed developmental delays (greater mean number of days to reach pre-emergent nymph stage at 362 mg Cl/L compared with lower treatments), we likely would have obtained similar results regarding weight if we ended the test at 14 d. Comparing endpoints is difficult because of the disparity in test duration, but another difference in the 2 test systems is that, again, Struewing et al. [15] fed their organisms a loose suspension of diatoms, whereas we fed ours live diatom biofilm scrapings. In our preliminary experiments, we were unable to bring mayflies to adulthood by using a refrigerated loose diatom suspension, even when plenty of excess food was available.

Table 6. Actual and relative acute to chronic ratios generated in the present study for three chronic toxicity tests with sodium salts[a]

	Cl test		N-NO_3 test		SO_4 test		
Endpoint	ACR	Relative ACR	ACR	Relative ACR	ACR	Relative ACR	Average relative ACR
% pre-emergent nymph WCF[b]	6.4	1.00	5.0	0.98	7.2	0.85	0.94
% survival to pre-emergent nymph stage	5.6	0.88	2.5	0.49	4.2	0.49	0.62
No. of days to pre-emergent nymph	4.0	0.63	5.0	0.98	2.3	0.27	0.63
% emergence	2.1	0.33	4.6	0.90	8.5	1.00	0.74
Weight[c]	NC	0	NC	0	NC	0	0
No. of eggs/female	NC	0	NC	0	NC	0	0
No. of eggs/original female	NC	0	5.1	1.00	4.4	0.52	0.51

[a] Relative acute-to-chronic ratios were calculated by dividing each individual acute-to-chronic ratio for a given test by the highest acute-to-chronic ratio for that test. A high relative acute-to-chronic ratio indicates high sensitivity (i.e., a larger difference between the chronic endpoint and the 96-h LC50).

[b] WCF = when controls finished (i.e., when the last control individual has reached pre-emergent nymph stage).

[c] Weight = pre-egg-laying live weight of adults.

ACR = acute to chronic ratio; WCF = when controls finished; NC = not calculated; LC50 = median lethal concentration.

The Stroud Water Research Center (J. Jackson, Stroud Water Research Center, Avondale, PA, USA, unpublished data) reported full-life chronic chloride toxicity to *N. triangulifer,* using naturally colonized periphyton plates as food. Their most sensitive endpoints were development time and instantaneous growth rate. Despite their testing at 20 °C, their maximum allowable toxicant concentrations in water with a similar hardness to ours (White Clay Creek) ranged from 177 Cl/L to 708 mg Cl/L. Thus, our full-life chronic method using laboratory-cultured food appears to produce chronic values similar to those produced using natural food sources.

In other chronic toxicity studies using this species or closely related mayflies with chloride dominated salts, Hassell et al. [16] obtained 21-d conductivity LC50s of 890 μS/cm to 2700 μS/cm in 2 different dilution waters at 15 °C, using wild-caught *Centroptilum* sp., and Johnson et al. [18] reported an EC20 of 672 μS/cm at 24.5 °C in a mesocosm study with *N. triangulifer,* using a combination of $CaCl_2$ and NaCl. Both of these tests were conducted with natural foods: conditioned leaves for the former and naturally colonizing periphyton for the latter. In our chloride test, we calculated a conductivity EC20 of 895 μS/cm (709–1129 μS/cm) for the most sensitive endpoint, so again, our test with cultured food can produce similar results to those with natural foods.

Other mayfly genera have been investigated for sodium chloride sensitivity in subchronic tests. For example, Diamond et al. [24] tested *Stenonema modestum* (Heptageniidae) at 12 °C in 14-d exposures. The maximum allowable toxicant concentrations for survival and molting endpoints ranged from 1410 mg Cl/L to 3798 mg Cl/L (calculated from salt concentrations). Echols et al. [3] observed a mean *Isonychia bicolor* 7-d NOEC of 855 mg Cl/L at 20 °C to 23 °C. Goetsch and Palmer [25] reported a 96-h LC50 between 1500 mg Cl/L and 2500 mg Cl/L for *Tricorythus* sp. in an unfed test at 10 °C to 16 °C. K. Allan recorded a 72-h conductivity LC50 of 12 600 μS/cm for the leptophlebiid mayfly *Nousia* sp. using sodium and chloride–dominated saline waters. A 7-d acclimation to higher salinity water did not significantly alter conductivity LC50s for that species (K. Allan, 2006, Master's thesis, University of Tasmania, Hobart, TAS, Australia). Although these effect levels are higher than those observed in our NaCl chronic test, comparisons are tenuous because of the differences in test temperatures, duration, endpoints, and the fact that all of these studies used wild-collected organisms.

Much less information is available in the literature on chronic toxicity of sulfate salts to *N. triangulifer* and related species. Kunz et al. [12] conducted static, nonrenewal exposures of first instar nymphs through adult emergence (~35 d) at ambient laboratory temperatures of 21 °C, using naturally colonized periphyton plates (from Stroud Water Research Center) as food and survival (emergence) and biomass as endpoints. They developed reconstituted waters to simulate major ion compositions typical of streams impacted by coal mining in Appalachian streams. One of the waters tested ("Upper Dempsey") was dominated by Na and SO_4, but in contrast to our findings, it did not cause significant toxicity at the highest test concentration (~640 mg SO_4/L). We observed EC20s ranging from 145 SO_4/L to 528 mg SO_4/L, but our test was conducted at a hardness of 95 mg/L, whereas the Kunz et al. [12] test was conducted at a hardness of 220 mg/L. Hardness has been shown to have a strong influence on sodium sulfate toxicity to other species [26,27]. In 2 other reconstituted waters having an ionic composition dominated by Ca, Mg, HCO_3, and SO_4, Kunz et al. [12] did observe significant toxicity to *N. triangulifer* at sulfate concentrations ranging from 386 mg/L ("Winding Shoals" water) to approximately 770 mg/L ("Boardtree" water; sulfate not measured). In terms of conductivity, the Winding Shoals and Boardtree waters were toxic to *N. triangulifer* at approximately 800 μS/cm and 1300 μS/cm, respectively [12]. We calculated a conductivity EC20 for our sodium sulfate test of 725 μS/cm.

Goetsch and Palmer [25] reported a 96-h LC50 of 532 mg SO_4/L for the mayfly *Tricorythus* sp. in sodium sulfate exposures at approximately 10 °C to 15 °C. This is substantially lower than our 96-h LC50 of 1227 mg SO_4/L, but in addition to using a different species with potentially different sensitivity to sulfate, the *Tricorythus* test was conducted at a moderately lower hardness (~70 mg/L as $CaCO_3$), and the test was not fed. Conversely, our conductivity EC20 (725 μS/cm) for *N. triangulifer* is quite similar to 7-d maximum allowable toxicant concentrations reported by Kennedy et al. [28] (737–773 μS/cm) for *Isonychia bicolor* exposed to a simulated mine effluent dominated by sodium and sulfate.

To our knowledge, these are the first acute and chronic nitrate toxicity values reported for *N. triangulifer*, but our lowest maximum allowable toxicant concentrations (36 mg N-NO_3/L) and EC20 (35 mg N-NO_3/L) are strikingly similar to a 20-d threshold effect concentration (equivalent to an maximum allowable toxicant concentration) reported for the New Zealand leptophlebiid *Deleatidium* sp. [29]. Our 96-h LC50 for *N. triangulifer* (179 mg N-NO_3/L) would place this species fifth in sensitivity among the invertebrate data previously compiled [30]. Our lowest chronic value is similar but nominally less sensitive than the mean maximum allowable toxicant concentration (22 mg N-NO_3/L, calculated from NOEC and least observable effect concentration values) reported for the water flea *Ceriodaphnia dubia* [31], but it is substantially more sensitive than *Daphnia magna* (mean maximum allowable toxicant concentration = 507 mg N-NO_3/L [31]), and the apple snail *Pomacea paludosa* (mean 14-d EC50 = 560 mg N-NO_3/L) [32].

For each of our 3 full-life chronic toxicity tests, we evaluated 7 endpoints: 2 involving survival to pre-emergent nymph stage, 1 involving development time, 1 involving adult emergence rates, and 3 involving adult weight or fecundity. Based on average relative acute-to-chronic ratio (Table 6), the most sensitive endpoints in our tests were those involved with survival, emergence, and development time. This suggests that exposure to sublethal concentrations of these sodium salts in the field may not affect somatic growth or fecundity directly, but could have phenological effects that may indirectly impact population growth. The weight and number of eggs per female endpoints were not sensitive in any of our 3 tests; however, others, using different testing scenarios, have found growth and biomass endpoints to be sensitive ([12,15,18]; J. Jackson, Stroud Water Research Center, Avondale, PA, USA, unpublished data). As stated previously, this disparity can in part be attributed to differences in when tests are ended, for example, based on our development time results (number of days to pre-emergent nymph stage), had we ended our tests at day 14 as in Struewing et al. [15], we likely would have seen differences among treatments in organism weights. Furthermore, we believe that even in a test conducted according to the method in our present study, weight and fecundity endpoints should be evaluated because chemicals with different modes of action than those tested in the present study may cause different types of effects.

In our chloride chronic test, nearly all mortality occurred by day 7, whereas in the nitrate chronic, mortality in the highest concentration occurred early in the test (beginning on day 2), but in the second highest concentration, no mortality occurred until pre-emergent nymph stages were appearing in other treatments (day 20; Figure 3). A similar pattern was observed in the sodium sulfate chronic. This suggests that the most sensitive life stage may be dependent on the contaminant, but it also supports the findings of others [19,33], who in field and laboratory studies exposing *N. triangulifer* to metals found that metamorphosis to the imago was a highly sensitive life-stage.

Based on our starvation test, acceptable control survival (≥90%) appears to not be possible in an unfed 48-h or 96-h acute toxicity test conducted at 25 °C. Similar results were reported for this species by Struewing et al. [15], who had 11% survival after 48-h in USEPA moderately hard reconstituted water with no food, and by the Stroud Water Research Center (J. Jackson, Stroud Water Research Center, Avondale, PA, USA, unpublished data), who in 4 of 5 waters tested had approximately 10% survival or less after 48 h. The ASTM International [4] method for conducting acute toxicity tests states that organisms should not be fed during acute toxicity tests, although it makes an exception for mysid shrimp, which are severely stressed if not fed. In the present study, the nature of the chemicals tested is such that the presence of food would not be expected to alter bioavailability of the contaminants, but the need to feed *N. triangulifer* in acute tests will be a factor to consider in tests with other contaminant types. Poteat and Buchwalter [34] contend that in the case of metals in particular, dietary uptake is a more ecologically valid measurement for aquatic insects than dissolved exposures, but in general, water quality criteria development still uses data from waterborne exposures, so this is an issue that may require some flexibility.

We compared the relationship between pre-egg-laying weight and the number of eggs produced per female in the present study with that reported in Weaver et al. [14], and although our fecundity numbers were consistently lower than those predicted by their equation (Figure 4), the slopes of the lines were quite similar whether we used only control data (Figure 4A) or both control and exposure data (Figure 4B). If we use our data to predict a minimum adult weight at which 1000 eggs or more would be produced in the same manner as that described in Weaver et al. [14], we obtain values of 2.95 mg for control-only data, and 2.72 for control plus exposure data. These numbers bracket the previously reported value of 2.8 mg [14]. In other words, using our "controls only" equation to predict number of eggs based on a weight of 2.0 mg results in a value that is 80% of the result using the Weaver et al. [14] equation, and at a weight of 3.8 mg (just above our maximum), our equation produced a number of eggs value that was 94% of that using the Weaver et al. [14] equation. These slight differences may be attributable to acclimation to different waters or different sources of within-laboratory error, but the overall similarity of these relationships obtained using similar methods of producing laboratory-cultured food indicates good potential for standardization.

In the process of adapting the Weaver et al. [14] method and developing our own full-life chronic toxicity method, a few of the lessons we learned are as follows. First, for culturing, we have obtained best results when seeding stocks and cages for diatom slides with fresh diatom stocks that have never been refrigerated. Second, further work should be done to specifically characterize ion and nutrient concentrations required for diatom slide and diatom stock media. We currently use dechlorinated tap water with an added nutrient and trace metal solution to culture diatom stocks and slides. Although we have analyzed tap water samples for ionic composition, this is likely variable over time because of the nature of municipal water supplies. One of the most variable aspects of the current method remains the quality of diatom slides, and better knowledge of specific ion requirements for diatoms will help to reduce this variability. Third, our results suggest that mayflies require live biofilms for robust development to adulthood. In numerous attempts, feeding concentrated mixed diatom suspensions that have been refrigerated did not result in development to pre-emergent nymph stage. Fourth, before feeding live biofilms to mayflies, a sample of the biofilm slide must be examined under a compound microscope to ensure that most of the cells are intact, and that the biofilm is not made up of amorphous material. Color of the biofilm does not appear to be a good predictor of food quality. Finally, having a higher percentage of emergence in controls during chronic toxicity tests would be desirable. One current thought is that because the lowest nitrate concentration had 90% emergence during that test, perhaps the addition of a low concentration of nitrate to culture medium and test dilution water would improve the food quality and therefore increase the rate of emergence.

Figure 4. Relationship between pre–egg-laying wet weight of *Neocloeon triangulifer* and number of eggs produced in (**A**) controls only and (**B**) all treatments of 3 chronic toxicity tests. Asterisks are number of eggs/female predicted by the equation from Weaver et al. [1] based on our mayfly weights. Dashed lines show 95% confidence intervals for the solid line described by the equation in each panel.

Mayflies have received much attention recently because of their apparent sensitivity to mining influences in Appalachian headwater streams, with declines attributed to elevated conductivity, a surrogate measure of major ion concentrations of total dissolved solids [35–38]. Until recently, a disconnect has been perceived between what major ion concentrations are toxic to organisms in the laboratory versus what concentrations cause impairment to endemic benthic macroinvertebrate communities. Although no laboratory exposure system can completely replicate exposure scenarios experienced by organisms in situ, our observation of effects at various life-stages and documentation of potential phenological impacts brings some environmental relevance in addition to the fact that this is an organism that better represents benthic macroinvertebrate communities than standard crustacean models. We believe that the continued use of this sensitive mayfly species in laboratory studies will help to close this gap in understanding.

SUPPLEMENTAL DATA

Table S1. (15 KB DOC).

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Data availability—Contact David Soucek at soucek@illinois.edu for available data.

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Influence of Dilution Water Ionic Composition on Acute Major Ion Toxicity to the Mayfly *Neocloeon triangulifer*

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Abstract: Field and laboratory studies have shown that mayflies (Ephemeroptera) tend to be relatively sensitive to elevated major ion concentrations, but little is known about how ionic composition influences these responses. The present study evaluated the acute toxicity of major ion salts to the mayfly *Neocloeon triangulifer* over a range of background water quality conditions. The mayfly was particularly sensitive to Na_2SO_4, with the median lethal concentration (LC50) of 1338 mg SO_4/L being lower than LC50s reported for 7 other species at that hardness. Increasing hardness of the dilution water from 30 to 150 mg/L (as $CaCO_3$) resulted in doubling of LC50s for sodium salts, and an approximately 1.5-fold increase in LC50 for $MgSO_4$. Potassium salt toxicity was not strongly influenced by hardness, consistent with findings for other species. When hardness was held constant but the Ca to Mg ratio was manipulated, the ameliorative effect on Na_2SO_4 and NaCl did not appear as strong as when hardness was varied; but for $MgSO_4$ the amelioration relative to Ca activity was similar between the 2 experiments. The toxicity of K salts to *N. triangulifer* was similar to Na salts on a millimolar basis, which contrasts with several other species for which K salts have been much more toxic. In addition, the toxicity of KCl to *N. triangulifer* was not notably affected by Na concentration, as has been shown for *Ceriodaphnia dubia*. Finally, plotting LC50s in terms of ion activity (Cl, SO_4, Na, Mg, or K) over the range of Ca activities in dilution water resulted in significant positive relationships, with comparable slopes to those previously observed for *C. dubia* over the same range of Ca activities. *Environ Toxicol Chem* 2018;37:1330–1339. © 2018 SETAC

Keywords: *Neocloeon triangulifer*; Mayfly; Acute toxicity; Major ion; Salinity

INTRODUCTION

The environmental impacts of elevated concentrations of major ions (i.e., Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, and HCO_3^-) have received considerable research attention in the 2 decades since it was recognized that major ion toxicity varies widely depending on the composition of the solution (Mount et al. 1997). The primary sources of elevated major ions in freshwaters were reviewed by Goodfellow et al. (2000), but much of the recent North American literature has focused on issues related to road salting and mining (Corsi et al. 2010; Palmer et al. 2010), with toxicity testing primarily focused on sodium chloride or sodium sulfate tested with standard test organisms, especially crustaceans (Soucek and Kennedy 2005; Davies and Hall 2007; Soucek 2007; Lasier and Hardin 2010; Elphick et al. 2011a, 2011b; Soucek et al. 2011; Mount et al. 2016; Wang et al. 2016).

A number of field studies have shown that mayflies (Ephemeroptera) appear to be more sensitive than other benthic macroinvertebrate taxa to elevated major ion concentrations in streams impacted by mining (Pond et al. 2008; Pond 2010; Cormier et al. 2013; Timpano et al. 2015; Boehme et al. 2016), and several recent laboratory studies have generated chronic toxicity data confirming this sensitivity (Kennedy et al. 2004; Hassell et al. 2006; Kunz et al. 2013; Johnson et al. 2015; Soucek and Dickinson 2015; Struewing et al. 2015; J. Jackson, Stroud Water Research Center, Avondale, PA, USA, personal communication). Until relatively recently, difficulties with culturing have precluded the use of mayflies as laboratory toxicity testing organisms to any great extent; however, researchers at Stroud Water Research Center developed a method for testing a parthenogenetic species (*Neocloeon triangulifer*) that readily reproduces under laboratory conditions (Sweeney et al. 1993). Furthermore, recent efforts to develop laboratory cultured diets and chronic toxicity testing methods for mayflies have been successful and will help us move toward standardization of methods for this species (Soucek and Dickinson 2015; Struewing et al. 2015; Weaver et al. 2015). In acute (96-h) exposures

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reported by Soucek and Dickinson (2015), sodium chloride median lethal concentrations (LC50s) for *N. triangulifer* were approximately equal to those reported for the cladoceran *Ceriodaphnia dubia* (Mount et al. 1997), but sodium sulfate LC50s were approximately half those for *C. dubia*. In a full-life chronic toxicity test, the percentage of emergence (the most sensitive endpoint in that test) 20% effect concentration for *N. triangulifer* exposed to sodium sulfate (Soucek and Dickinson 2015) was 15 to 23% of the lowest values reported for other invertebrates including *C. dubia*, *Lampsilis abrupta*, and *Chironomus dilutus* (Wang et al. 2016). The most sensitive chronic response level of *N. triangulifer* to sodium chloride (Soucek and Dickinson 2015) was 36 to 39% of those of *C. dubia* and *Daphnia magna* (Elphick et al. 2011a) and similar to the response of the chloride-sensitive mussel *Lampsilis siliquoidea* (Wang et al. 2015).

The fact that major ion toxicity to standard laboratory test organisms like cladocerans, amphipods, and fish is dependent on the ionic composition of a water or effluent has been well established; for example, several studies have shown that the toxicity of sodium sulfate and sodium chloride decrease with increasing water hardness (Mount et al. 1997, 2016; Soucek and Kennedy 2005; Davies and Hall 2007; Soucek 2007; Elphick et al. 2011a, 2011b; Soucek et al. 2011) and, more specifically, increasing Ca concentration (Davies and Hall 2007; Mount et al. 2016). It is also known that for crustaceans and fish, solutions tend to be more toxic when dominated by particular major ions; *C. dubia* is more sensitive to solutions dominated by K^+, Mg^+, and HCO_3^- than those dominated by Na^+, Cl^-, and SO_4^{2-}(Mount et al. 1997, 2016). Furthermore, recent work has shown that sodium concentration regulates the toxicity of potassium salts to *C. dubia* (Mount et al. 2016). Very little is known about how ionic composition influences responses of *N. triangulifer* to elevated major ions, although Kunz et al. (2013) observed differences in chronic responses of this species to Na^+ and SO_4^{2-}-dominated solutions compared to Ca^{2+}, Mg^{2+}, SO_4^{2-}, and HCO_3^- dominated solutions; and Stroud Water Research Center (J. Jackson, personal communication) noted differences in 48-h NaCl LC50s in 2 dilution waters with different hardnesses. Zalizniak et al. (2006) investigated the influence of ionic composition on salinity toxicity to the related Australian mayfly *Centroptilum* sp., but test concentrations were not sufficiently low to have confidence in LC50s. Because *N. triangulifer* appears to be more sensitive to some major ions than other laboratory cultured test organisms, greater knowledge of the influence of dilution water composition on major ion toxicity will be important to refining our ability to evaluate potential risks to aquatic communities.

Although most field studies (e.g., Pond et al. 2008; Cormier et al. 2013; Boehme et al. 2016) and some laboratory or mesocosm studies (Kefford et al. 2003; Hassell et al. 2006; Kunz et al. 2013; Johnson et al. 2015; Clements and Kotalik 2016) report responses to elevated major ions in terms of electrical conductivity, endpoints for major ion laboratory toxicity tests are most frequently reported in terms of anion concentrations (e.g., Soucek and Kennedy 2005; Davies and Hall 2007; Soucek 2007; Lasier and Hardin 2010; Elphick et al. 2011a, 2011b; Soucek et al. 2011; Wang et al. 2016). The focus on anions may be attributable in part to the finding of Mount et al. (1997) that Na and Ca were not significant factors in regressions used to model ion toxicity; however, more recent studies by Erickson et al. (2016) suggested that K, Mg, and Ca salt toxicities to *C. dubia* are related to the cations and that Na salt toxicity may be related to multiple ions or osmolarity (i.e., not necessarily the associated anion). These findings with *C. dubia* indicate the need to reexamine drivers of acute effects attributable to various major ion salts. In the present study, we report acute toxicity of various major ion salts in terms of both anion and cation concentrations, but a subsequent article will be devoted to more explicitly evaluating likely causes of toxicity to this species in major ion salt toxicity tests.

The primary goal of the present study was to determine how changes in ionic composition of dilution water impact responses of *N. triangulifer* to elevated major ions over a range of background water quality conditions. Building on the work of Mount et al. (2016) with *C. dubia*, we investigated how changes in hardness and, more specifically, changes in Ca impact acute toxicity of several major ion salts. We also tested the reciprocal influences of Na on KCl toxicity and K on NaCl toxicity.

METHODS

Culturing of test organisms and food

The parthenogenetic mayfly *N. triangulifer* (family Baetidae; McDunnough 1931) was originally described as *Cloeon triangulifer*, later transferred to the genus *Centroptilum* (McCafferty and Waltz 1990), and most recently assigned to *Neocloeon* (Jacobus and Wiersema 2014). The genetic strain we used was Stroud Water Research Center Clone #WCC-2^{TM}. It is reared in the laboratory on a diatom diet, and mayfly and diatom biofilm diet culturing methods were similar to those reported in Soucek and Dickinson (2015). Diatoms used to feed mayflies included *Mayamaea* sp., and *Nitzschia* sp. Both diatoms were obtained from Carolina Biological Supply, sold as *Navicula* sp. and *Synedra* sp., respectively. We had the genus-level identities taxonomically confirmed by an expert (S. Decelles) at the US Environmental Protection Agency (USEPA), Office of Research and Development (Cincinnati, OH).

Mixed diatom stocks. To culture diatoms, we autoclaved (30 min at 121 °C, liquid cycle) a 4-L flask containing 4 L of filtered (Whatman^{TM} 934-AH) dechlorinated tap water and a 2-inch long Teflon®-coated stir bar. After allowing to cool, a sterile technique was used to add 1.3 mL of Kent®–Proculture Professional F/2 Algal culture formula A, 1.3 mL of Kent®–Proculture Professional F/2 Algal culture formula B, 150 mg of sodium metasilicate ($Na_2SiO_3 \times 9H_2O$), and 200 mL of fresh diatom stock solution (just removed from the stir plate). Both diatom species were present in combination in stock cultures. The flasks were placed on stir plates with moderate to fast stirring (a large vortex was visible) in an environmental chamber set for a 16:8-h light:dark photoperiod and 25 °C. Light intensity in the chamber varied between 800 and 1200 lux depending on position in the chamber. Diatom stocks were allowed to grow for 5 d, then 200 mL of stock was used to seed the next flask and cages for mixed diatom slides (see *Mixed diatom slides* section). Allowing growth for much more than 5 d appeared to cause depletion of nutrients and cell death. Stocks were not refrigerated prior to seeding subsequent flasks or mixed diatom slide cages.

Mixed diatom slides. To culture mixed diatom slides, 15 fully frosted microscope slides (catalog no. 12-544-5CY, Fisher Scientific) were placed in a single layer (with frosted side facing up) on the bottom of a 7.2-L (189 × 297 × 128 mm) autoclavable polysulfone mouse cage (no. PC7115HT, Allentown, Inc., Allentown, PA) filled with 2.5 L of filtered (Whatman 934-AH) dechlorinated (carbon-filtered and aged) tap water. The container with the slides was autoclaved (30 min at 121 °C, liquid cycle) and allowed to cool. A sterile technique was used to add 1.3 mL of Kent–Proculture Professional F/2 Algal culture formula A, 1.3 mL of Kent–Proculture Professional F/2 Algal culture formula B, 150 mg of sodium metasilicate ($Na_2SiO_3 \times 9\ H_2O$; dissolved in a small amount of deionized water prior to addition), and 200 mL of fresh (never refrigerated) mixed diatom stock. The container with slides was covered with clear plastic wrap and placed in an environmental chamber set for a 16:8-h light: dark photoperiod and 25 °C. Light intensity at the level of the slides varied between 300 and 100 lux, depending on position in the chamber. Growth was allowed for 6 to 10 d. Soucek and Dickinson (2015) provided further details on assessing biofilm quality prior to feeding to mayflies.

Mayfly nymph rearing method. Mayflies were reared in an environmental chamber at 25 °C, a 16:8-h light: dark photoperiod, and light intensity of approximately 200 lux. Culture water was a reconstituted water (hereafter referred to as Duluth 100) with a nominal hardness of 100 mg/L as $CaCO_3$, prepared according to a formula developed at the USEPA laboratory in Duluth, Minnesota (Supplemental Data, Tables S1 and S2). This water recipe was designed with the goal of better mimicking the chemistry of "typical" North American freshwaters relative to other commonly used reconstituted waters. When eggs hatched, approximately 250 mL of culture water were added to a 300-mL clear glass jar. All water was filtered using Whatman #934-AH glass microfiber filters. One mixed diatom slide was added to the jar. Newly hatched mayfly larvae (hundreds to thousands) were then added to the jar, the lid was loosely replaced, and the jar was covered with aluminum foil to block direct overhead lighting. When mayflies were 4 to 8 d old (usually 6 or 7 d), 40 individuals were placed in a 1-L glass beaker containing 400 mL Duluth 100 reconstituted water and fed as described for the 300-mL glass jar. The diatom slide was placed in the beaker prior to adding mayflies, to avoid injury. Again, the container was covered with aluminum foil to block direct overhead lighting. When mayflies were 11 to 12 d old, 20 individuals were transferred to a 19 × 24 × 6.5–cm Pyrex casserole dish containing 1.5 L of Duluth 100 water and 5 mixed diatom slides. Slides were replaced when diatom biofilms were depleted, and water was changed twice per week or more if water appeared to be littered with loose diatoms and waste products. The container was covered loosely with foil. Using this method, aeration was not necessary at any point during mayfly culturing.

When preemergent nymph stages (determined by the presence of black wing pads) appeared (days 20–23), they were placed in a 300-mL I-chem jar containing culture water and a mixed diatom slide. A screened cover was placed on the jar to allow for emergence of subimagoes and molting to imago stage (within 24 h after preemergent nymph stage). To induce the imago to release its eggs, we held it by the wings with forceps and touched its abdomen to water held in a small Petri dish. This procedure was conducted with the aid of a dissecting microscope. Eggs were then pipetted into a scintillation vial; when possible, eggs of 3 females were combined in each vial. Eggs were either allowed to hatch or placed in an environmental chamber at 10 °C for later use.

General acute toxicity testing procedures

Static, nonrenewal, acute toxicity tests were conducted according to guidelines detailed in ASTM International (2014). Five concentrations spaced by 50% dilution were tested in addition to controls, with the highest test concentration made by adding the salt of interest to the dilution water of interest. Further details on test conditions are provided in Supplemental Data, Table S3. Eggs were transferred to test water prior to hatching so that organisms hatched into the water in which they would be tested, and organisms were <24 h old at the beginning of a test. Test chambers were fed by grasping a mixed diatom slide (cultured as described in the section *Mixed diatom slides*) with a forceps, scraping off an area of biofilm of approximately 5 to 10 × 25 mm with another clean microscope slide, and releasing the biofilm into each replicate test chamber. Test organisms were fed because in a previous study first-instar mayflies had 22% survival after 48 h with no food (Soucek and Dickinson 2015). This has been observed by others as well (Struewing et al. 2015; J. Jackson, Stroud Water Research Center, personal communication). Because all tests were conducted with major ions, food was not expected to impact dissolved concentrations of the contaminants, and analytical chemistry confirmed this (detailed in the *Quality assurance/quality control* section). Food was added to test chambers only on day 0 because previous testing demonstrated that one biofilm scraping was more than enough for the 96-h test duration. Mortality was assessed daily using a dissecting microscope. Individuals were considered dead if they did not respond to gentle prodding with a probe.

Temperature, pH, conductivity, and dissolved oxygen were measured in all treatments at the beginning and the end of each exposure period. Alkalinity and hardness were measured in the controls and the highest test treatment at the beginning of the test only. The pH measurements were made using an Accumet® (Fisher Scientific) model AB15 pH meter equipped with an Accumet gel-filled combination electrode (accuracy <±0.05 pH at 25 °C). Dissolved oxygen was measured using an air-calibrated Yellow Springs Instruments model 55 meter. Conductivity measurements were made using a Mettler Toledo® (Fisher Scientific) model MC226 conductivity/total dissolved solids meter. Alkalinity and hardness were measured by titration as described by the American Public Health Association (2005). Water samples from each treatment were collected at the beginning and end of tests and submitted to the Illinois State Water Survey analytical laboratory for verification of selected major ion concentrations using ion chromatography.

Experimental approach and dilution waters

We developed a testing approach that covered 3 basic objectives, which are detailed in this section. Recipes for and detailed chemistry of the 9 different dilution waters used are provided in Supplemental Data, Tables S1 and S2. Reagent-grade or certified American Chemical Society–grade salts were used to make all test waters. Solutions enriched with $MgSO_4$ were prepared using $MgSO_4 \times 7H_2O$ or $MgSO_4$ (anhydrous) but are reported as $MgSO_4$.

Hardness comparisons. The first set of tests determined the toxicity NaCl, Na_2SO_4, K_2SO_4, and $MgSO_4$ in dilution waters representing different hardnesses. Waters with nominal hardnesses of 30, 90, 150, and 210 mg/L (as $CaCO_3$) were prepared using recipes developed at the USEPA laboratory in Duluth, Minnesota, which varied all major ions to approximate average concentrations in US surface waters of a given hardness (Supplemental Data, Tables S1 and S2). These test waters differed from culture water (Duluth 100) in that $CaCO_3$ was the main source of carbonate rather than $NaHCO_3$. Carbon dioxide gas (99.9% CO_2) was bubbled through the solution (to pH ~5.2) to dissolve $CaCO_3$, then natural air was bubbled through the solution to bring the pH back to approximately 7.6 for testing.

Ca to Mg comparisons. While hardness tests evaluated the aggregate effect of varying all ions in dilution water, additional tests varying Ca to Mg ratios at constant hardness were used to evaluate the specific role of calcium. Toxicity of NaCl, Na_2SO_4, K_2SO_4, and $MgSO_4$ were tested in waters similar to Duluth 100 water but modified to "low Ca to Mg" (0.5 ratio on a mass basis) and "high Ca to Mg" (5.0 mass ratio) compositions with all other ions held constant (Supplemental Data, Tables S1 and S2). The "Ca to Mg" terminology is in reference to the original dilution water, so in the case of the Mg salt, the Ca to Mg ratio was in reality different from treatment to treatment because of the added Mg. From a practical perspective it was primarily Ca in the dilution water that was being altered.

Na/K interactions. The reciprocal influences of Na on KCl toxicity and K on NaCl toxicity were tested by comparing responses in Duluth 100 water (which served as a low-Na or low-K water) to responses in Duluth 100 water with high Na (150.3 mg Na/L added as NaCl) or high K (15.0 mg K/L added as KCl; see Supplemental Data, Table S2). These comparisons were spurred by the finding of Na-dependent toxicity of KCl in *C. dubia* (Mount et al. 2016) and K-dependent toxicity of Na_2SO_4 in fathead minnows (Wang et al. 2016).

Data analysis

For each acute toxicity test, the dominant anion concentrations were analytically verified in each treatment as described in the *General acute toxicity testing procedures* section. We calculated LC50s for each test in terms of measured dominant anion concentration (milligrams per liter) using the trimmed Spearman-Karber method (Hamilton et al. 1977). Then, based on measured anion concentration, calculated concentration of the associated cation (milligrams per liter), and nominal concentrations of other ions in the dilution water, we estimated all major ion concentrations (millimoles) at the LC50 value for each test (Table 1; Supplemental Data, Table S4). We then used Visual MINTEQ, Ver 3.0 (JP Gustafsson, Royal Institute of Technology, Department of Land and Water Resources Engineering, Stockholm, Sweden) to calculate the activity of all potential ionic species at the LC50 concentration for each test (Supplemental Data, Table S5). For activity modeling, we input fixed pH values based on the average measured pH for each test. Calcium was the most frequently manipulated major ion in dilution waters in the present study, and previous studies with *C. dubia* indicated that Ca influenced toxicity of Na and Mg salts (Mount et al. 2016). Therefore, to evaluate the influence of Ca on major ion toxicity, we created scatter plots with LC50s in terms of Cl, SO_4, Na, Mg, or K activities as dependent variables and Ca activity as the independent variable. For each dependent variable ion, we included all salts tested, for example, for SO_4 LC50s, we included K2SO4, $MgSO_4$, and Na_2SO_4. We also plotted K salt LC50s against Na activity as the independent variable because Na regulated K salt toxicity to *C. dubia* (Mount et al. 2016).

Quality assurance/quality control

Control survival was 100% in the majority of the acute toxicity tests reported and always ≥90%.

Across all acute toxicity tests conducted, the mean (± standard deviation) overall temperature, pH, and dissolved oxygen values were 25.0 ± 0.4 °C, 8.2 ± 0.3 standard units, and 7.7 ± 1.3 mg/L, respectively. The lowest dissolved oxygen concentration observed in any test was 6.4 mg/L. Specific conductivity, alkalinity, and hardness varied depending on dilution water and test treatment but were consistent with the nominal composition of the treatment.

For the acute toxicity tests with chloride salts, measured Cl^- concentrations averaged 104% of nominal (range, 92–118%). For the acute toxicity tests with sulfate salts, measured SO_4^{2-}concentrations averaged 103% of nominal (range 87–124%). Measured concentrations were used for calculating LC50s, and reported cation concentrations were calculated proportionally based on measured concentrations of anions.

RESULTS AND DISCUSSION

Hardness comparisons

When tested across dilution waters with different hardness, NaCl, Na_2SO_4, and $MgSO_4$ all decreased in toxicity with increasing hardness, though to varying degrees (Table 1 and Figure 1). For

TABLE 1: Median lethal concentrations at 96 h for *Neocloeon triangulifer* for single major ion salts in various dilution waters

Salt	Dilution water	Measured hardness (mg/L as $CaCO_3$)	LC50 (95% CI) (mg anion/L)	LC50[a] (mg cation/L)
Hardness comparisons				
NaCl	Recon 30	30	490 (424–566)	319
NaCl	Recon 90	88	837 (706–993)	545
NaCl	Recon 150	141	1128 (1059–1201)	735
NaCl	Recon 210	205	1116 (929–1341)	732
Na_2SO_4	Recon 30	31	728 (614–863)	349
Na_2SO_4	Recon 90	88	1338 (1166–1535)	639
Na_2SO_4	Recon 150	142	1758 (1511–2045)	837
Na_2SO_4	Recon 210	210	1822 (1583–2098)	865
K_2SO_4	Recon 30	30	1017 (887–1166)	821
K_2SO_4	Recon 90	87	1070 (918–1248)	854
K_2SO_4	Recon 150	141	1024 (857–1224)	803
K_2SO_4	Recon 210	205	1808 (1580–2069)	1418
K_2SO_4	Recon 210	212	1261 (1107–1437)	973
$MgSO_4$	Recon 30	28	1348 (1231–1478)	341
$MgSO_4$	Recon 90	86	1621 (unreliable)	411
$MgSO_4$	Recon 150	140	2112 (1814–2460)	536
$MgSO_4$	Recon 210	212	1836 (1571–2145)	463
Ca:Mg comparisons				
NaCl	Low Ca:Mg	95	905 (728–1125)	603
NaCl	High Ca:Mg	93	1086 (913–1293)	721
Na_2SO_4	Low Ca:Mg	94	1229 (1022–1477)	595
Na_2SO_4	High Ca:Mg	93	1427 (1211–1682)	689
K_2SO_4	Low Ca:Mg	95	1017 (789–1312)	785
K_2SO_4	High Ca:Mg	93	1164 (1062–1275)	904
$MgSO_4$	Low Ca:Mg	95	872 (758–1002)	224
$MgSO_4$	High Ca:Mg	94	1436 (1152–1790)	355
Na/K interactions				
KCl	Low Na (Duluth 100)	93	1226 (1043–1441)	1326
KCl	High Na	92	1221 (984–1516)	1123
NaCl	Low K (Duluth 100)	92	910 (719–1153)	606
NaCl	Low K (Duluth 100)	94	1153 (968–1374)	764
NaCl	High K	91	1012 (834–1228)	666

[a] Dilution water composition was considered in estimating cation concentration at LC50.
CI = confidence interval; LC50 = median lethal concentration.

both NaCl and Na_2SO_4, the LC50 more than doubled as hardness increased from approximately 30 to 150 mg/L (nominal hardnesses), whereas for $MgSO_4$ the increase was approximately 1.5-fold. Despite this rise, all 3 of these salts showed little difference in toxicity between 150 and 210 mg/L hardness. For K_2SO_4, the pattern was markedly different, with little difference among the 30, 90, and 150 mg/L waters. For the 210 hardness water, the initial test resulted in an LC50 of 1808 mg Cl/L, roughly 1.8 times above that in the lower 3 hardnesses. Because this pattern was unexpected, the test at 210 mg/L hardness was repeated, yielding an LC50 of 1261 mg Cl/L, much closer to the lower 3 (Table 1). Therefore, it is unclear whether K_2SO_4 toxicity is hardness-dependent, but it appears to be less so than the other 3 salts.

Hardness-dependent toxicities of NaCl and Na_2SO_4 have been reported for several other species previously. For NaCl, *C. dubia* (Soucek et al. 2011; Mount et al 2016), the snail *Gyraulus parvus* (Soucek et al. 2011), the unionid mussel *L. siliquoidea* (Gillis 2011), and the worm *Tubifex tubifex* (Soucek et al. 2011) have been observed to exhibit hardness-dependent acute sensitivity. In addition, Stroud Water Research Center (J. Jackson, personal communication) documented decreased NaCl toxicity to the mayflies *N. triangulifer*, *Procloeon rivulare*, and *Pseudocloeon frondale* in 48-h toxicity tests using a natural water with a hardness of 105 mg/L relative to a natural water with a hardness of 22 mg/L. Likewise for Na_2SO_4, hardness-dependent sensitivity has been observed for *C. dubia* (Soucek and Kennedy 2005; Mount et al. 2016), *D. magna* (Davies and Hall 2007), and *Hyalella azteca* (Davies and Hall 2007; Soucek 2007).

Ca to Mg comparisons

In the dilution waters used for hardness comparisons, all of the major ions increased with increasing hardness, not just Ca and Mg (Supplemental Data, Table S2). This was done purposefully to examine trends in waters that simulate how toxicity might vary in natural waters for which concentrations of all major ions generally covary with increasing hardness. Research on other organisms has suggested that the ameliorative effect of hardness is largely attributable to increased Ca specifically (Davies and Hall 2007; Mount et al. 2016); to evaluate this relationship for *N. triangulifer*, we tested the toxicity of the same 4 salts (NaCl, Na_2SO_4, K_2SO_4, and $MgSO_4$) in dilution waters with constant nominal hardness but different Ca to Mg ratios. Both waters had measured hardnesses of 93 to 95, but the low Ca to Mg ratio water had a Ca concentration similar to that in

FIGURE 1: Influence of Ca in dilution water on the acute toxicity of (**A**) NaCl, (**B**) Na_2SO_4, (**C**) K_2SO_4, and (**D**) $MgSO_4$ to the mayfly *Neocloeon triangulifer*. "All ions manipulation" are data from the "hardness comparisons" series of tests in the present study; Ca concentrations of 8, 25, 41, and 58 mg/L were waters with 30, 90, 150, and 210 mg/L as $CaCO_3$ nominal hardness, respectively. Error bars are 95% confidence limits. CL = confidence limits; LC50 = median lethal concentration.

the Recon 30 water (nominally 9.3 mg Ca/L), whereas Ca in the high Ca to Mg ratio water was more than 3 times higher (30.3 mg Ca/L), slightly higher than that in Recon 90 (Supplemental Data, Table S2). If the variations in LC50s in the "hardness comparisons" series were attributable only to Ca, we would expect shifts in LC50 from the Ca to Mg manipulation paralleling those in the hardness comparison studies.

In the case of the 2 sodium salts, the actual differences in LC50 from low Ca to Mg ratio to high Ca to Mg ratio were less than predicted by the "hardness comparison" tests (Table 1 and Figure 1). The fact that their LC50s increased less when manipulating only Ca to Mg rather than all ions suggests either that something aside from or in addition to calcium accounted for the decreased toxicity in the "hardness comparisons" series or that the higher overall ion concentrations in the low Ca to Mg water relative to 30 hardness water provided some benefit (Supplemental Data, Table S2). Conversely, the $MgSO_4$ results showed a similar increase in LC50 when only calcium was increased and when all ions increased proportionally as in the hardness comparison (Table 1 and Figure 1); this suggests that calcium accounted for most or all of the modification of toxicity attributable to hardness. The K_2SO_4 results were consistent with the previous tests in the hardness series, showing little, if any, effect of Ca or hardness on toxicity over the range present in the lower 3 hardness waters (Table 1 and Figure 1). The hardness/Ca dependence of toxicity for Na and Mg salts but not K salts, shown in the present study for *N. triangulifer*, is consistent with the findings of Mount et al. (2016) for *C. dubia*.

Na/K interactions

In the Na/K interactions series, we observed essentially no effect on KCl toxicity when Na varied from 34 mg/L (low Na = Duluth 100; Supplemental Data, Table S2) to 150 mg/L (high Na; Supplemental Data, Table S1 and S2), with 96-h LC50s of 1226 and 1221 mg Cl/L, respectively (Table 1). Likewise, the mean LC50 for the 2 NaCl tests conducted at low K (3.9 mg/L) was 1032 mg Cl/L, whereas the LC50 at high K (15 mg/L) was 1012 mg Cl/L (Table 1). The lack of influence of Na on the toxicity of KCl contrasts with the marked ameliorative effect of Na on KCl toxicity reported for *C. dubia* (Mount et al. 2016), whereas the lack of influence of K on toxicity of NaCl is parallel with that found for *C. dubia*.

Ion activities

For all of the tests in the present study, we calculated LC50s and dilution water compositions in terms of activity (millimoles; Supplemental Data, Table S5) to account for the fact that, in more concentrated solutions, ions behave as though their concentrations are lower than their total concentrations (Snoeyink and Jenkins 1980). The importance of considering activity in expressing exposure to major ion salts was further affirmed in analyses for *C. dubia* (Mount et al. 2016). Determining specific causes of toxicity (e.g., anions, cations, osmolarity) in major ion salt tests was beyond the scope of the present study, but Erickson et al. (2016) suggested that the toxicities of K, Mg, and Ca to *C. dubia* appear to be attributable

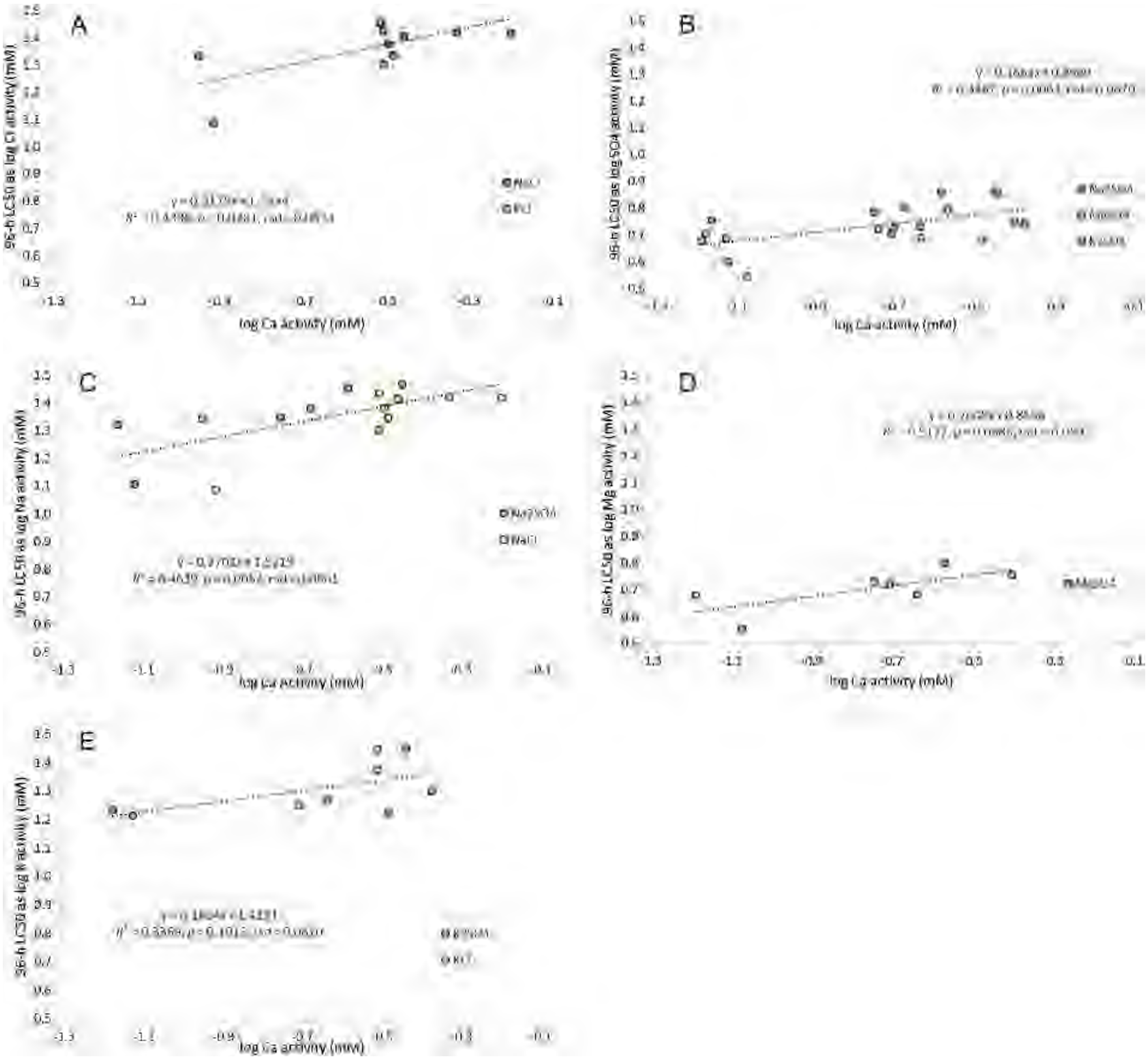

FIGURE 2: Influence of Ca activity on 96-h median lethal concentrations of various major ion salts for the mayfly *Neocloeon triangulifer* expressed as logs of (A) chloride activity, (B) sulfate activity, (C) sodium activity, (D) magnesium activity, and (E) potassium activity. LC50 = median lethal concentration; rsd = residual standard deviation.

to the cations rather than anions and that Na salt toxicity may be related to multiple ions or osmolarity (i.e., not necessarily the associated anion). We report toxicity data for the mayfly in terms of both anion and cation activities plotted against Ca activity because Ca was the most frequently manipulated major ion in dilution waters and was shown to be a primary influence on toxicity of Na and Mg salts to *C. dubia* (Mount et al. 2016).

As shown in Figure 2, the 96-h LC50s for Cl, SO_4, and Na were significantly positively correlated with Ca activity in the dilution water, whereas correlations for Mg and K with Ca were insignificant ($p > 0.05$). Although not statistically different from zero because of the inclusion of fewer tests, the slope for the Mg data was similar to those of the other ions and greater than the significantly positive slope for SO_4, which included more than double the number of tests. The chloride plot had the steepest slope, followed closely by Na. Mount et al. (2016) reported on the influence of Ca on Na salt toxicity (as Na activity) for *C. dubia* (Figure 3), but that study tested waters with substantially lower Ca concentrations than the present study did (as low as 0.04 mM compared to our lowest value of 0.2 mM). Much of the steepness in the curve in the *C. dubia* study (Mount et al. 2016) was below 0.2 mM Ca. Over the comparable range of Ca activities, the responses of the 2 organisms are similar; and if we had tested *N. triangulifer* in waters with even lower Ca concentrations, we might have observed a similarly stronger Ca dependence of Na toxicity. It is notable that in the case of the *C. dubia* data (Mount et al. 2016; Figure 3), there is distinct separation on the *y* axis between NaCl and Na_2SO_4; this was not observed for the mayfly.

Although the Ca slope for Mg activity was not statistically separable from zero, the Mg data for mayfly are generally consistent with the Ca slope found for *C. dubia* (Mount et al. 2016); and, as for the Na salts, the strongest effect of Ca on Mg salt toxicity

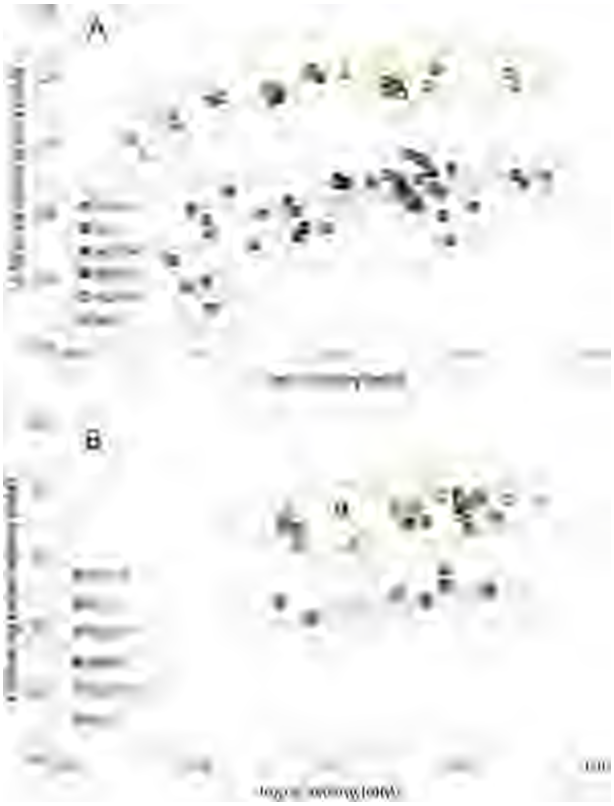

FIGURE 3: Influence of Ca activity on acute median lethal concentrations (LC50s) of various major ion salts for the (**A**) *Ceriodaphnia dubia* (from Mount et al. 2016) and (**B**) the mayfly *Neocloeon triangulifer*. All LC50s expressed in terms of cation activity for the given salt. Trendlines in (**A**) are linear fits for *C. dubia* data over the range of Ca activities tested with *Neocloeon*. The longer dashed yellow line fits Na_2SO_4 data, whereas the finer dotted line fits NaCl data. The same *C. dubia* trendlines are then superimposed with the *Neocloeon* data in (**B**).

for *C. dubia* occurred at Ca activities lower than those tested in the present study for mayfly (Figure 3). Another interesting trend apparent in Figure 3 is that the toxicities of Na and K are quite similar for *N. triangulifer* with Mg being substantially more toxic. This contrasts sharply with data for *C. dubia* and fathead minnows, for which K and Mg salts were substantially more toxic than Na salts (Mount et al. 1997, 2016). Unpublished data from C. Ivey et al. (2013, poster presented at Society of Environmental Toxicology and Chemistry) indicate an even greater disparity between Na and K toxicity to freshwater mussels, with KCl being on the order of 50-fold more toxic than NaCl on a millimolar basis.

Figure 4 in addition plots K salt LC50s against Na activity in dilution water for comparison with the finding of Mount et al. (2016) that Na regulates K salt toxicity to *C. dubia*. There is little slope evident in the data for the mayfly, further confirming our conclusion that in the case of this species Na appears to have little influence on K salt toxicity.

Sensitivity of N. triangulifer *relative to other organisms*

For the sodium salts, the range of LC50s we observed for *N. triangulifer* indicate that it is acutely sensitive relative to other species. The acute toxicity of NaCl in waters of 80 to 100 mg/L hardness has been reported for many aquatic species. The chloride LC50 for *N. triangulifer* (837 mg Cl/L at nominal hardness of 90; Table 1) in the present study is lower than values for 11 other species tested in this hardness range including cladocerans (*C. dubia*, *Daphnia ambigua*, *D. magna*; Mount et al. 1997; Harmon et al. 2003; Elphicket al. 2011a; Soucek et al. 2011), a mussel (*L. siliquoidea*; Gillis 2011), a rotifer (*Brachionus calyciflorus*; Elphick et al. 2011a), an amphipod (*H. Azteca*; Elphick et al 2011a; Soucek et al. 2013), 2 worms (*Lumbriculus variegatus* and *Tubifex tubifex*; Elphick et al. 2011a; Soucek et al. 2011), an insect (*C. dilutes*; Elphick et al. 2011a), and 2 fish (*Pimephales promelas* and *Oncorhynchus mykiss*; Mount et al. 1997; Elphick et al. 2011a). Notably, the mayfly was more sensitive than *C. dubia*, for which there are a number of published LC50s in this hardness range (average LC50 ~1138 mg Cl/L; Mount et al. 1997, 2016; Harmon et al. 2003; Elphick et al. 2011a; Soucek et al. 2011). Struewing et al. (2015) and Stroud Water Research Center (J. Jackson, personal communication) have reported NaCl LC50s for *N. triangulifer* previously, but those tests were 48 h in duration compared to the 96-h tests in the present study, so comparisons of effect levels with our data are tenuous. In the present study, 48-h LC50s were consistently approximately double the corresponding 96-h LC50s (data not shown). The first molt for the organism occurs within this time frame and might account for the sharp difference in 48- and 96-h LC50s (D.J. Soucek, personal observation). The glochidia of 3 mussel species (*Lampsilis fasciola*, *Epioblasma torulosa*, and *Lampsilis cardium*) have been found to be more sensitive than *N. triangulifer* (LC50s ranging 179–817 mg Cl/L; Gillis 2011).

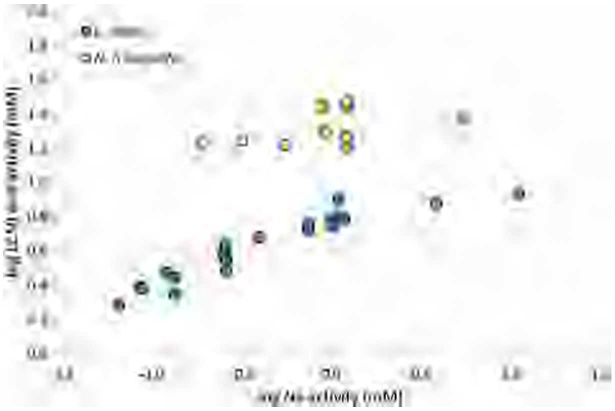

FIGURE 4: Potassium salt median lethal concentrations for the mayfly *Neocloeon triangulifer* and the cladoceran *Ceriodaphnia dubia* (from Mount et al. 2016) as a function of Na activity in dilution water. LC50 = median lethal concentration.

For Na_2SO_4, the LC50 for *N. triangulifer* at hardness 90 mg/L in the present study (1338 mg SO_4/L; Table 1) was lower than values for the 7 other species for which we found data at that approximate hardness: *H. azteca*, *Sphaerium simile*, *D. magna*, *P. promelas*, *C. dilutus*, *L. abrupta*, and *C. dubia* (Mount et al. 1997, 2016; Soucek and Kennedy 2005; Davies and Hall 2007; Soucek 2007; Wang et al. 2016). Reported LC50s for these species ranged from 1874 to 14 134 mg/L. Goetsch and Palmer (1997) generated an LC50 of 500 mg SO_4/L for the mayfly *Tricorythus* sp.

at a lower hardness (~69 mg/L), confirming the sensitivity of mayflies to Na_2SO_4. There is a published data point at a similar hardness for *H. azteca* of 512 mg SO_4/L (Soucek and Kennedy 2005), but the dilution water for that toxicity test had a chloride concentration lower than that considered sufficient for optimal health for that species (Soucek et al. 2015). Thus, in general, *N. triangulifer* appears to be relatively sensitive to sodium salts, especially sodium sulfate. There have been several studies published on the chronic responses of *N. triangulifer* to NaCl and Na_2SO_4 in a range of dilution waters, and these were reviewed by Soucek and Dickinson (2015).

Compared with the sodium salts, far fewer published data are available on the acute toxicity of Mg and K salts to other species (Mount et al. 1997, 2016; Van Dam et al. 2010). The Mg salt toxicity data for *C. dubia*, *D. magna*, and *P. promelas* reported by Mount et al. (1997), with LC50s ranging from 224 to 569 mg Mg/L, are similar to those reported in the present study for the mayfly at a similar hardness (224–411 mg Mg/L at hardness ~90 mg/L; Table 1). Conversely, *N. triangulifer* was substantially less sensitive to K than the 3 species tested by Mount et al. (1997), the former having LC50s ranging from 785 to 1326 mg K/L at hardness ~90 mg/L (Table 1) and the latter having LC50s of <305 to 462 mg K/L. Van Dam et al. (2010) tested the responses of a variety of Australian freshwater species from an area with background water containing extremely low Ca to $MgSO_4$, with most having median effect concentrations between 4.4 and 63 mg Mg/L (the exception being *Chlorella* at 1215 mg Mg/L). The lower values from that study are approximately an order of magnitude lower than those reported in the present study (Table 1), but the dilution water in the Van Dam et al. (2010) study had very low Ca (<0.8 mg/L), which could account for the difference given the effect of Ca on Mg toxicity observed in the present study.

CONCLUSIONS

In summary, the mayfly was in general relatively sensitive to NaCl, $MgSO_4$, and especially Na_2SO_4 compared with other species reported in the literature but relatively less so to K salts. Sodium and magnesium salt toxicity decreased with increased hardness, whereas the K salt toxicity did not, consistent with findings for other species. However, in Ca to Mg manipulations, it is not clear whether Ca activity alone can account for all of the "hardness" effect. Over the range of Ca activity tested for the mayfly, the slopes of the Ca effect on toxicity of Na and Mg salts to mayfly were similar to those reported elsewhere for *C. dubia*, though it is unclear whether the steeper slopes reported for *C. dubia* and lower Ca activities would also occur for *N. triangulifer*. Two other key differences between these 2 species were 1) that K salt toxicity to *N. triangulifer* was not modified by Na in dilution water, and 2) that K toxicity to mayflies was very similar to Na toxicity on a millimolar basis, instead of the much greater toxicity of K observed for *C. dubia*.

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Data availability—Data are available from the corresponding author (soucek@illinois.edu).

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Calculation of Aquatic Life Criteria for Chloride

These calculations are based on “Summary of Data Concerning the Acute Toxicity of Sodium Chloride to Aquatic Animals” dated 1-15-09 and “Summary of Data Concerning the Chronic Toxicity of Sodium Chloride to Aquatic Animals” dated 1-15-09. Except as noted (for example, see footnote a), these calculations are consistent with the 1985 Guidelines. GMAVs and SMAVs are normalized to hardness = 300 mg/L and sulfate = 65 mg/L. GMAVs and SMAVs are expressed as mg chloride/L.

Rank*	GMAV	Genus	Species	SMAV	SMACR
	-----	Agria	Damselfly, Agria sp.	-----	
23	17161	Anquilla	American eel, Anquilla rostrata	17160.6	
	16203	Cambarus	Crayfish, Cambarus sp.	16203.2	
	14897	Fundulus	Plains killifish, Fundulus kansae	14897.1	
	14843	Libellulidae**	Dragonfly, Libellulidae	14843.4	
	13453	Gasterosteus	Threespine stickleback, Gasterosteus aculeatus	13452.6	
	-----	Carassius	Goldfish, Carassius auratus	-----	
	9933	Gambusia	Mosquitofish,	9933.4	

		Gambusia affinis		
9157	Lepomis	Green sunfish, Lepomis cyanellus	9974.9	
		Bluegill, Lepomis macrochirus	8406.5e	
8971	Notropis	Red shiner, Notropis lutrensis	8971.1	
8043	Oncorhynchus	Rainbow trout, Oncorhynchus mykiss	8042.6	7.308
-----	Chironomus	Midge, Chironomus attenuatus	-----	
7442	Ameiurus	Black bullhead, Ameiurus melas	7442.4	
-----	Erpobdella	Leech, Erpobdella punctata	----	
6515	Pimephales	Fathead minnow, Pimephales promelas	6515.3f	15.17h
6219	Tubifex	Tubificid worm, Tubifex tubifex	6218.6	
5078	Hyalella	Amphipod, Hyalella azteca	5077.7	
-----	Asellus	Isopod,	-----	

		Asellus communis	
-----	Limnodrilus	Tubificid worm, Limnodrilus hoffmeisteri	-----
-----	Helisoma	Snail, Helisoma campanulata	-----
4686	Pseudacris	Chorus frog, Pseudacris sp.	4686.0
-----	Gammarus	Amphipod, Gammarus pseudolimnaeus	-----
-----	Crangonyx	Amphipod, Crangonyx sp.	-----
-----	Nemoura	Stonefly, Nemoura trispinosa	-----
-----	Lepidostoma	Caddisfly, Lepidostoma sp.	-----
-----	Parapsyche	Caddisfly, Parapsyche sp.	-----
3946	Diaptomus	Copepod, Diaptomus clavipes	3946.1
3891	Lirceus	Isopod, Lirceus fontinalis	3890.7
3728	Gyraulus	Snail,	-----

			Gyraulus circumstriatus		
			Snail, Gyraulus parvus	3727.7	
	3350	Physa	Snail, Physa gyrina	3350.0	
			Snail, Physa heterostropha	-----	
	3086	Villosa	Mussel, Villosa delumbis	3821.1	
			Mussel, Villosa iris	2491.6	
4	2835	Lampsilis	Mussel, Lampsilis fasciola	2907.1	
			Mussel, Lampsilis siliquoidea	2764.4	
3	2326	Daphnia	Cladoceran, Daphnia ambigua	1649.7	4.148
			Cladoceran, Daphnia magna	3773.1d	1.974
			Cladoceran, Daphnia pulex	2020.5g	3.952
2	1542	Ceriodaphnia	Cladoceran,	1542.3c	>2.470i

			Ceriodaphnia dubia	
1	1128	Sphaerium	Fingernail clam, Sphaerium simile	1127.9
			Fingernail clam, Sphaerium tenue	-----

* A “greater than” acute value for the brown trout (Salmo trutta) is not in this table because it is too low to be a useful “greater than” value.
** Name of family, not name of genus.

a. Section IV.I of the 1985 Guidelines says: “For each species for which at least one acute value is available, the Species Mean Acute Value should be calculated as the geometric mean of the results of all flow-through tests in which the concentrations of test material were measured. For a species for which no such result is available, the SMAV should be calculated as the geometric mean of all available acute values, i.e., results of flow-through tests in which the concentrations were not measured and results of static and renewal tests based on initial concentrations (nominal concentrations are acceptable for most test materials if measured concentrations are not available) of test material.” The guidance presented in section IV.I of the 1985 Guidelines seems inappropriate for chloride because chloride is different from most pollutants for which aquatic life criteria are derived. Chloride is very soluble in water, does not oxidize or reduce, is not volatile, does not degrade, does not sorb to test chambers, test organisms, food, or waste products, is not complexed by materials that commonly occur in water, is not involved in a pH-dependent equilibrium in water, and does not precipitate in waters in which aquatic organisms commonly occur.

   i. For chloride, as long as the concentration of dissolved oxygen is sufficiently high, it seems appropriate to give static and renewal acute tests the same weight as flow-through acute tests in the derivation of the SMAV for a species.

   ii. For chloride, it seems inappropriate to give measured acute tests a weight of 1 and unmeasured acute tests a weight of 0 when both are available for the derivation of the SMAV for a species. For example, if there is a choice between one measured acute test on chloride and three unmeasured acute tests in three different laboratories, the three tests are probably preferable to the one test, but if the choice is between one measured acute test and two unmeasured acute tests in two different laboratories, the one test is probably preferable. Thus, for a species for which both measured and unmeasured acute tests are available for chloride, it seems appropriate to give measured acute tests a weight of 2.5 and unmeasured acute tests a weight of 1 when the SMAV is calculated.

   The conclusions described above concerning chloride were developed during discussions among Charles Delos, Charles Stephan, and Glen Thursby. For other pollutants, different conclusions concerning the relative merits of static, renewal, and flow-through acute toxicity tests and the relative merits of measured and unmeasured acute toxicity tests are likely to be more appropriate.

b. For Ceriodaphnia dubia, the acute values from Hoke et al. (1992) are considered outliers. The geometric mean is 1468.1 for Mount et al. (1997), 1790.2 for U.S. EPA (1991), 1998.2 for WISLOH (2007), and 1457.3 for GLEC and INHS (2008).
SMAV = antilog ([log 1468.1 + log 1790.2 + log 1998.2 + log 1863.6 + 2.5(log 1311.1) + 2.5(log 1457.3)]/9) = 1542.3.

c. For Daphnia magna, the values of 3815.5 (Mount et al. 1997), <2785.1, (Anderson 1946), 2439.7 (Anderson 1948), and 3025.9 and 3357.4 (Biesinger and Christensen 1972) were not used. A geometric mean of 3906.7 was calculated from the limits given by Seymour et al. (1997). The geometric mean is 3208.8 for Hoke et al. (1992) and is 4054.2 for Davies and Hall (2007). The SMAV is 3773.1, which is the geometric mean of 3208.8, 5068.2, 3906.7, 2242.3, 4749.6, 3968.5, and 4054.2.

d. Bluegill: SMAV = antilog ([2.5(log 7702.3) + log 10461.6]/3.5) = 8406.5.

e. Fathead minnow: SMAV = antilog([log 2833.9 + 2.5(log 8665.1) + 2.5(log 8225.2) + log 5112.0 + log 5226.3]/8) = 6515.3.

f. Daphnia pulex: SMAV = antilog([2.5(log 1938.8) + log 2240.3]/3.5) = 2020.5.

g. Not used in calculations because, even though the acute and chronic tests were in the same document, different dilution waters were used in the tests.

h. The SMACR for Ceriodaphnia dubia is the geometric mean of 1.508, >3.841, and 2.601.

FAV = 1205 mg chloride/L

CMC = FAV/2 = 602.5 mg chloride/L

The five SMACRs (7.308, 4.148, 1.974, 3.952, and >2.438) that are available for use in calculations result in three GMACRs:

7.308	Oncorhynchus
3.187	Daphnia
>2.470	Ceriodaphnia

The 1985 Guidelines require ACRs for species in three different families, but Daphnia and Ceriodaphnia are in the same family. However, even though the ACR for the fathead minnow should not be used in calculations because the acute and chronic tests using the fathead minnow were performed in different dilution waters, the fathead minnow ACR can be considered a qualitative ACR and used to satisfy the MDRs because chloride is not likely to be complexed or sorbed or detoxified by organic or inorganic constituents of the dilution water.

The GMACRs for Oncorhynchus and Daphnia are consistent with the “greater than” GMACR for Ceriodaphnia and the GMACRs are within a factor of ten. Therefore, the Final ACR = 4.826, which is the geometric mean of the GMARCs for Oncorhynchus and Daphnia. This would give FCV = FAV/FACR = (1205 mg chloride/L)/4.826 = 249.7 mg chloride/L. However, this approach is contraindicated because the GMACRs (including the unused GMACR for Pimephales) indicate that the GMACR increases as the GMAV increases.

The GMACR for Daphnia is consistent with the “greater than” GMACR for Ceriodaphnia, so the GMACR for Daphnia can be used as the FACR. Therefore, FACR = 3.187 and FCV = FAV/FACR = (1205 mg chloride/L)/3.187 = 378.1 mg chloride/L.

CCC = FCV = 378.1 mg chloride/L.

The CMC and CCC given above are for hardness = 300 mg/L and sulfate = 65 mg/L. The equation that was used to normalize the acute values can be used to make the CMC and CCC dependent on hardness and sulfate. The resulting equations for the CMC and CCC are:

$$\begin{aligned} \text{CMC} &= (602.5\ \text{mg chloride/L})\ (\text{hardness}/300)^{0.205797}\ (\text{sulfate}/65)^{-0.07452} \\ &= (254.3\ \text{mg chloride/L})\ (\text{hardness})^{0.205797}\ (\text{sulfate})^{-0.07452} \end{aligned}$$

At hardness = 300 mg/L and sulfate = 65 mg/L, CMC = 602.5 mg chloride/L.

$$\begin{aligned} \text{CCC} &= (378.1\ \text{mg chloride/L})\ (\text{hardness}/300)^{0.205797}\ (\text{sulfate}/65)^{-0.07452} \\ &= (159.6\ \text{mg chloride/L})\ (\text{hardness})^{0.205797}\ (\text{sulfate})^{-0.07452} \end{aligned}$$

At hardness = 300 mg/L and sulfate = 65 mg/L, CCC = 378.1 mg chloride/L.

Derivation of an Alternative FCV

Even though the above derivation of FCV = 378.1 mg chloride /L follows the procedure described in the 1985 Guidelines, there is an alternative approach that is justified on the basis of the "good science" clause in section XII.B of the 1985 Guidelines. This approach is based on the fact that the four low SMACRs for chloride were obtained with invertebrates, whereas the high SMACR was obtained with a vertebrate. This can be interpreted to mean that vertebrates have a higher ACR, on the average, than invertebrates, especially because the qualitative ACR for the fathead minnow is 15.17. Therefore, a vertebrate ACR and an invertebrate ACR can be used with the GMAVs to calculate a predicted Genus Mean Chronic Value for each genus, and then a FCV can be calculated directly from the predicted GMCVs. This approach calculates and uses a predicted chronic value for each genus for which an acute value is available and probably does a better job of taking into account the chronic sensitivities of both vertebrates and invertebrates to chloride. The relevant data and calculations are presented on the next few pages.

The FACR of 3.187 derived above was derived from all of the acceptable ACRs for invertebrates. The only acceptable ACR for a vertebrate is 7.308. A predicted GMCV can be calculated from each GMAV by using 3.187 as the invertebrate ACR and using 7.308 as the vertebrate ACR.

Table of predicted GMCVs for Chloride

(GMAVs and pGMCVs are expressed as mg chloride/L)
(ranked according to predicted GMCVs)

Rank	GMAV	Genus	Species	pGMCV
	-----	Agria	Damselfly, Agria sp.	-----
23	16203	Cambarus	Crayfish, Cambarus sp.	5084
	14843	Libellulidae*	Dragonfly, Libellulidae	4657

17161	Anquilla	American eel, Anquilla rostrata	2348
6219	Tubifex	Tubificid worm, Tubifex tubifex	1951
-----	Chironomus	Midge, Chironomus attenuatus	-----
-----	Erpobdella	Leech, Erpobdella punctata	-----
13453	Gasterosteus	Threespine stickleback, Gasterosteus aculeatus	1841
14897	Fundulus	Plains killifish, Fundulus kansae	2038
-----	Carassius	Goldfish, Carassius auratus	-----
5078	Hyalella	Amphipod, Hyalella azteca	1593
----	Asellus	Isopod, Asellus communis	-----
-----	Limnodrilus	Tubificid worm, Limnodrilus hoffmeisteri	-----
-----	Helisoma	Snail, Helisoma campanulata	-----

8043	Oncorhynchus	Rainbow trout, Oncorhynchus mykiss	1101
9933	Gambusia	Mosquitofish, Gambusia affinis	1359
-----	Gammarus	Amphipod, Gammarus pseudolimnaeus	-----
-----	Crangonyx	Amphipod, Crangonyx sp.	-----
-----	Nemoura	Stonefly, Nemoura trispinosa	-----
-----	Lepidostoma	Caddisfly, Lepidostoma sp.	-----
-----	Parapsyche	Caddisfly, Parapsyche sp.	-----
3891	Lirceus	Isopod, Lirceus fontinalis	1221
9157	Lepomis	Green sunfish, Lepomis cyanellus	1253
		Bluegill, Lepomis macrochirus	
3350	Physa	Snail, Physa heterostropha	1051

		Snail, Physa gyrina	
3946	Diaptomus	Copepod, Diaptomus clavipes	1238
3086	Villosa	Mussel, Villosa delumbis	968.3
		Mussel, Villosa iris	
8971	Notropis	Red shiner, Notropis lutrensis	1228
3728	Gyraulus	Snail, Gyraulus circumstriatus	1170
		Snail, Guraulus parvus	
2835	Lampsilis	Mussel, Lampsilis fasciola	889.6
		Mussel, Lampsilis siliquoidea	
7442	Ameiurus	Black bullhead, Ameiurus melas	1018
6515	Pimephales	Fathead minnow, Pimephales promelas	891.5

4	2326	Daphnia	Cladoceran, Daphnia ambigua	729.8
			Cladoceran, Daphnia magna	
			Cladoceran, Daphnia pulex	
3	4686	Pseudacris	Chorus frog, Pseudacris sp.	641.2
2	1542	Ceriodaphnia	Cladoceran, Ceriodaphnia dubia	483.8
1	1128	Sphaerium	Fingernail clam, Sphaerium simile	353.9
			Fingernail clam, Sphaerium tenue	

* Name of family, not name of genus.

FCV based on predicted GMCVs = 382.7 mg chloride/L at hardness = 300 mg/L and sulfate = 65 mg/L.

CCC = (382.7 mg chloride/L) $(\text{hardness}/300)^{0.205797}$ $(\text{sulfate}/65)^{-0.07452}$
= (161.5 mg chloride/L) $(\text{hardness})^{0.205797}$ $(\text{sulfate})^{-0.07452}$

At hardness = 300 mg/L and sulfate = 65 mg/L, CCC = 382.7 mg chloride/L.

Summary of Data concerning the Chronic Toxicity of Sodium Chloride to Aquatic Animals

This summary is based on “Results of Literature Search concerning the Toxicity of Chloride to Aquatic Animals” dated 1-15-09. Except as noted, this summary is consistent with the 1985 Guidelines. All test results are expressed as mg chloride/L.

Fathead minnow

Birge et al. (1985) reported the results of a 33-day early life-stage test on sodium chloride in a stream water. The NOEC and LOEC were 252 and 352 mg/L, respectively, and the geometric mean was 298 mg/L. Birge et al. (1985) reported a 96-hr LC50 of 6570 mg/L, which would give an ACR of 22.05. However, it is not clear how the NOEC and LOEC were selected and the acute and chronic tests were performed in different dilution waters. The reported LOEC of 352 mg/L reduced survival by 9% compared to the control, whereas 533 mg/L reduced survival by 15%; neither concentration caused a substantial reduction in growth. It seems more appropriate to set the NOEC and LOEC at 352 and 533 mg/L, respectively, which gives a geometric mean of 433.1 mg/L. The ACR would be 15.17, but the acute and chronic tests were performed in different waters.

Diamond et al. (1992), Pickering et al. (1996) and the Wisconsin State Laboratory of Health (WISLOH 2007) performed 7-day tests on sodium chloride using the fathead minnow. Diamond et al. (1992) reported that the average geometric mean of the NOEC and LOEC was 1597 mg/L, whereas Pickering et al. (1996) reported a geometric mean subchronic value of 3458 mg/L. WISLOH (2007) reported a mean IC25 of 1752 mg/L. All three of these results are substantially higher than the geometric mean of 433.1 mg/L from Birge et al. (1985), which is not surprising because Table A19 in Birge et al. (1985) shows substantial mortality occurring between day 9 and day 33 in most of the treatments. Diamond et al. (1992) and Pickering et al. (1996) did not perform acute tests and so an ACR cannot be calculated; WISLOH (2007) obtained a mean 96-hr LC50 of 4143 mg/L, which would give an ACR of 2.365; this is substantially lower than the ACR of 15.17 from Birge et al. (1985). Although the 7-day test with the fathead minnow is not approved for use in the derivation of aquatic life criteria in the 1985 Guidelines, it could be used as a surrogate for the early life-stage test on a toxicant-specific basis if data demonstrated that the two tests give similar results. For sodium chloride, however, the data clearly indicate that the 7-day test misses much of the adverse effect observed in the 33-day early life-stage test. Because the early life-stage test is used as a surrogate for the life-cycle test, the comparison that is of most interest is a comparison of the 7-day test with the life-cycle test.

Rainbow trout

Birge et al. (1980) reported results of an early life-stage test on magnesium chloride.

Spehar (1986) reported the results of an 8-week exposure to NaCl that began with eggs. The highest tested concentration of 3,917 mg/L did not reduce egg hatchability, but killed all of the early juvenile fish. The next highest concentration of 1,924 mg/L did not reduce survival, but did reduce growth. Spehar (1987) reported the results of an early life-stage test on NaCl. The highest tested concentration of 2,740 mg/L killed 100%, 1,324 mg/L killed 46%, and 643 mg/L (and lower concentrations) killed less than 4%. The geometric mean of 1324 and 643 is 922.7 mg/L. The 96-hr LC50 was 6,743 mg/L and the ACR was 7.308.

Ceriodaphnia dubia

Sarma et al. (2006) reported that, on the basis of toxicity tests that studied population dynamics, all ten tested zooplankton species were adversely affected by 910 to 1820 mg/L; their data indicate that C. dubia was the most sensitive of the ten species.

Cowgill and Milazzo (1990) reported results of a life-cycle test on sodium chloride. If the geometric mean of the EC50 and the NOEC is used as the chronic value, it would be 925 mg/L, and the ACR would be 1395/925 = 1.508.

Cowgill and Milazzo (1991b) tested concentrations of chloride (as NaCl) from 8 to 283 mg/L. They found that reproduction peaked at 102 mg/L and all tested concentrations up to the maximum of 283 mg/L gave more reproduction than the control treatment.

Cowgill and Milazzo (1990) used calcium chloride extracted from a brine well to study the effect of “hardness as calcium carbonate” on C. dubia, whereas Cowgill and Milazzo (1991b) used reagent-grade calcium chloride to study the effect of “hardness as calcium carbonate” on C. dubia.

Diamond et al. (1992) reported that the average geometric mean of the NOEC and LOEC was 235 mg/L.

Wisconsin State Laboratory of Hygiene (WISLOH 2007) reported, for tests in hard reconstituted water (hardness = 169.5 mg/L and sulfate = 162.7 mg/L), a mean IC25 of 385.2 mg/L and a mean acute LC50 of 1499 mg/L. A 25% reduction is too high for a chronic value, so in hard water the chronic value is <385.2 mg/L and the ACR is >3.891. For tests in moderately hard reconstituted water (hardness = 84.8 mg/L and sulfate = 81.4 mg/L), WISLOH reported a mean IC25 of 442.2 mg/L and a mean acute LC50 of 1677 mg/L. Because a 25% reduction is too high for a chronic value, in moderately hard water the chronic value is <442.2 mg/L and the ACR is >3.792. The geometric mean ACR is >3.841.

Lasier et al. (2004) reported an IC50 of 563 mg/L and an IC25 of 340 mg/L. A 25% reduction is too high for a chronic value, so the chronic value is <340 mg/L.

Lasier et al. (2006) reported that the chronic toxicity of chloride is reduced by hardness cations, but they did not give any quantitative information.

From an interlaboratory study of the Ceriodaphnia dubia life-cycle test DeGraeve et al. (1992) reported that fifteen 7-day LC50s ranged from 170 to 1179 mg/L and sixteen IC50s based on reproduction ranged from 182 to 1153 mg/L. A 50% reduction is too much adverse effect to use LC50s or IC50s as chronic values, but they could be used as upper limits on chronic values.

Aragao and Pereira (2003) performed twelve 7-day life-cycle tests on NaCl; six were started with young from 16 to 24 hr old and six were started with young from 6 to 30 hr old. Of the twelve NOECs, based on reproduction, six were 303 mg/L, four were 152 mg/L, and two were <152 mg/L. The twelve 7-day IC50s based on reproduction ranged from 346 to 685 mg/L. If the geometric mean of the NOEC and the IC50 is used as the chronic value, the twelve chronic values would be:

NOEC	IC50	Chronic Value
303	685	456
303	558	411
152	455	263
303	582	420
303	667	450
303	594	424
<152	431	<256
152	412	250
152	346	229
<152	370	<237
152	437	258
303	406	351

The twelve chronic values range from <237 to 456 mg/L, and the geometric mean is <322 mg/L.

Cooney et al. (1992) performed 18 life-cycle tests on sodium chloride using C. dubia. For each of the 18 tests, the survival NOEC was 1092 mg/L and the survival LOEC was 1456 mg/L. The 18 reproduction NOECs ranged from <455 to 819 mg/L, whereas the 18 reproduction LOECs ranged from 455 to 1092 mg/L. If the geometric mean of the reproduction NOEC and the reproduction LOEC is used as the chronic value, the 18 chronic values would be:

NOEC	LOEC	Chronic Value
455	607	525
607	819	705
819	1092	946
819	1092	946
607	819	705
819	1092	946
455	607	525
819	1092	946

455	607	525
<455	455	<455
<455	455	<455
<455	455	<455
607	819	705
607	819	705
607	819	705
<455	455	<455
455	607	525
455	607	525

The 18 chronic values range from <455 to 946 mg/L, and the geometric mean is <629 mg/L.

Harmon et al. (2003) reported results of a life-cycle test on sodium chloride. The chronic EC20 based on reproduction was 370.6 mg/L. The ACR is 964/370.6 = 2.601.

Daphnia ambigua

Harmon et al. (2003) reported results of a life-cycle test on sodium chloride. The chronic EC20 based on reproduction was 292.4 mg/L. The ACR is 1213/292.4 = 4.148.

Daphnia magna

Leblanc and Surprenant (1984) conducted a life-cycle test on one concentration of potassium chloride and Cowgill and Milazzo (1990) conducted a life-cycle test on calcium chloride extracted from brine wells.

Biesinger and Christensen (1972) reported that 1573 mg/L caused a 50% reduction in reproduction, whereas 1049 mg/L caused a 16% reduction in reproduction in a life-cycle test. If the chronic value is set at 1111 mg/L because this is the estimated EC20, the ACR would be 2.276 using the unfed acute test and 2.526 using the fed acute test, but this is not an acceptable chronic test because the concentrations of the toxicant in the test solutions were not measured.

> NOTE: Many years ago when ASTM had a task group on life-cycle tests with Daphnia magna, most of the people on the task group were having a low percentage of successful chronic tests using the Biesinger methodology because survival was too low in the control treatment. The group met in Duluth to talk to Biesinger about his methodology. When it was explained that people were having a low success rate due to low survival in the control treatment, Biesinger said that his success rate was about 50%; he would start a test on a toxicant as often as necessary in order to eventually get a successful test on that toxicant. People complained to Biesinger that he did not say this in his publication (Biesinger and Christensen 1972), and Biesinger said that he would state the success rate in a subsequent publication concerning the development of the methodology; Biesinger never published a methodology paper. A variety of people tested a variety of foods and eventually found that the success rate could be greatly increased by using a food that was better than the one that Biesinger used. This leads me to think that the

healthiness of the daphnids used by Biesinger and Christensen (1972) is suspect and that all of the acute and chronic data presented in Biesinger and Christensen (1972) are suspect; however, this does not mean that these test results are incorrect.

Cowgill and Milazzo (1990) reported results of a life-cycle test on sodium chloride; they reported an EC50 of 2597 mg/L and a NOEC of 2184 mg/L. If the geometric mean of the EC50 and the NOEC is used as the chronic value, it would be 2382 mg/L, and the ACR would be 4701/2382 = 1.974.

Cowgill and Milazzo (1991b) tested concentrations of chloride (as NaCl) from 8 to 283 mg/L. They found that reproduction peaked at 37 mg/L and all tested concentrations up to the maximum of 283 mg/L gave as much reproduction as the control treatment.

Cowgill and Milazzo (1990) used calcium chloride extracted from a brine well to study the effect of “hardness as calcium carbonate” on D. magna, whereas Cowgill and Milazzo (1991b) used reagent-grade calcium chloride to study the effect of “hardness as calcium carbonate” on D. magna.

Daphnia pulex

Birge et al. (1985) reported that the most sensitive adverse effect observed in a 21-day life-cycle test was a 27% reduction in reproduction at 441 mg/L, with no reduction at 314 mg/L. The chronic value was 372 mg/L (the geometric mean of 441 and 314 mg/L) and the ACR was 1470/372 = 3.952.

Stenonema modestum (a mayfly)

Diamond et al. (1992) reported that NOECs and LOECs based on survival and number of molts in 14-day tests ranged from 1213 to 4246 mg/L. These are not acceptable chronic tests, but they might be used to estimate an upper limit on the chronic value for this species.

Musculium securis (a clam)

The test results reported by Mackie (1978) are not acceptable because the treatments were prepared by adding sodium chloride to a mixture of soil, leaves, and deionized water.

Musculium transversum (a clam)

Anderson et al. (1978) reported results of chronic tests on KCl.

Rana sylvatica (a frog)

Sanzo and Hecnar (2006) reported that 625 mg/L caused a 62% reduction in the survival of wood frog tadpoles during a 90-day exposure, whereas 47 mg/L did not reduce survival. Although this is not an acceptable chronic test, 625 mg/L can be used as an upper limit on the chronic value for this species.

Gammarus pseudopinmaeus (an amphipod)

Williams et al. (2000) reported that neither 1000 nor 2000 mg/L reduced survival in a two-month exposure in spring water, but this is not an acceptable chronic test.

Physa sp. (a snail)

Williams et al. (2000) reported that neither 1000 nor 2000 mg/L reduced survival in a two-month exposure in spring water, but this is not an acceptable chronic test.

Orconectes limosus (a crayfish)

Boutet and Chaisemartin (1973) reported the results of unacceptable chronic tests on potassium chloride and magnesium chloride.

The acceptable chronic values available for chloride are:

Fathead minnow	433.1 mg/L	Birge et al. 1985
Rainbow trout	922.7 mg/L	Spehar 1987
Ceriodaphnia dubia	925 mg/L	Cowgill and Milazzo 1990
Ceriodaphnia dubia	235 mg/L	Diamond et al. 1992
Ceriodaphnia dubia	<442.2 mg/L	WISLOH 2007 (mod. hard water)
Ceriodaphnia dubia	<385.2 mg/L	WISLOH 2007 (hard water)
Ceriodaphnia dubia	<340 mg/L	Lasier et al. 2004
Ceriodaphnia dubia	<322 mg/L	Aragao and Pereira 2003
Ceriodaphnia dubia	<629 mg/L	Cooney et al. 1992
Ceriodaphnia dubia	370.6 mg/L	Harmon et al. 2003
Daphnia ambigua	292.4 mg/L	Harmon et al. 2003
Daphnia magna	2382 mg/L	Cowgill and Milazzo 1990
Daphnia pulex	372 mg/L	Birge et al. 1985
Rana sylvatica	<625 mg/L	Sanzo and Hecnar 2006

These result in the following Genus Mean Chronic Values:

Pimephales	433.1 mg/L
Oncorhynchus	922.7 mg/L
Ceriodaphnia	<418.7 mg/L
Daphnia	637.5 mg/L
Rana	<625 mg/L

The acceptable ACRs available for chloride are:

Rainbow trout	7.308	Spehar 1987
Ceriodaphnia dubia	1.508	Cowgill and Milazzo 1990
Ceriodaphnia dubia	>3.841	WISLOH 2007
Ceriodaphnia dubia	2.601	Harmon et al. 2003
Daphnia ambigua	4.148	Harmon et al. 2003
Daphnia magna	1.974	Cowgill and Milazzo 1990
Daphnia pulex	3.952	Birge et al. 1985

The following ACR is also available for chloride but it should not be used in calculations because the acute and chronic tests were performed in different waters:

Fathead minnow	15.17	Birge et al. 1985

Summary of Data Concerning the Acute Toxicity of Sodium Chloride to Aquatic Animals

This summary is based on "Results of Literature Search concerning the Toxicity of Chloride to Aquatic Animals" dated 1-15-09. Except as noted, this summary is consistent with the 1985 Guidelines.

Acute Values and Normalized Acute Values are expressed as mg chloride/L. Normalized Acute Values were calculated by normalizing the Acute Values to hardness = 300 mg/L and sulfate = 65 mg/L using the following equation:

$$\text{NAV} = (\text{AV})\,(300/\text{Hardness})^{0.205797}\,(65/\text{Sulfate})^{-0.07452}$$

This equation is based on the equation presented in "Multiple Regression Equation for Chloride" dated 1-15-09. The hardness of 300 mg/L and the sulfate concentration of 65 mg/L are arbitrary; any other values for hardness and sulfate would have worked equally well. NAVs could not be calculated for all AVs because assumed values were not used for hardness or sulfate. Some of the values of hardness and sulfate are nominal, not measured, values.

Species	Method	Test Material	Hardness (mg/L)	Sulfate (mg/L)	Acute Value	Normalized Acute Value	Reference
Tubificid worm, Limnodrilus hoffmeisteri	S,U	Sodium chloride	100	----	3761	------	Wurtz and Bridges 1961
Tubificid worm, Tubifex tubifex	S,M	Sodium chloride	52	57.9	4278	6083.2	GLEC and INHS 2008
			220	58.9	6008	6357.1	
Leech, Erpobdella punctata	S,U	Sodium chloride	100	----	4550	------	Wurtz and Bridges 1961
Mussel, juvenile Villosa delumbis	S,M	Sodium chloride	169.5	162.7	3173	3821.1	Bringolf et al. 2007
Mussel, juvenile Villosa iris	R,M	Sodium chloride	169.5	162.7	2069	2491.6	Wang 2007

Mussel, juvenile Lampsilis fasciola	S,M	Sodium chloride	169.5	162.7	2414	2907.1	Bringolf et al. 2007
Mussel, juvenile Lampsilis siliquoidea	R,M	Sodium chloride	169.5	162.7	1905	2294.1	Wang 2007
Mussel, juvenile Lampsilis siliquoidea	S,M	Sodium chloride	169.5	162.7	2766	3331.0	Bringolf et al. 2007
Fingernail clam, Sphaerium simile	S,M	Sodium chloride	51 192	59.9 61.7	740 1100	1059.2 1201.1	GLEC and INHS 2008
Fingernail clam, Sphaerium tenue	S,U	Sodium chloride	100 20	---- ----	667 698	------ ------	Wurtz and Bridges 1961
Snail, Physa gyrina	F,M	Sodium chloride	84.8	81.4	2540	3350.0	Birge et al. 1985
Snail, Physa heterostropha	S,U	Sodium chloride	100 100 100 20	---- ---- ---- ----	2123 3094 3761 2487	------ ------ ------ ------	Wurtz and Bridges 1961
Snail, Physa sp.	S,M	Sodium chloride	22	15	3247hp	4983.6	Clemens and Jones 1954
Snail, Physa sp.	S,U	Sodium chloride	----	----	>3000p	------	Williams et al. 2000
Snail, Gyraulus circumstriatus	S,U	Sodium chloride	100	----	1941	------	Wurtz and Bridges 1961
Snail,	S,M	Sodium	56	60.9	3078	4326.9	GLEC and INHS 2008

Gyraulus parvus		chloride	212	59.7	3009	3211.4	
Snail, Helisoma campanulata	S,U	Sodium chloride	100	----	3731	------	Wurtz and Bridges 1961
Cladoceran, Ceriodaphnia dubia	S,U	Sodium chloride	84.8	81.4	1189brt	1568.2	Mount et al. 1997
					1042brt	1374.3	
Cladoceran, Ceriodaphnia dubia	R,U	Sodium chloride	74.1	----	1395	------	Cowgill and Milazzo 1990
Cladoceran, Ceriodaphnia dubia	S,U	Sodium chloride	39.2	4.6	507	632.7	Hoke et al. 1992
					447	557.8	
Cladoceran, Ceriodaphnia dubia	S,U	Sodium chloride	39.2	4.6	1395	1740.8	USEPA 1991
			39.2	4.6	1638	2044.1	
			39.2	4.6	1274	1589.8	
			39.2	4.6	1395	1740.8	
			339.0	325.4	1698	1867.0	
Cladoceran, Ceriodaphnia dubia	S,U	Sodium chloride	84.8	81.4	1677c	2211.8	WISLOH 2007
			169.5	162.7	1499c	1805.2	
Cladoceran, Ceriodaphnia dubia	S,U	Sodium chloride	84.8	81.4	1413e	1863.6	Valenti et al. 2007
Cladoceran, Ceriodaphnia dubia	S,M	Sodium chloride	67.1	64.4q	964	1311.1	Harmon et al. 2003
Cladoceran, Ceriodaphnia dubia	S,M	Sodium chloride	30	78.7	947	1542.9	GLEC and INHS 2008
			44	75.9	955	1434.1	
			96	73.7	1130	1442.1	
			180	67.7	1609	1792.8	

			400	78.7	1491	1425.5	
			570	76.2	1907	1690.9	
			800	75.5	1764	1457.7	
			25	69.9	1007	1688.4	
			49	67.8	767	1117.1	
			95	70.3	1369	1744.7	
			194	69.9	1195	1314.3	
			375	68.9	1687	1618.3	
			560	68.3	1652	1458.2	
			792	70.9	1909	1573.5	
			280	28.1	1400	1334.0	
			280	59.6	1720	1733.4	
			280	117	1394	1477.2	
			280	239	1500	1676.5	
			280	482	1109	1306.0	
			280	729	1206	1464.7	
			279	22.9	1311	1231.2	
			276	49.7	1258	1254.4	
			283	107	1240	1302.5	
			281	229	1214	1351.5	
			290	461	1199	1397.2	
			278	694	1179	1428.8	
Cladoceran, Daphnia ambigua	S,M	Sodium chloride	67.1	64.4q	1213	1649.7	Harmon et al. 2003.
Cladoceran, Daphnia magna	S,U	Sodium chloride	84.8	81.4	2893brs	3815.5	Mount et al. 1997
Cladoceran, Daphnia magna	S,U	Sodium chloride	240	----	621	------	Khangarot and Ray 1989
Cladoceran,	S,U	Sodium	39.2	4.6	3038	3791.1	Hoke et al. 1992

Daphnia magna		chloride	39.2 39.2	4.6 4.6	2726 2053	3401.8 2561.9	
Cladoceran, Daphnia magna	-,-	Sodium chloride	----	----	1008k 3319m	------ ------	Cowgill 1987
Cladoceran, Daphnia magna	S,U	Sodium chloride	108.7	13	<2548	<2785.1	Anderson 1946
Cladoceran, Daphnia magna	S,U	Sodium chloride	108.7	13	2232i	2439.7	Anderson 1948
Cladoceran, Daphnia magna	S,U	Sodium chloride	41.5	31.2	3563	5068.2	Dowden and Bennett 1965
Cladoceran, Daphnia magna	S,M	Sodium chloride	45.3	3.9v	2529a,f 2806b,f	3025.9 3357.4	Biesinger and Christensen 1972
Cladoceran, Daphnia magna	S,U	Sodium chloride	169.5	162.7	>2669 <3943d	>3214.2 <4748.4	Seymour et al. 1997
Cladoceran, Daphnia magna	S,U	Sodium chloride	46	3.9v	1880	2242.3	USEPA 1991
Cladoceran, Daphnia magna	S,U	Sodium chloride	169.5	162.7	3944c	4749.6	WISLOH 2007
Cladoceran, Daphnia magna	S,U	Sodium chloride	84.8	81.4	3009e	3968.5	Valenti et al. 2007
Cladoceran, Daphnia magna	S,U	Sodium chloride	106	102	3136 3222 3137	4017.4 4127.5 4018.6	Davies and Hall 2007

Cladoceran, Daphnia pulex	S,M	Sodium chloride	84.8	81.4	1470	1938.8	Birge et al. 1985
Cladoceran, Daphnia pulex	S,U	Sodium chloride	84.8	81.4	1159	1528.6	Palmer et al. 2004
			84.8	81.4	1775	2341.0	
			84.8	81.4	1805	2380.6	
			84.8	81.4	2242	2956.9	
Copepod, Diaptomus clavipes	S,M	Sodium chloride	22	15	2571h	3946.1	Clemens and Jones 1954
Isopod, Asellus communis	S,U	Sodium chloride	100	----	5004	------	Wurtz and Bridges 1961
			20	----	3094	------	
Isopod, Lirceus fontinalis	F,M	Sodium chloride	84.8	81.4	2950	3890.7	Birge et al. 1985
Amphipod, Hyalella azteca	S,U	Sodium chloride	102.5	98.4	3947	5077.7	Lasier et al. 1997
Amphipod, Gammarus pseudolimnaeus	S,U	Sodium chloride	----	----	>3000	------	Williams et al. 2000
Amphipod, Crangonyx sp.	S,U	Sodium chloride	----	----	>3000	------	Williams et al. 2000
Crayfish, Cambarus sp.	S,M	Sodium chloride	22	15	10557h	16203.2	Clemens and Jones 1954
Dragonfly, Libellulidae	S,M	Sodium chloride	22	15	9671h	14843.4	Clemens and Jones 1954
Damselfly,	S,U	Sodium	100	----	14558	------	Wurtz and Bridges 1961

Agria sp.		chloride	20	----	13952	------	
Stonefly, Nemoura trispinosa	S,U	Sodium chloride	----	----	>3000	------	Williams et al. 2000
Caddisfly, Lepidostoma sp.	S,U	Sodium chloride	----	----	>3000	------	Williams et al. 2000
Caddisfly, Parapsyche sp.	S,U	Sodium chloride	----	----	>3000	------	Williams et al. 2000
Midge, Chironomus attenuatus	S,U	Sodium chloride	----	----	4850	------	Thornton and Sauer 1972
American eel, Anquilla rostrata	S,U	Sodium chloride	42.4	40.7	10846	15667.3	Hinton and Eversole 1978
American eel, Anquilla rostrata	S,U	Sodium chloride	42.4	40.7	13012	18796.2	Hinton and Eversole 1979
Goldfish, Carassius auratus	S,M	Sodium chloride	148.8	----	9465	------	Threader and Houston 1983
Red shiner, Notropis lutrensis	S,M	Sodium chloride	22	15	5771g 5920g	8857.5 9086.2	Clemens and Jones 1954
Fathead minnow, Pimephales promelas	S,U	Sodium chloride	39.2 39.2 339.0	4.6 4.6 325.4	2790 2123 2244	3481.7 2649.3 2467.3	USEPA 1991
Fathead minnow, Pimephales promelas	F,M	Sodium chloride	84.8	81.4	6570	8665.1	Birge et al. 1985

Fathead minnow, Pimephales promelas	S,M	Sodium chloride	22	15	5288g 5431g	8116.2 8335.7	Clemens and Jones 1954
Fathead minnow, Pimephales promelas	S,U	Sodium chloride	84.8	81.4	3876br	5112.0	Mount et al. 1997
Fathead minnow, Pimephales promelas	S,U	Sodium chloride	84.8 169.5	81.4 162.7	4167c 4127c	5495.8 4970.0	WISLOH 2007
Black bullhead, Ameiurus melas	S,M	Sodium chloride	22	15	4849g	7442.4	Clemens and Jones 1954
Rainbow trout, Oncorhynchus mykiss	S,U	Sodium chloride	22.4	----	>485j	------	Camargo and Tarazona 1991
Rainbow trout, Oncorhynchus mykiss	F,M	Sodium chloride	46	3.9v	6743	8042.6	Spehar 1986,1987
Rainbow trout, Oncorhynchus mykiss	R,U	Sodium chloride	284	----	12363	------	Vosyliene et al. 2006
Brown trout, Salmo trutta	S,U	Sodium chloride	22.4	----	>607j	------	Camargo and Tarazona 1991
Plains killifish, Fundulus kansae	S,M	Sodium chloride	22	15	9706g	14897.1	Clemens and Jones 1954
Mosquitofish, Gambusia affinis	S,M	Sodium chloride	22	15	6472g	9933.4	Clemens and Jones 1954
Mosquitofish, Gambusia affinis	S,U	Sodium chloride	----	14.9	9099	------	Al-Daham and Bhatti 1977

Threespine stickleback, Gasterosteus aculeatus	R,M	Sodium chloride	84.8	81.4	10200b	13452.6	Garibay and Hall 2004
Green sunfish, Lepomis cyanellus	S,M	Sodium chloride	22	15	6499g	9974.9	Clemens and Jones 1954
Bluegill, Lepomis macrochirus	F,M	Sodium chloride	84.8	81.4	5840	7702.3	Birge et al. 1985
Bluegill (3.7 g), Lepomis macrochirus	S,U	Sodium chloride	44.3	15.5	7853	10461.6	Academy of Natural Sciences 1960; Patrick et al. 1968; Trama 1954
Chorus frog, Pseudacris sp.	R,M	Sodium chloride	84.8	81.4	3553	4686.0	Garibay and Hall 2004

a = not fed. (All tests not marked “a” or “b” were unfed tests.)
b = fed.
c = mean of at least 15 LC50s.
d = range of several toxicity tests.
e = mean of 32 tests.
f = not used because there is reason to suspect that the daphnids might have been unhealthy.
g = tables 4, 7, and 9, except for tests at 28C in table 4.
h = tables 8 and 11; Daphnia pulex tests were not used because test duration was 96 hr.
i = test duration was 64 hr.
j = no deaths in 196 hr.
k = selenium deficient.
m = selenium sufficient.
p = not used in calculation of GMAV because the species is unknown and so it is not known how to combine this acute value with the acute values for which the species are known.
q = calculated using the formula for reconstituted water and the reported average measured hardness.
r = concentrations were measured in stock solutions.
s = not acclimated to the dilution water.

t = might not have been acclimated to the dilution water.
v = based on analyses of samples of Lake Superior water taken in the spring and fall of 2008.

Supplementary information concerning the results of toxicity tests on chloride

1. The atomic weights used are those given on the website of the National Institute of Standards and Technology:
    Calcium = 40.078
    Carbon = 12.011
    Chlorine = 35.453
    Hydrogen = 1.008
    Iron = 55.845
    Magnesium = 24.305
    Nitrogen = 14.007
    Oxygen = 15.999
    Potassium = 39.098
    Sodium = 22.990
    Sulfur = 32.065

2. The molecular weights used are:
    $CaCl_2$ = 110.984 (63.89 % chloride) (36.11 % calcium) (Cl/Ca = 1.769)
    $CaCl_2{\cdot}2H_2O$ = 147.014 (27.26 % calcium) (48.23 % chloride)
    $CaCO_3$ = 100.086 (40.04 % calcium)
    CaO = 56.077 (71.47 % calcium)
    $Ca(NO_3)_2$ = 164.086 (24.42 % calcium)
    $Ca(NO_3)_2{\cdot}4H_2O$ = 236.146 (16.97 % calcium)
    $CaSO_4$ = 136.139 (70.56 % sulfate)
    $CaSO_4{\cdot}2H_2O$ = 172.169 (55.79 % sulfate) (23.28 % calcium) (20.93 % water)
    $FeCl_3{\cdot}6H_2O$ = 270.294 (39.35 % chloride)
    H_2O = 18.015
    KCl = 74.551 (47.56 % chloride) (52.44 % potassium) (Cl/K = 0.9068)
    K_2SO_4 = 174.257 (55.13 % sulfate)
    $MgCl_2$ = 95.211 (74.47 % chloride) (25.53 % magnesium) (Cl/Mg = 2.917)
    $MgSO_4$ = 120.366 (79.81 % sulfate) (20.19 % magnesium)
    $MgSO_4{\cdot}7H_20$ = 246.471 (38.97 % sulfate) (9.86 % magnesium)
    NaCl = 58.443 (60.66 % chloride) (39.34 % sodium) (Cl/Na = 1.542)
    Na_2SO_4 = 142.041 (67.63 % sulfate)

$Na_2SO_4 \cdot 10H_2O$ = 322.191   (29.81 % sulfate)
SO_4 = 96.061

3. Hardness (as $CaCO_3$)   = (100.086/40.078)(Ca) + (100.086/24.305)(Mg) = 2.497(Ca) + 4.118(Mg)

4. Trama (1954), Cairns and Scheier (1959), Academy of Natural Sciences (1960), and Patrick et al. (1968) all reported results of toxicity tests that were performed at the Academy of Natural Sciences of Philadelphia with the bluegill in dilution waters that were very similar:

	mg/L
KCl	20
Na_2SiO_3	20
$NaHCO_3$	40
$MgSO_4 \cdot 7H_2O$	40
$Ca(NO_3)_2$	30*
$CaCO_3$	10
K_2HPO_4	10 or 2
Fe^{+++} (as ferric citrate)	4 or 0.4

Ca = 11.3 mg/L
Mg = 3.9 mg/L
Hardness = 44.3 mg/L as $CaCO_3$
Chloride = 9.5 mg/L
Sulfate = 15.5 mg/L

*Long after the tests of concern were performed, this was reported to be 40 mg/L of $Ca(NO_3)_2 \cdot 4H_20$.

5. Freeman (1953), Freeman and Fowler (1953), Fairchild (1955), Dowden (1960), Dowden (1962), and Dowden and Bennett (1965) all contained information regarding toxicity tests performed at Louisiana State University in Baton Rouge, but several different dilution waters were used. Dowden and Bennett (1965) tried to clarify the most important dilution waters used, but the citations given for the waters on page 1310 need to be clarified. Reference 4 is the correct citation for “Standard Reference Water” (SRW), but the correct citation for “Reference Dilution Water” (RDW) is reference 6 (not reference 3) and reference 3 should be cited for “glass-wool filtered University Lake Water (ULW). ULW is considered an unacceptable dilution water because it is from “a small drainpipe-fed lake on the campus of Louisiana State University” (Dowden 1960). The compositions of SRW and RDW are:

SRW	mg/L
$MgSO_4 \cdot 7H_2O$	71.

K_2SO_4	6.5
$MnSO_4 \cdot 4H_2O$	0.2
$CaCl_2 \cdot 2H_2O$	18.6
$NaHCO_3$	25.
NH_4NO_3	3.
$K_2HPO_4 \cdot 3H_2O$	1.1
CaO	32.2
$Na_2SiO_3 \cdot 9H_2O$	62.6
$FeCl_3 \cdot 6H_2O$	1.2

Ca = 23.0 mg/L
Mg = 7.0 mg/L
Hardness = 86.2 mg/L as $CaCO_3$
Chloride = 9.4 mg/L
Sulfate = 31.2 mg/L

RDW	mg/L
$CaCl_2$	110
$NaHCO_3$	110
$NaCl$	100
$MgSO_4 \cdot 7H_2O$	60
KCl	20

Ca = 39.7 mg/L
Mg = 5.9 mg/L
Hardness = 123.4 mg/L as $CaCO_3$
Chloride = 140.4 mg/L
Sulfate = 23.4 mg/L

6. When known, the concentration of chloride in dilution water was negligible in tests on chloride.

7. Karraker (2007) says that “road salt” contains sodium chloride, sodium ferrocyanide, heavy metals, and often sand or cinder. Results of toxicity tests on “road salt” were not used.

8. Hyalella azteca appears to be especially sensitive to some pollutants when the concentration of chloride is low, e.g., lower than 25 mg/L. This should not affect the sensitivity of this species to chloride.

9. Mount et al. (1997) reported that the toxicities of sodium and calcium salts to C. dubia, D. magna, and the fathead minnow are primarily attributable to the corresponding anion.

10. Some data presented in Table 1 of USEPA (1988) have been changed:
    a. Several values differ because of roundoff differences.
    b. USEPA (1988) used results of short acute tests for the reasons given on pages 2 and 3 (see also Lowell et al. 1995), but results of short tests are not used here because short acute tests sometimes give higher LC50s than standard tests.
    c. Data from Dowden (1961, which should be 1960) and Kostecki and Jones (1983) are not used here because of the dilution water used in the tests.
    d. The test results from Trama (1954) are also given in Academy of Natural Sciences (1960) and Patrick et al. (1968).
    e. Hamilton et al. (1975) did not adequately acclimate the midges.
    f. Fed acute tests were not used in USEPA (1988), but fed acute tests are used here and are given preference over unfed acute tests when the test organisms are cladocerans.

Description of the Review of Results of Toxicity Tests on Chloride

Sections II.B, II.C, II.D, II.E, II.F, IV.B, IV.C, IV.D, IV.E, IV.H, VI.B, VI.C, VI.D, and VI.E of the 1985 Guidelines give reasons why some results of toxicity tests should not be used, should be rejected, or should not be used in calculations, whereas sections II.G, X, XI.C, XII.A.14, and XII.B allow the use of "questionable data" and "other data" in some situations. An easy-to-understand way of explaining the material given in the sections listed above is to say that sections II.B, II.C, II.D, II.E, II.F, IV.B, IV.C, IV.D, IV.E, IV.H, VI.B, VI.C, VI.D, and VI.E give reasons why some results of toxicity tests using aquatic animals should not be directly used in the derivation of a FAV or a FCV, whereas sections II.G, X, XI.C, XII.A.14, and XII.B describe other possible uses of test results with aquatic animals that should not be directly used in the derivation of a FAV or a FCV.

The 1985 Guidelines say the following concerning the use of results of toxicity tests using aquatic animals:

1. General guidance
    a. All data should be available in typed, dated, and signed hard copy (publication, manuscript, letter, memorandum, etc.) with enough supporting information to indicate that acceptable test procedures were used and that the results are probably reliable. (see section II.B)
    b. Information that is confidential or privileged or otherwise not available for distribution should not be used. (see section II.B)
    c. Questionable data, whether published or unpublished, should not be used. For example, a test result should usually be rejected if it is from:
        i. a test that did not contain a control treatment.
        ii. a test in which too many organisms in the control treatment died or showed signs of stress or disease.
        iii. a test in which distilled or deionized water was used as the dilution water without addition of appropriate salts.

        (see section II.C)
    d. A result of a test on technical-grade material may be used if appropriate, but a result of a test on a formulated mixture or an emulsifiable concentrate of the test material should not be used. (see section II.D)
    e. For some highly volatile, hydrolyzable, or degradable materials it is probably appropriate to use only results of flow-through tests in which the concentrations of test material in the test solutions were measured often enough using acceptable analytical methods. (see section II.E)
    f. Data should be rejected if they were obtained using:
        i. Brine shrimp
        ii. A species that does not have a reproducing wild population in North America.

        iii. Organisms that were previously exposed to substantial concentrations of the test material or other contaminants.

        (see section II.F)

2. Guidance specifically regarding results of acute tests
    g. Acute toxicity tests should have been conducted using acceptable procedures. (see section IV.B)
    h. Except for tests using saltwater annelids and mysids, results of acute tests during which the test organisms were fed should not be used, unless data indicate that the food did not affect the toxicity of the test material. (see section II.C)
    i. Results of acute tests conducted in unusual dilution water, e.g., dilution water in which total organic carbon or particulate matter exceeded 5 mg/L, should not be used, unless a relationship is developed between acute toxicity and organic carbon or particulate matter or unless data show that organic carbon, particulate matter, etc., do not affect toxicity. (see section IV.D)
    j. Acute values should be based on endpoints which reflect the total severe acute adverse impact of the test material on the organisms used in the test. Therefore, only the following kinds of data on acute toxicity to aquatic animals should be used:
        1. Tests with daphnids and other cladocerans should be started with organisms less than 24 hours old and tests with midges should be started with second- or third-instar larvae. The result should be the 48-hr EC50 based on percentage of organisms immobilized plus percentage of organisms killed. If such an EC50 is not available from a test, the 48-hr LC50 should be used in place of the desired 48-hr EC50. An EC50 or LC50 of longer than 48 hr can be used as long as the animals were not fed and the control animals were acceptable at the end of the test.
        2. The result of a test with embryos and larvae of barnacles, bivalve molluscs (clams, mussels, oysters, and scallops), sea urchins, lobsters, crabs, shrimp, and abalones should be the 96-hr EC50 based on the percentage of organisms with incompletely developed shells plus the percentage of organisms killed. If such an EC50 is not available from a test, the lower of the 96-hr EC50 based on the percentage of organisms with incompletely developed shells and the 96-hr LC50 should be used in place of the desired 96-hr EC50. If the duration of the test was between 48 and 96-hr, the EC50 or LC50 at the end of the test should be used.
        3. The acute values from tests with all other freshwater and saltwater animal species and older life stages of barnacles, bivalve molluscs, sea urchins, lobsters, crabs, shrimps, and abalones should be the 96-hr EC50 based on the percentage of organisms exhibiting loss of equilibrium plus the percentage of organisms immobilized plus the percentage of organisms killed. If such an EC50 is not available from a test, the 96-hr LC50 should be used in place of the desired 96-hr EC50.
        4. Tests with single-celled organisms are not considered acute tests, even if the duration was 96 hours or less.
        5. If the tests were conducted properly, acute values reported as “greater than” values and those which are above the solubility of the test material should be used,

because rejection of such acute values would unnecessarily lower the Final Acute Value by eliminating acute values for resistant species.

(see section IV.E)

k. The agreement of the data within and between species should be considered. Acute values that appear to be questionable in comparison with other acute and chronic data for the same species and for other species in the same genus probably should not be used in the calculation of a SMAV. For example, if the acute values available for a species or genus differ by more than a factor of 10, some or all of the values probably should not be used in calculations. (see section IV.H)

3. Guidance specifically regarding results of chronic tests

l. Chronic values should be based on results of flow-through (except renewal is acceptable for daphnids) chronic tests in which the concentrations of test material in the test solutions were properly measured at appropriate times during the test. (see section VI.B)

m. Results of chronic tests in which survival, growth, or reproduction in the control treatment was unacceptably low should not be used. The limits of acceptability will depend on the species. (see section VI.C)

n. Results of chronic tests conducted in unusual dilution water, e.g., dilution water in which total organic carbon or particulate matter exceeded 5 mg/L, should not be used, unless a relationship is developed between chronic toxicity and organic carbon or particulate matter or unless data show that organic carbon, particulate matter, etc., do not affect toxicity. (see section VI.D)

o. Chronic values should be based on endpoints and lengths of exposure appropriate to the species. Therefore, only data on chronic toxicity to aquatic animals that satisfy the species-specific requirements given in sections VI.E.1, VI.E.2, and VI.E.3 should be used.

4. Guidance regarding other possible uses of results of toxicity tests using aquatic animals

p. Questionable data, data on formulated mixtures and emulsifiable concentrates, and data obtained with non-resident species or previously exposed organisms may be used to provide auxiliary information but should not be used in the derivation of criteria. (see section II.F)

q. Pertinent information that could not be used in earlier sections might be available concerning adverse effects on aquatic organisms and their uses. The most important of these are data on cumulative and delayed toxicity, flavor impairment, reduction in survival, growth, or reproduction, or any other adverse effect that has been shown to be biologically important. Especially important are data for species for which no other data are available. Data from behavioral, biochemical, physiological, microcosm, and field studies might also be available. Data might be available from tests conducted in unusual dilution water, from chronic tests in which the concentrations were not measured, from tests with previously exposed organisms, and from tests on formulated mixtures or emulsifiable concentrates. Such data might affect a criterion if the data were obtained with an important species, the test concentrations were measured, and the endpoint was biologically important. (see section X)

r. The CCC is equal to the lowest of the FCV, FPV, and FRV, unless other data show that a lower value should be used. (see section XI.C)
s. Are any of the other data important? (see section XII.A.14)
t. On the basis of all available pertinent laboratory and field information, determine if the criterion is consistent with sound scientific information. If it is not, another criterion, either higher or lower, should be derived using appropriate modifications of these Guidelines. (see section XII.B)

In addition, the following aquatic life criteria documents published by U.S. EPA in 1985, 1986, 1987, and 1988 gave a variety of reasons for classifying specific test results as "unused":

U.S. EPA. 1985. Ambient Water Quality Criteria for Cadmium - 1984. EPA 440/5-84-032. U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA. 1985. Ambient Water Quality Criteria for Chlorine - 1984. EPA 440/5-84-030. U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA. 1985. Ambient Water Quality Criteria for Copper - 1984. EPA 440/5-84-031. U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA. 1985. Ambient Water Quality Criteria for Lead - 1984. EPA 440/5-84-027. U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA. 1985. Ambient Water Quality Criteria for Mercury - 1984. EPA 440/5-84-026. U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA. 1986. Ambient Water Quality Criteria for Chlorpyrifos - 1986. EPA 440/5-86-005. U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA. 1986. Ambient Water Quality Criteria for Parathion - 1986. EPA 440/5-86-007. U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA. 1986. Ambient Water Quality Criteria for Pentachlorophenol - 1986. EPA 440/5-86-009. U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA. 1986. Ambient Water Quality Criteria for Toxaphene - 1986. EPA 440/5-86-006. U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA. 1987. Ambient Water Quality Criteria for Selenium - 1987. EPA 440/5-87-006. U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA. 1987. Ambient Water Quality Criteria for Zinc - 1987. EPA 440/5-87-003. U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA. 1988. Ambient Water Quality Criteria for Chloride - 1988. EPA 440/5-88-001. U.S. Environmental Protection Agency, Washington, DC.

The following is a compilation of the reasons, although some have been paraphrased. Most of these reasons can be considered to be based on items a through o listed above.

1. The document is a secondary publication of the test result.
2. The test procedures, test material, dilution water, and/or results were not adequately described.
3. The test species is not resident in North America.
4. The test species was not obtained in North America and was not identified well enough to determine whether it is resident in North America.
5. The test organisms were not identified beyond, for example, “crayfish” or “minnows.”
6. There is reason to believe that the test organisms were possibly stressed by disease or parasites.
7. The test organisms were exposed to elevated concentrations of the test material before the test and/or the control organisms contained high concentrations of the test material.
8. The test organisms were obtained from a sewage oxidation pond.
9. By the end of the test, the test organisms had not been fed for too long a period of time.
10. The water quality varied too much during the test.
11. The test was conducted with brine shrimp, which species are from a unique saltwater environment.
12. The exposed biological material was an enzyme, excised or homogenized tissue, tissue extract, plasma, or cell culture.
13. The test organisms were not acclimated to the dilution water for a sufficiently long time.
14. The test organisms were exposed to the test material via gavage, injection, or food.
15. There is reason to believe that the test organisms were probably crowded during the test.
16. The test organisms reproduced during an acute test and the young could not be distinguished from the old at the end of the test.
17. The test material was a component of a mixture, effluent, fly ash, sediment, drilling mud, sludge, or formulation.
18. In a test on zinc, the dilution water contained a phosphate buffer.
19. The test material was chlorine and it was not measured acceptably during the test.
20. The test chamber contained sediment.
21. The test was conducted in plastic test chambers without measurement of the test material.
22. The test was a field study and the concentration of test material was not measured adequately.
23. A known volume of stock solution was placed on a wall of the test chamber and evaporated and then dilution water was placed in the test chamber; the investigators assumed that all of the test material dissolved in the dilution water, but the concentrations of the test material in the test solutions were not measured.
24. The test only studied metabolism of the test material.
25. The only effects studied were biochemical, histological, and/or physiological.
26. The data concerned the selection, adaptation, or acclimation of organisms for increased resistance to the test material.

27. The percent survival in the control treatment was too low.
28. The concentration of solvent in some or all of the test solutions was too high.
29. The study was a microcosm study.
30. The concentration of test material fluctuated too much during the exposure.
31. Too few test organisms were used in the test.
32. The dilution factor was ten.
33. There was no control treatment.
34. The pH was below 6.5.
35. The dilution water was chlorinated or “tap” water.
36. The dilution water contained an excessive amount of a chelating agent such as EDTA or other organic matter.
37. The acceptability of the dilution water is questionable because of its origin or content.
38. The dilution water was distilled or deionized water without addition of appropriate salts.
39. The measured test temperature fluctuated too much.
40. Neither raw data nor a clearly defined endpoint was reported.
41. The results were not adequately presented or could not be interpreted.
42. The results were only presented graphically.
43. The test was a chronic test and the concentration of test material was not measured.

Nevertheless, the things that the 1985 Guidelines say regarding other possible uses of test results with aquatic animals that should not be directly used in the derivation of a FAV or a FCV makes it clear that “unused” test results can be used in a criteria document, if appropriate. They should not be directly used in the derivation of a FAV or a FCV, but they can be used in other ways in some situations.

Further, several additional considerations were taken into account when results of aquatic toxicity tests on chloride were reviewed:

a. Review of results of toxicity tests on such pollutants as chloride should take additional considerations into account because chloride is different from most pollutants for which aquatic life criteria are derived. Chloride is very soluble in water, does not oxidize or reduce, is not volatile, does not degrade, does not sorb to test chambers, test organisms, food, or waste products, is not complexed by materials that commonly occur in water, is not involved in a pH-dependent equilibrium in water, and does not precipitate in waters in which aquatic organisms commonly occur. Toxicity tests on most pollutants are subject to one or more of these potential complications and so it is more important to be concerned about test methodology when considering other pollutants.
b. Section IV.C of the 1985 Guidelines says: “Except for tests with saltwater annelids and mysids, results of acute tests during which the test organisms were fed should not be used, unless data indicate that the food did not affect the toxicity of the test material.” Section XII.B of the 1985 Guidelines says: “On the basis of all available pertinent laboratory and field information, determine if the criterion is consistent with sound scientific evidence. If it is not, another criterion, either higher or lower, should be derived using appropriate modifications of these Guidelines.” Appendix 1 below demonstrates that (i) results of acute toxicity tests on chloride should not be rejected just because the test organisms were fed

during the tests and (ii) results of fed acute tests should be given preference over results of unfed acute tests when the test organisms are cladocerans.

c. If tests in a document using some species had acceptable control mortalities but other tests in the same document using other species had unacceptable control mortalities, the tests using species with unacceptable control mortalities were not used. The general policy is that tests in a document using different species should be evaluated on a species-by-species basis, not on a document-by-document basis.

d. Results of 96-hr toxicity tests using juvenile freshwater mussels were used if the methodology, etc., were acceptable, but results of tests using glochidia of freshwater mussels were not used. Acute (e.g., 96-h) toxicity tests using juvenile freshwater mussels are rather standard acute toxicity tests and can be evaluated using, for example, ASTM Standard E729. In contrast, acute toxicity tests using glochidia of freshwater mussels require special consideration because free-living glochidia must attach to a host in order to survive, and they typically attach within seconds to days. In addition, for a specific species and a specific toxicant, the glochidia 6-h EC50 might be substantially higher than the glochidia 24-h EC50. It is known that free-living glochidia of several species can remain viable for up to about ten days, depending on the species and on the percent viability that is considered acceptable. However, it is not known how fast glochidia of individual species usually attach to a host, and this is important because the glochidia EC50 for some toxicants and species is quite dependent on the duration of the toxicity test. Thus, a very important question is "What species-specific toxicity-test duration is ecologically relevant for glochidia?"

e. USEPA is tending more toward a weight-of-evidence approach to the derivation of aquatic life criteria than when the 1985 Guidelines were written. The 1985 Guidelines provided very little guidance concerning a variety of issues regarding the review of test results and a weight-of-evidence approach benefits from the consideration of more data; early rejection of test results is not a good idea when a weight-of-evidence approach is used.

f. When a potential problem is identified regarding the quality of the result of a toxicity test, it usually means that the test result is questionable, not that it is incorrect. It is certainly appropriate to note test results that are questionable, but is not necessarily appropriate to immediately reject a test result that is questionable.

g. There is uncertainty regarding the results of all scientific studies. Even detailed reports of the results of toxicity tests do not necessarily allow the identification of all potential problems, as demonstrated by the range of results that is sometimes found in round-robin tests. For example, the quality of food is usually unknown, regardless of what information is given regarding the food used. (Even humans occasionally receive unhealthy food from grocery stores and restaurants.)

h. The most important issue regarding the quality of the result of a toxicity test is replication. Questions concerning the validity of a test result are best addressed by repeating the test, especially if the test is repeated in a different laboratory.

i. Test results should be rejected only for sound scientific reasons, not merely on the basis of suspicions or concerns.

j. If a test result is rejected, there is little impetus for anybody to repeat the test.

Appendix 1: Fed and Unfed Acute Tests on Chloride Salts

The table on the last page of this attachment compares the results of fed and unfed acute toxicity tests on NaCl, $CaCl_2$, $MgCl_2$, and KCl. The table contains all of the sets of data from each document that contained comparable acute toxicity data regarding three or more of the four salts listed above.

It is quite possible that different ions, especially chloride and potassium, have different modes of action, have different relative toxicities to different species, and possibly are affected differently by an increase in hardness, calcium, and/or magnesium. (It is possible to obtain an LC50 for NaCl or KCl in soft water, but it is not possible to obtain an LC50 for $CaCl_2$ or $MgCl_2$ in soft water because the test material increases hardness.)

The Mount et al. fed tests with C. dubia and D. magna are at the top of the table with the Biesinger and Christensen D. magna fed tests. The two fed tests with D. magna agree very well for NaCl and for $MgCl_2$ and are almost within a factor of two for $CaCl_2$ and KCl. For all four salts the B&C fed tests gave lower values than the Mount et al. fed tests. Also for all four salts, the B&C unfed tests gave lower results than the B&C fed tests. This might indicate that Mount et al. used a better food than B&C.

For all four salts C. dubia was more sensitive than D. magna in the Mount et al. fed tests. The five ratios are similar for C. dubia and D. magna, but the C. dubia ratio is always equal to or higher than the D. magna ratio. The ratios from the Mount et al. fed tests with C. dubia and D. magna are similar to the ratios from fed and unfed tests using the three species of fish at the bottom of the table:

Ca/Na (n=5): from 0.57 to 0.98 (High/Low = 1.7)
Mg/Na (n = 3): from 0.34 to 0.55 (H/L = 1.6)
K/Na (n = 5): from 0.11 to 0.25 (H/L = 2.3)
Ca/K (n = 5): from >2.19 to 7.12 (H/L = <3.25)
Ca/Mg (n = 3): from 1.78 to 1.87 (H/L = 1.1)

It is interesting that the five ratios are very similar for cladocerans and fishes. It is also interesting that there is no overlap among the Ca/Na, Mg/Na, and K/Na ratios and there is no overlap among the Ca/Na, Ca/K, and Ca/Mg ratios. The apparent toxicity of chloride depends on the other ions that are in the test solutions in a quite reproducible manner.

These data clearly indicate that results of fed tests on these four salts should not be rejected just because the test organisms were fed during the tests. In addition, the best data regarding D. magna are probably from the Mount et al. fed tests. The fed-unfed comparisons by Biesinger and Christensen and Mount et al. demonstrate that either better foods should be developed for cladocerans and/or cladocerans should be fed during all acute tests. If cladocerans are fed during acute toxicity tests, it is possible that the duration of the tests could be increased from 48 hr to 96 hr.

There are several considerations regarding the feeding of cladocerans during acute toxicity tests on NaCl, $CaCl_2$, $MgCl_2$, and KCl:

1. The better the food used, the more healthy the organisms will be at the beginning of the test.
2. Feeding during the test will reduce the possibility of stress due to lack of food.
3. Lower quality organisms and stressed organisms might be more sensitive to these four salts.
4. Because these four salts are not very toxic, the concentrations used in toxicity tests are so high that contamination by one or more heavy metals might be sufficient to stress or kill cladocerans during acute toxicity tests. If contamination by one or more metals causes stress or death, food might sorb or complex the metals and reduce their toxicities.
    a. Cladocerans are more sensitive to some metals than fathead minnows, bluegills, and mosquitofish.
    b. EDTA will complex and detoxify several heavy metals.
5. If food affects the results of acute toxicity tests, it also affects the acute-chronic ratio.
6. All species are fed during chronic toxicity tests.
7. Chloride is not likely to be sorbed or complexed by food.

	Acute Value (mg chloride/L)				Ratio of Acute Values				
	NaCl	CaCl2	MgCl2	KCl	Ca/Na	Mg/Na	K/Na	Ca/K	Ca/Mg
C. dubia Mount fed	1189	1169	655	300	0.98	0.55	0.25	3.90	1.78
D. magna Mount fed	2893	1770	990	314	0.61	0.34	0.11	5.64	1.78
D. magna B&C fed	2806	821	939	150	0.29	0.33	0.05	5.47	0.87
D. magna B&C unfed	2529	92	408	84	0.04	0.16	0.03	1.10	0.23
D. magna* K&R unfed	621	679		128	1.09		0.21	5.30	
D. magna Anderson unfed (64-hr tests)	2232	588	551	205	0.26	0.25	0.09	2.87	1.07
D. magna D&B unfed	3563	1920	2755		0.54	0.77			0.70
T. tubifex** Khangarot	1204	497		737	0.41		0.61	0.67	
Fathead minnow Mount fed	3876	2958	1579	418	0.76	0.41	0.11	7.08	1.87
Mosquitofish A&B unfed	9099	5196		<2378	0.57		<0.26	>2.19	
Bluegill Trama unfed	7853	6804		956	0.87		0.12	7.12	

* This acute value for NaCl is unusually low compared to the other acute values for D. magna.

** These results were considered not acceptable because test temperature was high and the acute value for D. magna in the same water was unusually low.

C. Stephan

Summary of Data Concerning the Acute Toxicity of Sodium Chloride to Aquatic Animals

This summary is based on “Results of Literature Search concerning the Toxicity of Chloride to Aquatic Animals” dated 1-15-09. Except as noted, this summary is consistent with the 1985 Guidelines.

Acute Values and Normalized Acute Values are expressed as mg chloride/L. Normalized Acute Values were calculated by normalizing the Acute Values to hardness = 300 mg/L and sulfate = 65 mg/L using the following equation:

$$\text{NAV} = (\text{AV})\,(300/\text{Hardness})^{0.205797}\,(65/\text{Sulfate})^{-0.07452}$$

This equation is based on the equation presented in “Multiple Regression Equation for Chloride” dated 1-15-09. The hardness of 300 mg/L and the sulfate concentration of 65 mg/L are arbitrary; any other values for hardness and sulfate would have worked equally well. NAVs could not be calculated for all AVs because assumed values were not used for hardness or sulfate. Some of the values of hardness and sulfate are nominal, not measured, values.

Species	Method	Test Material	Hardness (mg/L)	Sulfate (mg/L)	Acute Value	Normalized Acute Value	Reference
Tubificid worm, Limnodrilus hoffmeisteri	S,U	Sodium chloride	100	----	3761	------	Wurtz and Bridges 1961
Tubificid worm, Tubifex tubifex	S,M	Sodium chloride	52	57.9	4278	6083.2	GLEC and INHS 2008
			220	58.9	6008	6357.1	
Leech, Erpobdella punctata	S,U	Sodium chloride	100	----	4550	------	Wurtz and Bridges 1961
Mussel, juvenile Villosa delumbis	S,M	Sodium chloride	169.5	162.7	3173	3821.1	Bringolf et al. 2007
Mussel, juvenile Villosa iris	R,M	Sodium chloride	169.5	162.7	2069	2491.6	Wang 2007

Mussel, juvenile Lampsilis fasciola	S,M	Sodium chloride	169.5	162.7	2414	2907.1	Bringolf et al. 2007
Mussel, juvenile Lampsilis siliquoidea	R,M	Sodium chloride	169.5	162.7	1905	2294.1	Wang 2007
Mussel, juvenile Lampsilis siliquoidea	S,M	Sodium chloride	169.5	162.7	2766	3331.0	Bringolf et al. 2007
Fingernail clam, Sphaerium simile	S,M	Sodium chloride	51	59.9	740	1059.2	GLEC and INHS 2008
			192	61.7	1100	1201.1	
Fingernail clam, Sphaerium tenue	S,U	Sodium chloride	100	----	667	------	Wurtz and Bridges 1961
			20	----	698	------	
Snail, Physa gyrina	F,M	Sodium chloride	84.8	81.4	2540	3350.0	Birge et al. 1985
Snail, Physa heterostropha	S,U	Sodium chloride	100	----	2123	------	Wurtz and Bridges 1961
			100	----	3094	------	
			100	----	3761	------	
			20	----	2487	------	
Snail, Physa sp.	S,M	Sodium chloride	22	15	3247hp	4983.6	Clemens and Jones 1954
Snail, Physa sp.	S,U	Sodium chloride	----	----	>3000p	------	Williams et al. 2000
Snail, Gyraulus circumstriatus	S,U	Sodium chloride	100	----	1941	------	Wurtz and Bridges 1961
Snail,	S,M	Sodium	56	60.9	3078	4326.9	GLEC and INHS 2008

Gyraulus parvus		chloride	212	59.7	3009	3211.4	
Snail, Helisoma campanulata	S,U	Sodium chloride	100	----	3731	------	Wurtz and Bridges 1961
Cladoceran, Ceriodaphnia dubia	S,U	Sodium chloride	84.8	81.4	1189brt	1568.2	Mount et al. 1997
					1042brt	1374.3	
Cladoceran, Ceriodaphnia dubia	R,U	Sodium chloride	74.1	----	1395	------	Cowgill and Milazzo 1990
Cladoceran, Ceriodaphnia dubia	S,U	Sodium chloride	39.2	4.6	507	632.7	Hoke et al. 1992
					447	557.8	
Cladoceran, Ceriodaphnia dubia	S,U	Sodium chloride	39.2	4.6	1395	1740.8	USEPA 1991
			39.2	4.6	1638	2044.1	
			39.2	4.6	1274	1589.8	
			39.2	4.6	1395	1740.8	
			339.0	325.4	1698	1867.0	
Cladoceran, Ceriodaphnia dubia	S,U	Sodium chloride	84.8	81.4	1677c	2211.8	WISLOH 2007
			169.5	162.7	1499c	1805.2	
Cladoceran, Ceriodaphnia dubia	S,U	Sodium chloride	84.8	81.4	1413e	1863.6	Valenti et al. 2007
Cladoceran, Ceriodaphnia dubia	S,M	Sodium chloride	67.1	64.4q	964	1311.1	Harmon et al. 2003
Cladoceran, Ceriodaphnia dubia	S,M	Sodium chloride	30	78.7	947	1542.9	GLEC and INHS 2008
			44	75.9	955	1434.1	
			96	73.7	1130	1442.1	
			180	67.7	1609	1792.8	

			400	78.7	1491	1425.5	
			570	76.2	1907	1690.9	
			800	75.5	1764	1457.7	
			25	69.9	1007	1688.4	
			49	67.8	767	1117.1	
			95	70.3	1369	1744.7	
			194	69.9	1195	1314.3	
			375	68.9	1687	1618.3	
			560	68.3	1652	1458.2	
			792	70.9	1909	1573.5	
			280	28.1	1400	1334.0	
			280	59.6	1720	1733.4	
			280	117	1394	1477.2	
			280	239	1500	1676.5	
			280	482	1109	1306.0	
			280	729	1206	1464.7	
			279	22.9	1311	1231.2	
			276	49.7	1258	1254.4	
			283	107	1240	1302.5	
			281	229	1214	1351.5	
			290	461	1199	1397.2	
			278	694	1179	1428.8	
Cladoceran, Daphnia ambigua	S,M	Sodium chloride	67.1	64.4q	1213	1649.7	Harmon et al. 2003.
Cladoceran, Daphnia magna	S,U	Sodium chloride	84.8	81.4	2893brs	3815.5	Mount et al. 1997
Cladoceran, Daphnia magna	S,U	Sodium chloride	240	----	621	------	Khangarot and Ray 1989
Cladoceran,	S,U	Sodium	39.2	4.6	3038	3791.1	Hoke et al. 1992

Daphnia magna		chloride	39.2 39.2	4.6 4.6	2726 2053	3401.8 2561.9	
Cladoceran, Daphnia magna	-,-	Sodium chloride	----	----	1008k 3319m	------ ------	Cowgill 1987
Cladoceran, Daphnia magna	S,U	Sodium chloride	108.7	13	<2548	<2785.1	Anderson 1946
Cladoceran, Daphnia magna	S,U	Sodium chloride	108.7	13	2232i	2439.7	Anderson 1948
Cladoceran, Daphnia magna	S,U	Sodium chloride	41.5	31.2	3563	5068.2	Dowden and Bennett 1965
Cladoceran, Daphnia magna	S,M	Sodium chloride	45.3	3.9v	2529a,f 2806b,f	3025.9 3357.4	Biesinger and Christensen 1972
Cladoceran, Daphnia magna	S,U	Sodium chloride	169.5	162.7	>2669 <3943d	>3214.2 <4748.4	Seymour et al. 1997
Cladoceran, Daphnia magna	S,U	Sodium chloride	46	3.9v	1880	2242.3	USEPA 1991
Cladoceran, Daphnia magna	S,U	Sodium chloride	169.5	162.7	3944c	4749.6	WISLOH 2007
Cladoceran, Daphnia magna	S,U	Sodium chloride	84.8	81.4	3009e	3968.5	Valenti et al. 2007
Cladoceran, Daphnia magna	S,U	Sodium chloride	106	102	3136 3222 3137	4017.4 4127.5 4018.6	Davies and Hall 2007

Cladoceran, Daphnia pulex	S,M	Sodium chloride	84.8	81.4	1470	1938.8	Birge et al. 1985
Cladoceran, Daphnia pulex	S,U	Sodium chloride	84.8	81.4	1159	1528.6	Palmer et al. 2004
			84.8	81.4	1775	2341.0	
			84.8	81.4	1805	2380.6	
			84.8	81.4	2242	2956.9	
Copepod, Diaptomus clavipes	S,M	Sodium chloride	22	15	2571h	3946.1	Clemens and Jones 1954
Isopod, Asellus communis	S,U	Sodium chloride	100	----	5004	------	Wurtz and Bridges 1961
			20	----	3094	------	
Isopod, Lirceus fontinalis	F,M	Sodium chloride	84.8	81.4	2950	3890.7	Birge et al. 1985
Amphipod, Hyalella azteca	S,U	Sodium chloride	102.5	98.4	3947	5077.7	Lasier et al. 1997
Amphipod, Gammarus pseudolimnaeus	S,U	Sodium chloride	----	----	>3000	------	Williams et al. 2000
Amphipod, Crangonyx sp.	S,U	Sodium chloride	----	----	>3000	------	Williams et al. 2000
Crayfish, Cambarus sp.	S,M	Sodium chloride	22	15	10557h	16203.2	Clemens and Jones 1954
Dragonfly, Libellulidae	S,M	Sodium chloride	22	15	9671h	14843.4	Clemens and Jones 1954
Damselfly,	S,U	Sodium	100	----	14558	------	Wurtz and Bridges 1961

Agria sp.		chloride	20	----	13952	------	
Stonefly, Nemoura trispinosa	S,U	Sodium chloride	----	----	>3000	------	Williams et al. 2000
Caddisfly, Lepidostoma sp.	S,U	Sodium chloride	----	----	>3000	------	Williams et al. 2000
Caddisfly, Parapsyche sp.	S,U	Sodium chloride	----	----	>3000	------	Williams et al. 2000
Midge, Chironomus attenuatus	S,U	Sodium chloride	----	----	4850	------	Thornton and Sauer 1972
American eel, Anquilla rostrata	S,U	Sodium chloride	42.4	40.7	10846	15667.3	Hinton and Eversole 1978
American eel, Anquilla rostrata	S,U	Sodium chloride	42.4	40.7	13012	18796.2	Hinton and Eversole 1979
Goldfish, Carassius auratus	S,M	Sodium chloride	148.8	----	9465	------	Threader and Houston 1983
Red shiner, Notropis lutrensis	S,M	Sodium chloride	22	15	5771g	8857.5	Clemens and Jones 1954
					5920g	9086.2	
Fathead minnow, Pimephales promelas	S,U	Sodium chloride	39.2	4.6	2790	3481.7	USEPA 1991
			39.2	4.6	2123	2649.3	
			339.0	325.4	2244	2467.3	
Fathead minnow, Pimephales promelas	F,M	Sodium chloride	84.8	81.4	6570	8665.1	Birge et al. 1985

Fathead minnow, Pimephales promelas	S,M	Sodium chloride	22	15	5288g 5431g	8116.2 8335.7	Clemens and Jones 1954
Fathead minnow, Pimephales promelas	S,U	Sodium chloride	84.8	81.4	3876br	5112.0	Mount et al. 1997
Fathead minnow, Pimephales promelas	S,U	Sodium chloride	84.8 169.5	81.4 162.7	4167c 4127c	5495.8 4970.0	WISLOH 2007
Black bullhead, Ameiurus melas	S,M	Sodium chloride	22	15	4849g	7442.4	Clemens and Jones 1954
Rainbow trout, Oncorhynchus mykiss	S,U	Sodium chloride	22.4	----	>485j	------	Camargo and Tarazona 1991
Rainbow trout, Oncorhynchus mykiss	F,M	Sodium chloride	46	3.9v	6743	8042.6	Spehar 1986,1987
Rainbow trout, Oncorhynchus mykiss	R,U	Sodium chloride	284	----	12363	------	Vosyliene et al. 2006
Brown trout, Salmo trutta	S,U	Sodium chloride	22.4	----	>607j	------	Camargo and Tarazona 1991
Plains killifish, Fundulus kansae	S,M	Sodium chloride	22	15	9706g	14897.1	Clemens and Jones 1954
Mosquitofish, Gambusia affinis	S,M	Sodium chloride	22	15	6472g	9933.4	Clemens and Jones 1954
Mosquitofish, Gambusia affinis	S,U	Sodium chloride	----	14.9	9099	------	Al-Daham and Bhatti 1977

Threespine stickleback, Gasterosteus aculeatus	R,M	Sodium chloride	84.8	81.4	10200b	13452.6	Garibay and Hall 2004
Green sunfish, Lepomis cyanellus	S,M	Sodium chloride	22	15	6499g	9974.9	Clemens and Jones 1954
Bluegill, Lepomis macrochirus	F,M	Sodium chloride	84.8	81.4	5840	7702.3	Birge et al. 1985
Bluegill (3.7 g), Lepomis macrochirus	S,U	Sodium chloride	44.3	15.5	7853	10461.6	Academy of Natural Sciences 1960; Patrick et al. 1968; Trama 1954
Chorus frog, Pseudacris sp.	R,M	Sodium chloride	84.8	81.4	3553	4686.0	Garibay and Hall 2004

a = not fed. (All tests not marked “a” or “b” were unfed tests.)
b = fed.
c = mean of at least 15 LC50s.
d = range of several toxicity tests.
e = mean of 32 tests.
f = not used because there is reason to suspect that the daphnids might have been unhealthy.
g = tables 4, 7, and 9, except for tests at 28C in table 4.
h = tables 8 and 11; Daphnia pulex tests were not used because test duration was 96 hr.
i = test duration was 64 hr.
j = no deaths in 196 hr.
k = selenium deficient.
m = selenium sufficient.
p = not used in calculation of GMAV because the species is unknown and so it is not known how to combine this acute value with the acute values for which the species are known.
q = calculated using the formula for reconstituted water and the reported average measured hardness.
r = concentrations were measured in stock solutions.
s = not acclimated to the dilution water.

t = might not have been acclimated to the dilution water.
v = based on analyses of samples of Lake Superior water taken in the spring and fall of 2008.

Supplementary information concerning the results of toxicity tests on chloride

1. The atomic weights used are those given on the website of the National Institute of Standards and Technology:
    Calcium = 40.078
    Carbon = 12.011
    Chlorine = 35.453
    Hydrogen = 1.008
    Iron = 55.845
    Magnesium = 24.305
    Nitrogen = 14.007
    Oxygen = 15.999
    Potassium = 39.098
    Sodium = 22.990
    Sulfur = 32.065

2. The molecular weights used are:
    $CaCl_2$ = 110.984 (63.89 % chloride) (36.11 % calcium) (Cl/Ca = 1.769)
    $CaCl_2 \cdot 2H_2O$ = 147.014 (27.26 % calcium) (48.23 % chloride)
    $CaCO_3$ = 100.086 (40.04 % calcium)
    CaO = 56.077 (71.47 % calcium)
    $Ca(NO_3)_2$ = 164.086 (24.42 % calcium)
    $Ca(NO_3)_2 \cdot 4H_2O$ = 236.146 (16.97 % calcium)
    $CaSO_4$ = 136.139 (70.56 % sulfate)
    $CaSO_4 \cdot 2H_2O$ = 172.169 (55.79 % sulfate) (23.28 % calcium) (20.93 % water)
    $FeCl_3 \cdot 6H_2O$ = 270.294 (39.35 % chloride)
    H_2O = 18.015
    KCl = 74.551 (47.56 % chloride) (52.44 % potassium) (Cl/K = 0.9068)
    K_2SO_4 = 174.257 (55.13 % sulfate)
    $MgCl_2$ = 95.211 (74.47 % chloride) (25.53 % magnesium) (Cl/Mg = 2.917)
    $MgSO_4$ = 120.366 (79.81 % sulfate) (20.19 % magnesium)
    $MgSO_4 \cdot 7H_20$ = 246.471 (38.97 % sulfate) (9.86 % magnesium)
    $NaCl$ = 58.443 (60.66 % chloride) (39.34 % sodium) (Cl/Na = 1.542)
    Na_2SO_4 = 142.041 (67.63 % sulfate)

$Na_2SO_4 \cdot 10H_2O$ = 322.191 (29.81 % sulfate)
SO_4 = 96.061

3. Hardness (as $CaCO_3$) = (100.086/40.078)(Ca) + (100.086/24.305)(Mg) = 2.497(Ca) + 4.118(Mg)

4. Trama (1954), Cairns and Scheier (1959), Academy of Natural Sciences (1960), and Patrick et al. (1968) all reported results of toxicity tests that were performed at the Academy of Natural Sciences of Philadelphia with the bluegill in dilution waters that were very similar:

	mg/L
KCl	20
Na_2SiO_3	20
$NaHCO_3$	40
$MgSO_4 \cdot 7H_2O$	40
$Ca(NO_3)_2$	30*
$CaCO_3$	10
K_2HPO_4	10 or 2
Fe^{+++} (as ferric citrate)	4 or 0.4

Ca = 11.3 mg/L
Mg = 3.9 mg/L
Hardness = 44.3 mg/L as $CaCO_3$
Chloride = 9.5 mg/L
Sulfate = 15.5 mg/L

*Long after the tests of concern were performed, this was reported to be 40 mg/L of $Ca(NO_3)_2 \cdot 4H_20$.

5. Freeman (1953), Freeman and Fowler (1953), Fairchild (1955), Dowden (1960), Dowden (1962), and Dowden and Bennett (1965) all contained information regarding toxicity tests performed at Louisiana State University in Baton Rouge, but several different dilution waters were used. Dowden and Bennett (1965) tried to clarify the most important dilution waters used, but the citations given for the waters on page 1310 need to be clarified. Reference 4 is the correct citation for “Standard Reference Water” (SRW), but the correct citation for “Reference Dilution Water” (RDW) is reference 6 (not reference 3) and reference 3 should be cited for “glass-wool filtered University Lake Water (ULW). ULW is considered an unacceptable dilution water because it is from “a small drainpipe-fed lake on the campus of Louisiana State University” (Dowden 1960). The compositions of SRW and RDW are:

SRW	mg/L
$MgSO_4 \cdot 7H_2O$	71.

K_2SO_4	6.5
$MnSO_4 \cdot 4H_2O$	0.2
$CaCl_2 \cdot 2H_2O$	18.6
$NaHCO_3$	25.
NH_4NO_3	3.
$K_2HPO_4 \cdot 3H_2O$	1.1
CaO	32.2
$Na_2SiO_3 \cdot 9H_2O$	62.6
$FeCl_3 \cdot 6H_2O$	1.2

Ca = 23.0 mg/L
Mg = 7.0 mg/L
Hardness = 86.2 mg/L as $CaCO_3$
Chloride = 9.4 mg/L
Sulfate = 31.2 mg/L

RDW	mg/L
$CaCl_2$	110
$NaHCO_3$	110
NaCl	100
$MgSO_4 \cdot 7H_2O$	60
KCl	20

Ca = 39.7 mg/L
Mg = 5.9 mg/L
Hardness = 123.4 mg/L as $CaCO_3$
Chloride = 140.4 mg/L
Sulfate = 23.4 mg/L

6. When known, the concentration of chloride in dilution water was negligible in tests on chloride.

7. Karraker (2007) says that “road salt” contains sodium chloride, sodium ferrocyanide, heavy metals, and often sand or cinder. Results of toxicity tests on “road salt” were not used.

8. Hyalella azteca appears to be especially sensitive to some pollutants when the concentration of chloride is low, e.g., lower than 25 mg/L. This should not affect the sensitivity of this species to chloride.

9. Mount et al. (1997) reported that the toxicities of sodium and calcium salts to C. dubia, D. magna, and the fathead minnow are primarily attributable to the corresponding anion.

10. Some data presented in Table 1 of USEPA (1988) have been changed:
    a. Several values differ because of roundoff differences.
    b. USEPA (1988) used results of short acute tests for the reasons given on pages 2 and 3 (see also Lowell et al. 1995), but results of short tests are not used here because short acute tests sometimes give higher LC50s than standard tests.
    c. Data from Dowden (1961, which should be 1960) and Kostecki and Jones (1983) are not used here because of the dilution water used in the tests.
    d. The test results from Trama (1954) are also given in Academy of Natural Sciences (1960) and Patrick et al. (1968).
    e. Hamilton et al. (1975) did not adequately acclimate the midges.
    f. Fed acute tests were not used in USEPA (1988), but fed acute tests are used here and are given preference over unfed acute tests when the test organisms are cladocerans.

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Multiple Regression Equation for Chloride

Grizzle and Mauldin (1995; 24-hr tests using striped bass) and Lasier et al. (2006; 7-day survival tests using Ceriodaphnia dubia) reported that calcium and/or magnesium reduced the toxicity of chloride, whereas MacGregor et al. (1986) reported that the toxicity of NaCl was not significantly affected by hardness. Davies and Hall (2007) reported that the Ca-Mg ratio did not affect the toxicity of either NaCl or KCl to D. magna. Because of these incomplete and potentially conflicting reports, U.S. EPA hired the Great Lakes Environmental Center (GLEC) and the Illinois Natural History Survey (INHS) to perform toxicity tests concerning the effect of hardness and sulfate on the toxicity of chloride to selected aquatic animals.

The equations presented in Figures 1 through 7 of the GLEC and INHS (2008) report can be used to make the aquatic life criterion for chloride dependent on both hardness and sulfate. However, although the LC50s used in Figures 1 through 7 are based on measured concentrations of chloride, the hardnesses and the concentrations of sulfate are nominal, not measured, values. Nevertheless, for Ceriodaphnia dubia Figure 3 shows that a log-log plot gives a straighter line than the plots given in Figures 1 and 2. A straight line fits the sulfate data in Figures 4, 5, and 6 equally well. Because a log-log plot will be used for the hardness data, a log-log plot will be used for the sulfate data.

Figure 7 gives log-log hardness equations for three other species, based on nominal hardnesses. The intercepts of the four log-log hardness equations differ because the species have different sensitivities. For the purpose of making a chloride criterion dependent on hardness, the important differences between the four equations are the exponents.

C. dubia	0.2144
S. simile	0.286
G. parvus	-0.0164
T. tubifex	0.245

The exponent for C. dubia is based on seven LC50s from GLEC and seven LC50s from INHS, whereas each of the other exponents is based on two LC50s from one laboratory. In addition, the exponent for C. dubia is close to the mean of the other three exponents. Therefore, it seems reasonable to set the chloride hardness exponent equal to the chloride hardness exponent obtained with C. dubia.

Multiple regression was performed on the data presented on pages 29 and 36 of GLEC and INHS (2008) concerning the effects of hardness and sulfate on the toxicity of chloride to C. dubia. The analysis was performed using the natural logs of the hardnesses, concentrations of sulfate, and LC50s and using the assumption that the effects of hardness and sulfate on the toxicity of chloride are proportional effects, not additive effects. The following hardnesses, concentrations of sulfate, and LC50s were used:

Hardness	Sulfate	LC50
30	78.7	947
44	75.9	955
96	73.7	1130
180	67.7	1609
400	78.7	1491
570	76.2	1907
800	75.5	1764
25	69.9	1007
49	67.8	767
95	70.3	1369
194	69.9	1195
375	68.9	1687
560	68.3	1652
792	70.9	1909
280	28.1	1400
280	59.6	1720
280	117	1394
280	239	1500
280	482	1109
280	729	1206
279	22.9	1311
276	49.7	1258
283	107	1240
281	229	1214
290	461	1199
278	694	1179

The resulting equation for C. dubia is:

$$\text{LC50} = (616.67 \text{ mg chloride/L})(\text{hardness})^{0.205797}(\text{sulfate})^{-0.07452}$$

where the units are:

LC50: mg chloride/L
hardness: mg $CaCO_3$/L
sulfate: mg sulfate/L.

As noted above, the C. dubia data concerning the effect of hardness on the chloride LC50 are supported by data in GLEC and INHS (2008) for three other species: a fingernail clam (Sphaerium simile), a tubificid worm (Tubifex tubifex), and a snail (Gryaulus parvus). However, no data for other species were presented in GLEC and INHS (2008) supporting the C. dubia data concerning the effect of sulfate on the chloride LC50.

WISLOH (2007) and USEPA (1991) present the following chloride LC50s in low-hardness, low-sulfate dilution water and in high-hardness, high-sulfate dilution water for C. dubia and for the fathead minnow (see “Summary of Data concerning the Acute Toxicity of Sodium Chloride to Aquatic Animals” dated 1-15-09):

Species	Hardness (mg/L)	Sulfate (mg/L)	Acute Value (mg Cl/L)	Reference
Cladoceran	39.2	4.6	1395	USEPA 1991
C. dubia	39.2	4.6	1638	
	39.2	4.6	1274	
	39.2	4.6	1395	
	339.0	325.4	1698	
Cladoceran	84.8	81.4	1677	WISLOH 2007
C. dubia	169.5	162.7	1499	
Fathead minnow	39.2	4.6	2790	USEPA 1991
P. promelas	39.2	4.6	2123	
	339.0	325.4	2244	
Fathead minnow	84.8	81.4	4167	WISLOH 2007
P. promelas	169.5	162.7	4127	

To facilitate use of these data, geometric mean LC50s were calculated from replicate LC50s.

If the multiple-regression equation based on the C. dubia data in GLEC and INHS (2008) is used to adjust the low-hardness, low-sulfate LC50 to the corresponding high hardness and high concentration of sulfate, the results are:

	Predicted LC50	Measured LC50	M - P*	Percent Difference**
C. dubia				
WISLOH (2007):	1837	1499	-338	-20.3%
USEPA (1991):	1612	1698	+ 86	5.2%
Fathead minnow				
WISLOH (2007):	4563	4127	-436	-10.0%
USEPA (1991):	2762	2244	-518	-20.7%

* M - P = (Measured LC50) - (Predicted LC50)

** Percent Difference = 100 (Measured - Predicted)/([Measured + Predicted]/2)

Each predicted LC50 is within a factor of two of the corresponding measured LC50 and so all four differences could be due to experimental variation. However, three of the four differences are in the same direction and the difference in the other direction is smaller.

There is a more direct way to relate the WISLOH (2007) and USEPA (1991) data for C. dubia and the fathead minnow to the multiple-regression equation. The relationship regarding the effect of hardness on the chloride LC50 is supported by data for four species, so it is reasonable to use this relationship to adjust the low-hardness LC50 to the high hardness. Because the low-hardness LC50 is at low sulfate, the predicted high-hardness LC50 is also at low sulfate. Therefore, the predicted high hardness, low sulfate LC50 and the measured high hardness, high sulfate LC50 can be used to calculate an exponent for sulfate. (An exponent in an arithmetic equation is a slope in a log-log plot.) The resulting sulfate exponents are:

C. dubia
    WISLOH (2007): sulfate exponent = -0.368
    USEPA (1991): sulfate exponent = -0.062

Fathead minnow
    WISLOH (2007): sulfate exponent = -0.220
    USEPA (1991): sulfate exponent = -0.123

The sulfate exponent from the multiple-regression analysis of the GLEC and INHS (2008) C. dubia data was -0.07452. This is in the range of the new C. dubia exponents, but is substantially lower than one of the new C. dubia exponents and is lower than both of the new fathead minnow exponents; the mean of the four new exponents is -0.193. Charlie Delos and Chuck Stephan interpret these exponents to mean that WISLOH (2007) and USEPA (1991) provide data for both C. dubia and the fathead minnow that support the concepts that (i) 0.205797 is a reasonable value for the hardness exponent, (ii) an increase in the concentration of sulfate causes a decrease in the chloride LC50 and (iii) the sulfate exponent might be more negative than indicated by the GLEC and INHS (2008) data.

The above calculations use multiple regression; covariance analysis is not used for several reasons. Data are available for C. dubia from two labs whereas data are available for the other three species from only one lab. Covariance analysis would do a good job of weighting the data by species, but it is not clear that it would do a good job of taking into account the fact that data for C. dubia are available from two labs. Also, many more data points are available for C. dubia than for all of the other species combined, so covariance analysis would give much more weight to C. dubia than to the other three species, which is what the above calculations do. Further, the exponent for C. dubia is close to the mean of the exponents for the other three species. For these reasons it is likely that covariance analysis would give a pooled exponent that is close to the exponent for C. dubia. In addition, data concerning sulfate are available only for C. dubia, so it seems desirable to do multiple regression of the C. dubia data rather than doing covariance analysis of the hardness data and then trying to find a way to integrate the sulfate exponent with the hardness exponent. It is possible that multiple regression and covariance analysis could be used together, but a better approach might be to use GLM or GLiM. However, it is possible that the available data do not satisfy the assumptions of any of these techniques.

Calculation of Aquatic Life Criteria for Chloride

These calculations are based on “Summary of Data Concerning the Acute Toxicity of Sodium Chloride to Aquatic Animals” dated 1-15-09 and “Summary of Data Concerning the Chronic Toxicity of Sodium Chloride to Aquatic Animals” dated 1-15-09. Except as noted (for example, see footnote a), these calculations are consistent with the 1985 Guidelines. GMAVs and SMAVs are normalized to hardness = 300 mg/L and sulfate = 65 mg/L. GMAVs and SMAVs are expressed as mg chloride/L.

Rank*	GMAV	Genus	Species	SMAV	SMACR
	-----	Agria	Damselfly, Agria sp.	-----	
23	17161	Anquilla	American eel, Anquilla rostrata	17160.6	
	16203	Cambarus	Crayfish, Cambarus sp.	16203.2	
	14897	Fundulus	Plains killifish, Fundulus kansae	14897.1	
	14843	Libellulidae**	Dragonfly, Libellulidae	14843.4	
	13453	Gasterosteus	Threespine stickleback, Gasterosteus aculeatus	13452.6	
	-----	Carassius	Goldfish, Carassius auratus	-----	
	9933	Gambusia	Mosquitofish,	9933.4	

		Gambusia affinis		
9157	Lepomis	Green sunfish, Lepomis cyanellus	9974.9	
		Bluegill, Lepomis macrochirus	8406.5e	
8971	Notropis	Red shiner, Notropis lutrensis	8971.1	
8043	Oncorhynchus	Rainbow trout, Oncorhynchus mykiss	8042.6	7.308
-----	Chironomus	Midge, Chironomus attenuatus	-----	
7442	Ameiurus	Black bullhead, Ameiurus melas	7442.4	
-----	Erpobdella	Leech, Erpobdella punctata	----	
6515	Pimephales	Fathead minnow, Pimephales promelas	6515.3f	15.17h
6219	Tubifex	Tubificid worm, Tubifex tubifex	6218.6	
5078	Hyalella	Amphipod, Hyalella azteca	5077.7	
-----	Asellus	Isopod,	-----	

		Asellus communis	
-----	Limnodrilus	Tubificid worm, Limnodrilus hoffmeisteri	-----
-----	Helisoma	Snail, Helisoma campanulata	-----
4686	Pseudacris	Chorus frog, Pseudacris sp.	4686.0
-----	Gammarus	Amphipod, Gammarus pseudolimnaeus	-----
-----	Crangonyx	Amphipod, Crangonyx sp.	-----
-----	Nemoura	Stonefly, Nemoura trispinosa	-----
-----	Lepidostoma	Caddisfly, Lepidostoma sp.	-----
-----	Parapsyche	Caddisfly, Parapsyche sp.	-----
3946	Diaptomus	Copepod, Diaptomus clavipes	3946.1
3891	Lirceus	Isopod, Lirceus fontinalis	3890.7
3728	Gyraulus	Snail,	-----

			Gyraulus circumstriatus		
			Snail, Gyraulus parvus	3727.7	
	3350	Physa	Snail, Physa gyrina	3350.0	
			Snail, Physa heterostropha	-----	
	3086	Villosa	Mussel, Villosa delumbis	3821.1	
			Mussel, Villosa iris	2491.6	
4	2835	Lampsilis	Mussel, Lampsilis fasciola	2907.1	
			Mussel, Lampsilis siliquoidea	2764.4	
3	2326	Daphnia	Cladoceran, Daphnia ambigua	1649.7	4.148
			Cladoceran, Daphnia magna	3773.1d	1.974
			Cladoceran, Daphnia pulex	2020.5g	3.952
2	1542	Ceriodaphnia	Cladoceran,	1542.3c	>2.470i

			Ceriodaphnia dubia	
1	1128	Sphaerium	Fingernail clam, Sphaerium simile	1127.9
			Fingernail clam, Sphaerium tenue	-----

* A “greater than” acute value for the brown trout (Salmo trutta) is not in this table because it is too low to be a useful “greater than” value.
** Name of family, not name of genus.

a. Section IV.I of the 1985 Guidelines says: “For each species for which at least one acute value is available, the Species Mean Acute Value should be calculated as the geometric mean of the results of all flow-through tests in which the concentrations of test material were measured. For a species for which no such result is available, the SMAV should be calculated as the geometric mean of all available acute values, i.e., results of flow-through tests in which the concentrations were not measured and results of static and renewal tests based on initial concentrations (nominal concentrations are acceptable for most test materials if measured concentrations are not available) of test material.” The guidance presented in section IV.I of the 1985 Guidelines seems inappropriate for chloride because chloride is different from most pollutants for which aquatic life criteria are derived. Chloride is very soluble in water, does not oxidize or reduce, is not volatile, does not degrade, does not sorb to test chambers, test organisms, food, or waste products, is not complexed by materials that commonly occur in water, is not involved in a pH-dependent equilibrium in water, and does not precipitate in waters in which aquatic organisms commonly occur.
   i. For chloride, as long as the concentration of dissolved oxygen is sufficiently high, it seems appropriate to give static and renewal acute tests the same weight as flow-through acute tests in the derivation of the SMAV for a species.
   ii. For chloride, it seems inappropriate to give measured acute tests a weight of 1 and unmeasured acute tests a weight of 0 when both are available for the derivation of the SMAV for a species. For example, if there is a choice between one measured acute test on chloride and three unmeasured acute tests in three different laboratories, the three tests are probably preferable to the one test, but if the choice is between one measured acute test and two unmeasured acute tests in two different laboratories, the one test is probably preferable. Thus, for a species for which both measured and unmeasured acute tests are available for chloride, it seems appropriate to give measured acute tests a weight of 2.5 and unmeasured acute tests a weight of 1 when the SMAV is calculated.

The conclusions described above concerning chloride were developed during discussions among Charles Delos, Charles Stephan, and Glen Thursby. For other pollutants, different conclusions concerning the relative merits of static, renewal, and flow-through acute toxicity tests and the relative merits of measured and unmeasured acute toxicity tests are likely to be more appropriate.

b. For Ceriodaphnia dubia, the acute values from Hoke et al. (1992) are considered outliers. The geometric mean is 1468.1 for Mount et al. (1997), 1790.2 for U.S. EPA (1991), 1998.2 for WISLOH (2007), and 1457.3 for GLEC and INHS (2008).
SMAV = antilog ([log 1468.1 + log 1790.2 + log 1998.2 + log 1863.6 + 2.5(log 1311.1) + 2.5(log 1457.3)]/9) = 1542.3.

c. For Daphnia magna, the values of 3815.5 (Mount et al. 1997), <2785.1, (Anderson 1946), 2439.7 (Anderson 1948), and 3025.9 and 3357.4 (Biesinger and Christensen 1972) were not used. A geometric mean of 3906.7 was calculated from the limits given by Seymour et al. (1997). The geometric mean is 3208.8 for Hoke et al. (1992) and is 4054.2 for Davies and Hall (2007). The SMAV is 3773.1, which is the geometric mean of 3208.8, 5068.2, 3906.7, 2242.3, 4749.6, 3968.5, and 4054.2.

d. Bluegill: SMAV = antilog ([2.5(log 7702.3) + log 10461.6]/3.5) = 8406.5.

e. Fathead minnow: SMAV = antilog([log 2833.9 + 2.5(log 8665.1) + 2.5(log 8225.2) + log 5112.0 + log 5226.3]/8) = 6515.3.

f. Daphnia pulex: SMAV = antilog([2.5(log 1938.8) + log 2240.3]/3.5) = 2020.5.

g. Not used in calculations because, even though the acute and chronic tests were in the same document, different dilution waters were used in the tests.

h. The SMACR for Ceriodaphnia dubia is the geometric mean of 1.508, >3.841, and 2.601.

FAV = 1205 mg chloride/L

CMC = FAV/2 = 602.5 mg chloride/L

The five SMACRs (7.308, 4.148, 1.974, 3.952, and >2.438) that are available for use in calculations result in three GMACRs:

7.308	Oncorhynchus
3.187	Daphnia
>2.470	Ceriodaphnia

The 1985 Guidelines require ACRs for species in three different families, but Daphnia and Ceriodaphnia are in the same family. However, even though the ACR for the fathead minnow should not be used in calculations because the acute and chronic tests using the fathead minnow were performed in different dilution waters, the fathead minnow ACR can be considered a qualitative ACR and used to satisfy the MDRs because chloride is not likely to be complexed or sorbed or detoxified by organic or inorganic constituents of the dilution water.

The GMACRs for Oncorhynchus and Daphnia are consistent with the “greater than” GMACR for Ceriodaphnia and the GMACRs are within a factor of ten. Therefore, the Final ACR = 4.826, which is the geometric mean of the GMARCs for Oncorhynchus and Daphnia. This would give FCV = FAV/FACR = (1205 mg chloride/L)/4.826 = 249.7 mg chloride/L. However, this approach is contraindicated because the GMACRs (including the unused GMACR for Pimephales) indicate that the GMACR increases as the GMAV increases.

The GMACR for Daphnia is consistent with the “greater than” GMACR for Ceriodaphnia, so the GMACR for Daphnia can be used as the FACR. Therefore, FACR = 3.187 and FCV = FAV/FACR = (1205 mg chloride/L)/3.187 = 378.1 mg chloride/L.

CCC = FCV = 378.1 mg chloride/L.

The CMC and CCC given above are for hardness = 300 mg/L and sulfate = 65 mg/L. The equation that was used to normalize the acute values can be used to make the CMC and CCC dependent on hardness and sulfate. The resulting equations for the CMC and CCC are:

$$\begin{aligned}\text{CMC} &= (602.5\ \text{mg chloride/L})\ (\text{hardness}/300)^{0.205797}\ (\text{sulfate}/65)^{-0.07452}\\ &= (254.3\ \text{mg chloride/L})\ (\text{hardness})^{0.205797}\ (\text{sulfate})^{-0.07452}\end{aligned}$$

At hardness = 300 mg/L and sulfate = 65 mg/L, CMC = 602.5 mg chloride/L.

$$\begin{aligned}\text{CCC} &= (378.1\ \text{mg chloride/L})\ (\text{hardness}/300)^{0.205797}\ (\text{sulfate}/65)^{-0.07452}\\ &= (159.6\ \text{mg chloride/L})\ (\text{hardness})^{0.205797}\ (\text{sulfate})^{-0.07452}\end{aligned}$$

At hardness = 300 mg/L and sulfate = 65 mg/L, CCC = 378.1 mg chloride/L.

Derivation of an Alternative FCV

Even though the above derivation of FCV = 378.1 mg chloride /L follows the procedure described in the 1985 Guidelines, there is an alternative approach that is justified on the basis of the “good science” clause in section XII.B of the 1985 Guidelines. This approach is based on the fact that the four low SMACRs for chloride were obtained with invertebrates, whereas the high SMACR was obtained with a vertebrate. This can be interpreted to mean that vertebrates have a higher ACR, on the average, than invertebrates, especially because the qualitative ACR for the fathead minnow is 15.17. Therefore, a vertebrate ACR and an invertebrate ACR can be used with the GMAVs to calculate a predicted Genus Mean Chronic Value for each genus, and then a FCV can be calculated directly from the predicted GMCVs. This approach calculates and uses a predicted chronic value for each genus for which an acute value is available and probably does a better job of taking into account the chronic sensitivities of both vertebrates and invertebrates to chloride. The relevant data and calculations are presented on the next few pages.

The FACR of 3.187 derived above was derived from all of the acceptable ACRs for invertebrates. The only acceptable ACR for a vertebrate is 7.308. A predicted GMCV can be calculated from each GMAV by using 3.187 as the invertebrate ACR and using 7.308 as the vertebrate ACR.

Table of predicted GMCVs for Chloride

(GMAVs and pGMCVs are expressed as mg chloride/L)
(ranked according to predicted GMCVs)

Rank	GMAV	Genus	Species	pGMCV
	-----	Agria	Damselfly, Agria sp.	-----
23	16203	Cambarus	Crayfish, Cambarus sp.	5084
	14843	Libellulidae*	Dragonfly, Libellulidae	4657

17161	Anquilla	American eel, Anquilla rostrata	2348
6219	Tubifex	Tubificid worm, Tubifex tubifex	1951
-----	Chironomus	Midge, Chironomus attenuatus	-----
-----	Erpobdella	Leech, Erpobdella punctata	-----
13453	Gasterosteus	Threespine stickleback, Gasterosteus aculeatus	1841
14897	Fundulus	Plains killifish, Fundulus kansae	2038
-----	Carassius	Goldfish, Carassius auratus	-----
5078	Hyalella	Amphipod, Hyalella azteca	1593
----	Asellus	Isopod, Asellus communis	-----
-----	Limnodrilus	Tubificid worm, Limnodrilus hoffmeisteri	-----
-----	Helisoma	Snail, Helisoma campanulata	-----

8043	Oncorhynchus	Rainbow trout, Oncorhynchus mykiss	1101
9933	Gambusia	Mosquitofish, Gambusia affinis	1359
-----	Gammarus	Amphipod, Gammarus pseudolimnaeus	-----
-----	Crangonyx	Amphipod, Crangonyx sp.	-----
-----	Nemoura	Stonefly, Nemoura trispinosa	-----
-----	Lepidostoma	Caddisfly, Lepidostoma sp.	-----
-----	Parapsyche	Caddisfly, Parapsyche sp.	-----
3891	Lirceus	Isopod, Lirceus fontinalis	1221
9157	Lepomis	Green sunfish, Lepomis cyanellus	1253
		Bluegill, Lepomis macrochirus	
3350	Physa	Snail, Physa heterostropha	1051

		Snail, Physa gyrina	
3946	Diaptomus	Copepod, Diaptomus clavipes	1238
3086	Villosa	Mussel, Villosa delumbis	968.3
		Mussel, Villosa iris	
8971	Notropis	Red shiner, Notropis lutrensis	1228
3728	Gyraulus	Snail, Gyraulus circumstriatus	1170
		Snail, Guraulus parvus	
2835	Lampsilis	Mussel, Lampsilis fasciola	889.6
		Mussel, Lampsilis siliquoidea	
7442	Ameiurus	Black bullhead, Ameiurus melas	1018
6515	Pimephales	Fathead minnow, Pimephales promelas	891.5

4	2326	Daphnia	Cladoceran, Daphnia ambigua	729.8
			Cladoceran, Daphnia magna	
			Cladoceran, Daphnia pulex	
3	4686	Pseudacris	Chorus frog, Pseudacris sp.	641.2
2	1542	Ceriodaphnia	Cladoceran, Ceriodaphnia dubia	483.8
1	1128	Sphaerium	Fingernail clam, Sphaerium simile	353.9
			Fingernail clam, Sphaerium tenue	

* Name of family, not name of genus.

FCV based on predicted GMCVs = 382.7 mg chloride/L at hardness = 300 mg/L and sulfate = 65 mg/L.

$$\text{CCC} = (382.7 \text{ mg chloride/L})\ (\text{hardness}/300)^{0.205797}\ (\text{sulfate}/65)^{-0.07452}$$
$$= (161.5 \text{ mg chloride/L})\ (\text{hardness})^{0.205797}\ (\text{sulfate})^{-0.07452}$$

At hardness = 300 mg/L and sulfate = 65 mg/L, CCC = 382.7 mg chloride/L.

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Acute toxicity of sodium chloride and potassium chloride to a unionid mussel (*Lampsilis siliquoidea*) in water exposures

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Abstract

Freshwater mussels (order Unionoida) are one of the most imperiled groups of animals in the world. However, many ambient water quality criteria and other environmental guideline values do not include data for freshwater mussels, in part because mussel toxicity test methods are comparatively new and data may not have been available when criteria and guidelines were derived. The objectives of the present study were to evaluate the acute toxicity of sodium chloride (NaCl) and potassium chloride (KCl) to larvae (glochidia) and/or juveniles of a unionid mussel (fatmucket, *Lampsilis siliquoidea*) and to determine the potential influences of water hardness (50, 100, 200, and 300 mg/L as $CaCO_3$) and other major ions (Ca, K, SO_4, or HCO_3) on the acute toxicity of NaCl to the mussels. From the KCl test, the 50% effect concentration (EC50) for fatmucket glochidia was 30 mg K/L, similar to or slightly lower than the EC50s for juvenile fatmucket (37–46 mg K/L) tested previously in our laboratory. From the NaCl tests, the EC50s for glochidia increased from 441 to 1597 mg Cl/L and the EC50s for juvenile mussels increased from 911 to 3092 mg Cl/L with increasing water hardness from 50 to 300 mg/L. Increasing K from 0.4 to 1.9 mg/L, SO_4 from 13 to 40 mg/L, or HCO_3 from 44 to 200 mg/L in the 50 mg/L hardness water did not substantially change the NaCl EC50s for juvenile mussels, whereas increasing Ca from 9.9 to 42 mg/L increased the EC50s by a factor of 2. The overall results indicate that glochidia were equally or more sensitive to NaCl and KCl compared with juvenile mussels and that the increased water hardness ameliorated the acute toxicity of NaCl to glochidia and juveniles. These responses rank fatmucket among the most acutely sensitive freshwater organisms to NaCl and KCl. *Environ Toxicol Chem* 2018;37:3041–3049. © 2018 SETAC. This article is a US government work and, as such, is in thepublic domain in the United States of America.

Citing Literature

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Environmental Toxicology and Chemistry

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EVALUATING CHRONIC TOXICITY OF SODIUM CHLORIDE OR POTASSIUM CHLORIDE TO A UNIONID MUSSEL (*LAMPSILIS SILIQUOIDEA*) IN WATER EXPOSURES USING STANDARD AND REFINED TOXICITY TESTING METHODS

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Table S1. Summary of test conditions for conducting chronic toxicity tests with fatmucket (*Lampsilis siliquoidea*) in basic accordance with ASTM (2017)

Parameter	Conditions
Test chemical	KCl or NaCl
Test type	Flow through
Test Duration	4, 8, or 12 weeks
Temperature	20°C (2012 NaCl test only) or 23°C (see text)
Light	Ambient laboratory light; about 500 lux; 16L:8D
Test chamber	300-ml beaker with 200 mL of water (5 ml of sand in exposures with sand; see text)
Water renewal	125 ml of additional water/4 h
Age of test organism	About 1-week, 1-month, or 2-month-old juveniles (see text)
Organisms/chamber	10
Replicates/conc.	4 or 8
Feeding	2 mL of algal mixture 2 to 6 times daily (see text)
Dilution water	Diluted well water (see text)
Test concentrations	5 concentrations plus control; 50% serial dilution (see text)
Chemical analyses	Water samples for Cl or K analyses at the beginning and end of the test and once every week (Cl) or once every 2 weeks (K)
Water quality	Dissolved oxygen, pH, conductivity, hardness, and alkalinity measured weekly in the control, medium, and/or high exposure concentrations. Major cations and anions in control waters measured periodically (see text)
Endpoint	Survival, length, dry weight, biomass
Test acceptability criteria	≥ 80% control survival

Table S2. Mean measured water quality characteristics (standard deviation in parenthesis)[a] in a chronic 4-week KCl test and 4 chronic 4- to 12-week NaCl toxicity tests with different ages of juvenile fatmucket (*Lampsilis siliquoidea*), feeding, and with or without a sand substrate

			Water quality						Major cations and anions (mg/L)						
Test	Test duration (week)	Sand	Number of samples	Dissolved oxygen (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Total ammonia (mg N/L)	Number of samples	Ca	K	Mg	Na	Cl	SO_4
2012 NaCl exposure started with 2-month-old juveniles and fed 2 mL of algal mixture 2 times daily															
Cl-1a[b]	4	No	24-30	8.9 (0.5)	8.2 (0.1)	105 (1.7)	91 (2.3)	0.05 (0.03)	2-3	26 (0.1)	1.1 (0.0)	9.3 (0.0)	13 (0.1)	11 (0.3)	18 (0.4)
Cl-1b[b]	4	Yes													
2013 KCl exposure started with 2-month-old juveniles and fed 2 mL of algal mixture 2 times daily															
K-1a[b]	4	No	17	8.1 (0.3)	8.3 (0.2)	105 (3.5)	94 (2.9)	0.07 (0.03)	1-3	26 (0.8)	1.1 (0.1)	9.2 (0.4)	10 (0.7)	12	18
K-1b[b]	4	Yes													
2014 NaCl exposure started with 2-month-old juveniles and fed 2 mL of algal mixture 6 times daily [c]															
Cl-2a	4	Yes	24-60	8.0 (0.5)	8.1 (0.2)	103 (2.7)	93 (2.6)	0.07 (0.05)	2-10	25 (0.1)	1.1 (0.1)	9.1 (0.1)	12 (0.1)	15 (1.1)	21 (0.0)
Cl-2b	8	Yes	39-108	7.8 (0.5)	8.1 (0.2)	103 (3.7)	95 (3.7)	0.09 (0.05)	4-18	25 (0.2)	1.1 (0.1)	9.0 (0.1)	12 (0.3)	15 (1.2)	20 (0.4)
Cl-2c	12	Yes	63-132	7.8 (0.5)	8.0 (0.2)	104 (4.6)	96 (3.5)	0.09 (0.05)	6-22	26 (0.3)	1.1 (0.1)	9.0 (0.1)	12 (0.4)	15 (1.2)	20 (0.9)
2015 NaCl exposure started with 1-month-old juveniles and fed 2 mL of algal mixture 6 times daily															
Cl-3	4	Yes	4-12	7.8 (0.1)	8.1 (0.1)	98 (1.6)	97 (2.5)	0.10 (0.02)	1	25	1.1	9.0	12	12	19
2015 NaCl exposure started with 1-week-old juveniles and fed 2 mL of algal mixture 6 times daily [c]															
Cl-4a	4	Yes	28-36	7.9 (0.2)	8.1 (0.1)	105 (4.3)	97 (4.5)	0.06 (0.02)	1-5	26	1.1	9.1	12	11 (0.9)	18
Cl-4b	12	Yes	65-79	7.8 (0.3)	8.0 (0.2)	103 (3.5)	96 (4.3)	0.08 (0.03)	2-12	25 (0.3)	1.1 (0.1)	9.0 (0.1)	12 (0.4)	15 (1.8)	18 (0.0)

[a] Water quality was measured in the control, medium, and high exposure concentrations. Major ions measured in the control water.

[b] Composite samples from replicate beakers with or without sand.

[c] Feeding rate was increased by 50% every 4 weeks.

Table S3. Measured potassium concentrations, mean survival (standard deviation in parentheses), and 50% effect concentration (EC50) during the first 4 days of the 4-week KCl toxicity exposure started with 2-month-old juvenile fatmucket (*Lampsilis siliquoidea*)

Nominal (mg K/L)	Measured (mg K/L)	Survival (%; n=4)
Control	1.0	100 (0)
6.3	3.9	100 (0)
13	12	100 (0)
25	24	100 (0)
50	45	15 (5.8)
100	105	0 (0)
EC50 (95% confidence limits)		37 (34-40)[a]

[a] The data did not meet the requirements of the Gaussian distribution model (at least 2 partial responses) in the Toxicity Relationship Analysis Program (version 1.30a, Erickson 2015), Spearman-Karber method was used to determine the EC50 following the flowchart recommended by the USEPA (2002) using TOXSTAT® software (version 3.5, Western EcoSystems Technology).

References:

Erickson RJ. 2015. Toxicity Relationship Analysis Program (TRAP), Ver 1.30a. EPA/600/C-11/002. U.S. Environmental Protection Agency, Washington, DC.

U.S. Environmental Protection Agency. 2002. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 5th ed. EPA-821-R-02-012. Office of Water, Washington, DC.

Table S4. Mean measured water quality characteristics[a] (standard deviation in parenthesis) during the first 4 days of the chronic 4-week KCl toxicity test started with 2-month-old juvenile fatmucket (*Lampsilis siliquoidea*)[a]

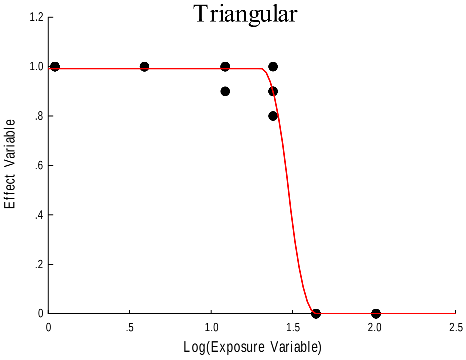
Dissolved oxygen (mg/L; n=6)	pH (n=6)	Hardness (mg/L as $CaCO_3$; n=6)	Alkalinity (mg/L as $CaCO_3$; n=6)	Major cation and anion (mg/L; n=1) Ca	K	Mg	Na	Cl	SO_4
8.0 (0.1)	8.4 (0.1)	104 (2.6)	93 (1.0)	25	1.0	8.7	9	12	18

[a] Water quality was measured in the control, medium, and high exposure concentrations at the beginning and end of tests. Major ions were measured in the control water at the beginning of the test.

Figure S1. Regressions of estimating 20% effect concentrations (EC20s; mg K/L) and 95% confidence limits for the responses of fatmucket (*Lampsilis siliquoidea*) in 4-week KCl water exposures with or without sand substrate in 2013. Each circle indicates the measured response in each of the 4 replicates, and the line represents the nonlinear regression fit. The EC20 and regression line are highlighted in red when insufficient data were available to support adequate analysis by Toxicity Relationship Analysis Program

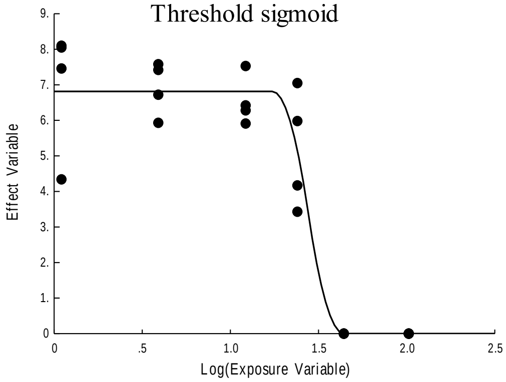
Exposure without sand

EC20 based on survival: 26 (no CL)



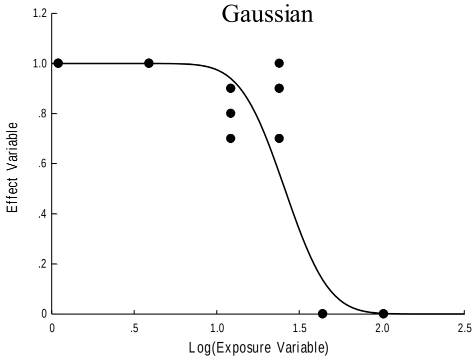
EC20 based on dry weight: NE
do not meet the conditions of any regression models.

EC20 based on biomass: 23 (21-26)

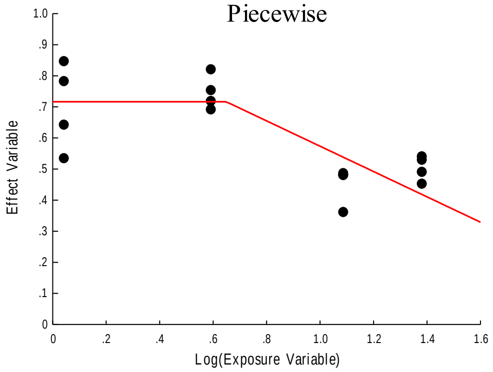


Exposure with sand

EC20 based on survival: 17 (16-18)



EC20 based on dry weight: 10 (3.9-25)



EC20 based on biomass: 8.7 (4.3-18)

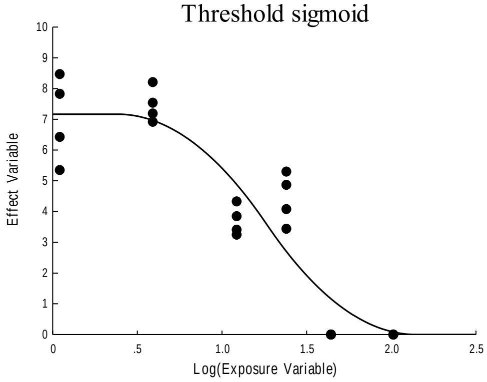
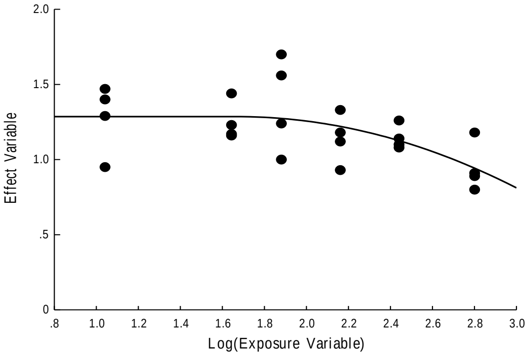


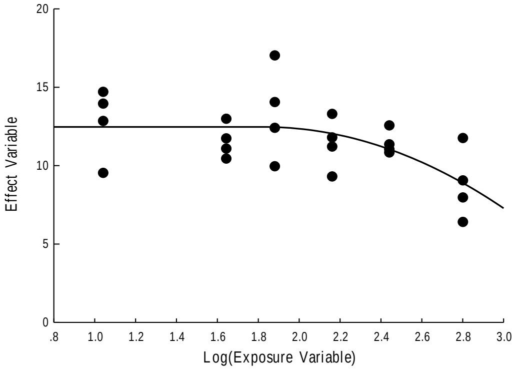
Figure S2. Regressions of estimating 20% effect concentrations (EC20s; mg Cl/L) and 95% confidence limits for the responses of fatmucket (*Lampsilis siliquoidea*) in 4-week NaCl test started with 2-month-old juveniles in water exposure with or without sand substrate in 2012. Each circle indicates the measured response in each of the 4 replicates, and the line represents the nonlinear regression (threshold sigmoidal) fit

Exposure without sand

EC20 based on dry weight: 445 (202-979)

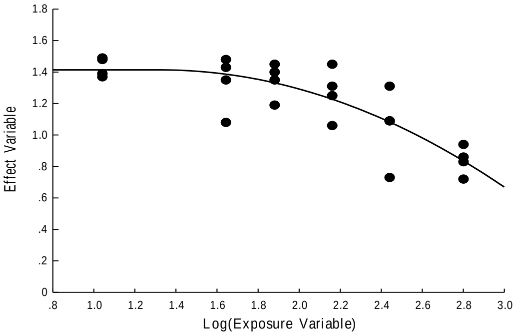


EC20 based on biomass: 437 (221-862)



Exposure with sand

EC20 based on dry weight: 227 (117-442)



EC20 based on biomass: 264 (151-461)

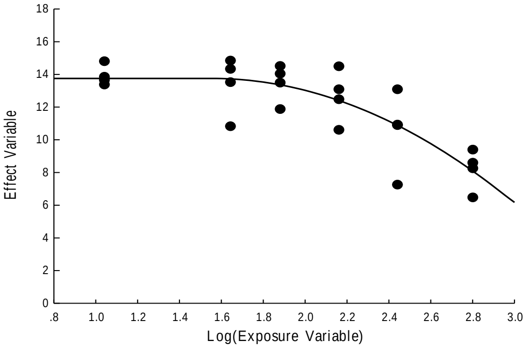
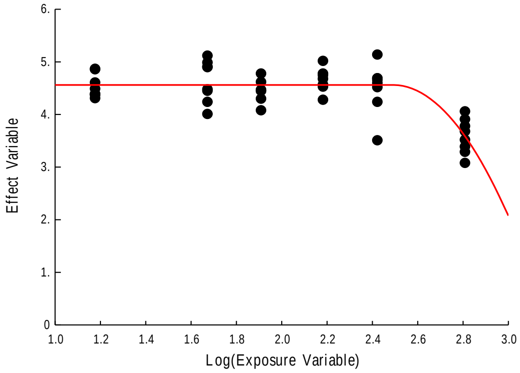
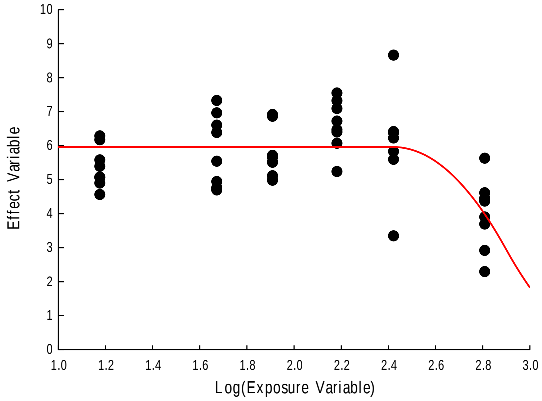


Figure S3. Regressions of estimating 20% effect concentrations (EC20s; mg Cl/L) and 95% confidence limits for the responses of fatmucket (*Lampsilis siliquoidea*) in 12-week NaCl test started with 2-month-old juveniles in water exposure with sand substrate in 2014. Each circle indicates the measured response in each of the 8 replicates, and the line represents the nonlinear regression (threshold sigmoidal) fit. The EC20 and regression line are highlighted in red when insufficient data were available to support adequate analysis by Toxicity Relationship Analysis Program

EC20 based on length: 628 (no CL)



EC20 based on dry weight: 527 (no CL)



EC20 based on biomass: 482 (320-724)

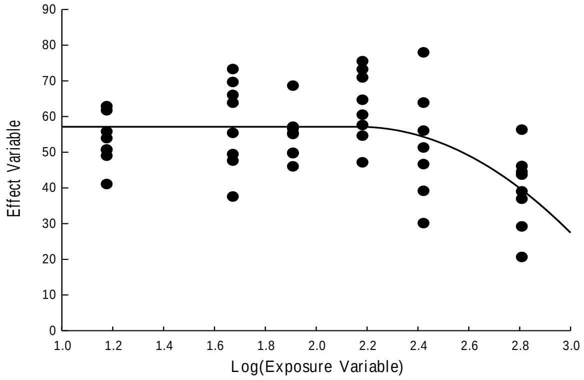
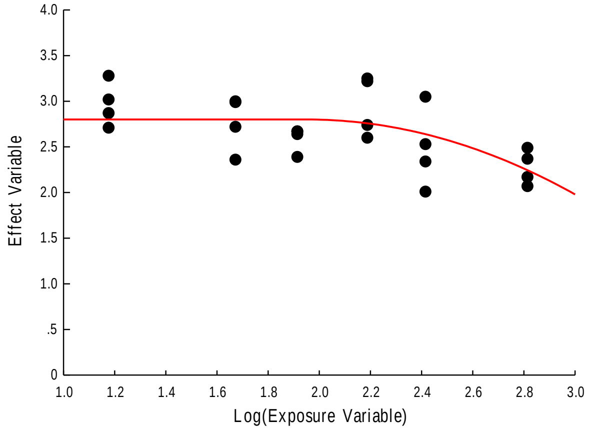
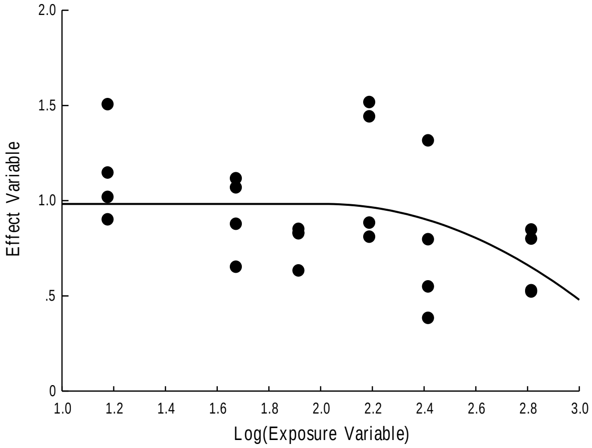


Figure S4. Regressions of estimating 20% effect concentrations (EC20s; mg Cl/L) and 95% confidence limits for the responses of fatmucket (*Lampsilis siliquoidea*) in chronic 4-week NaCl test started with 1-month-old juveniles in water exposures with sand substrate in 2015. Each circle indicates the measured response in each of the 4 replicates, and the line represents the nonlinear regression (threshold sigmoidal) fit. The EC20 and regression line are displayed in red if insufficient data were available to support adequate analysis by Toxicity Relationship Analysis Program

EC20 based on length: 656 (348-1238)



EC20 based on dry weight: 425 (147-1231)



EC20 based on biomass: 403 (142-1145)

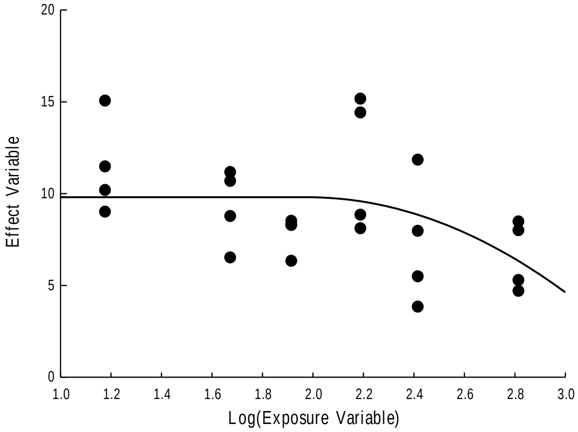
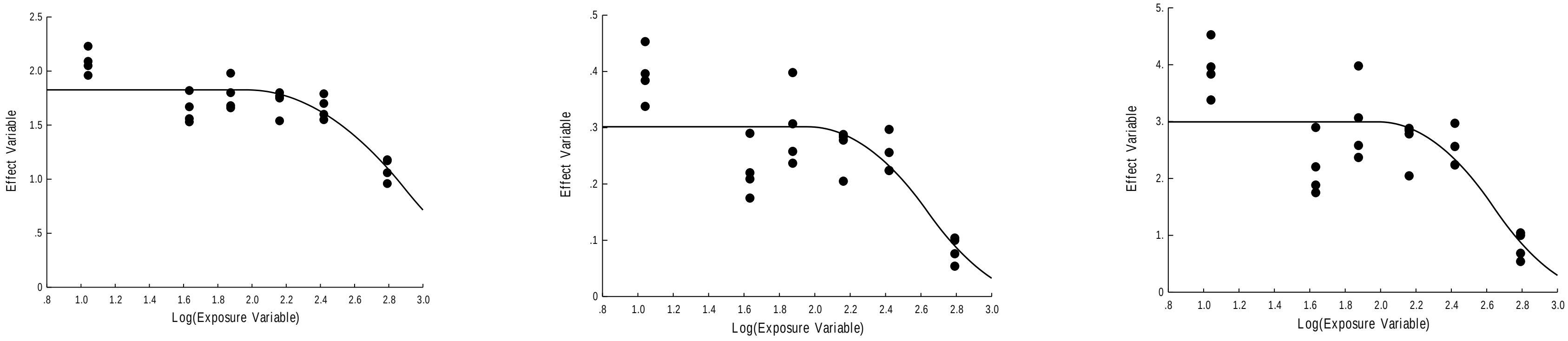


Figure S5. Regressions of estimating 20% effect concentrations (EC20s; mgCl/L) and 95% confidence limits for the responses of fatmucket (*Lampsilis siliquoidea*) in chronic in 4- and 12-week NaCl test started with 1-week-old juveniles in water exposure with sand substrate in 2015. Each circle indicates the measured response in each of the 4 replicates, and the line represents the nonlinear regression (threshold sigmoidal) fit

4-week responses

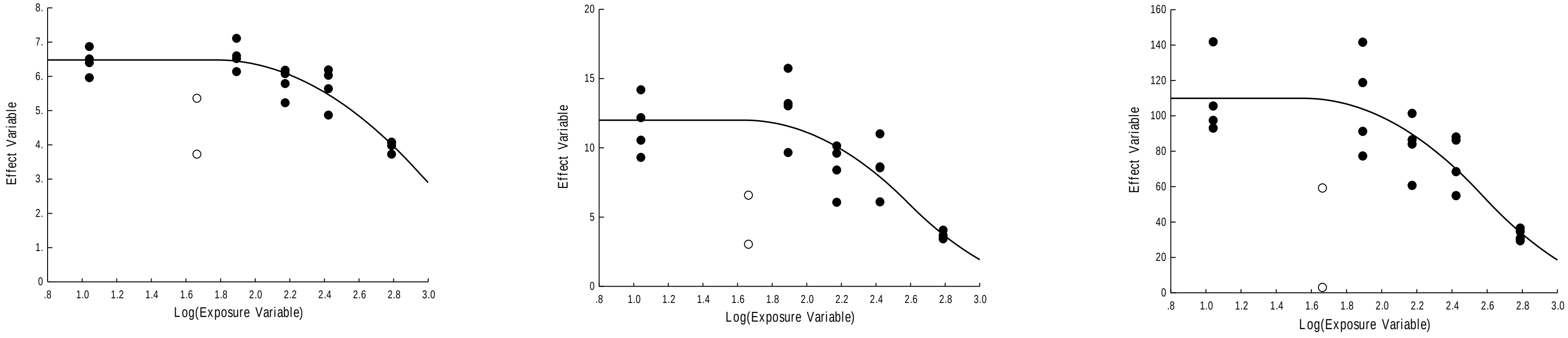


EC20 based on length: 358 (257-499)

EC20 based on dry weight:244 (137-436)

EC20 based on biomass: 251 (140-449)

12-week responses



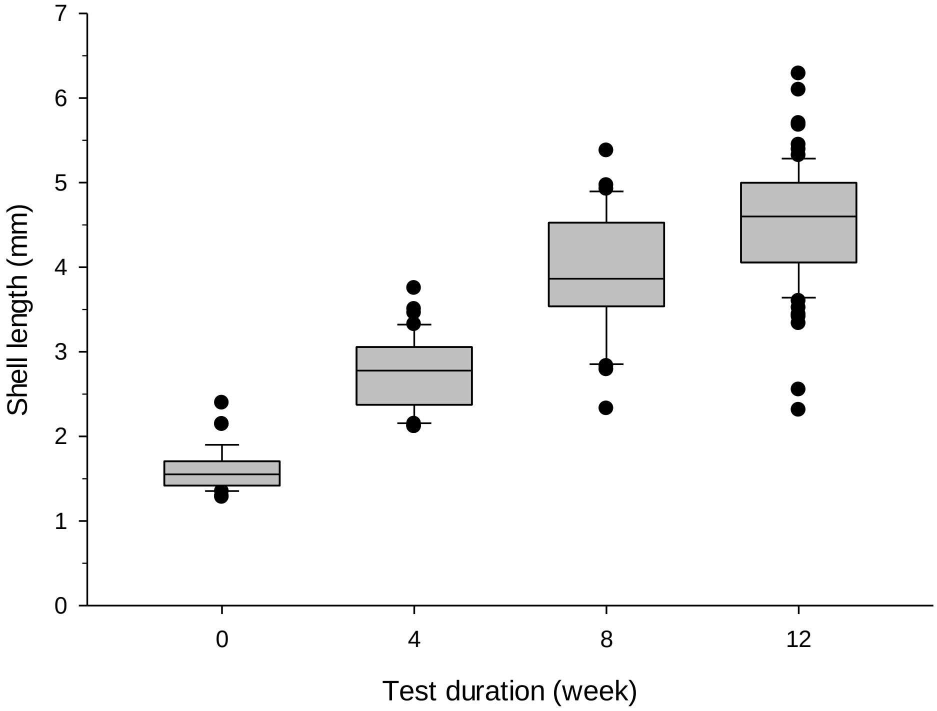
EC20 based on length: 323 (234-444)

EC20 based on dry weight: 173 (87-345)

EC20 based on biomass: 158 (78-321)

* The growth values with open circles were not included for the analyses due to the high mortality, including 2 replicates with 100% mortality, in the treatment.

Figure S6. The variability in shell lengths of juvenile fatmucket (*Lampsilis siliquoidea*) at the beginning of the test and at weeks 4, 8, and 12 in the control treatment of the 2014 4- to 12-week NaCl toxicity test. The boundary of the box indicates the 25th and 75th percentiles, a line within the box shows the median, and the error bars indicate 10th and 90th percentiles (n=40).



Supplemental Data, Section S1

REFINING METHODS FOR CONDUCTING CHRONIC TOXICITY TESTS WITH FRESHWATER MUSSELS IN WATER EXPOSURES

BACKGROUND

The ASTM International standard for conducting chronic toxicity testing with mussels was first published in 2005 (ASTM 2017) and is in need of refinement (Wang et al. 2011). Although the survival of mussels in controls in previous chronic tests typically exceeded the test acceptability criterion of ≥80% survival (ASTM 2017), the growth of the control mussels has been inconsistent among chronic toxicity tests (Ingersoll et al. 2015). Further studies are necessary to optimize feeding and other test conditions for assessing the mussel growth endpoint (Wang et al. 2011, Ingersoll et al. 2015). The objective of the present study was to evaluate survival and growth of juvenile mussels (Fatmucket, *Lampsilis siliquoidea*) in 4- to 12 week water exposures under control conditions (1) with and without a sand substrate, (2) with or without sand and test chamber replacement every 2 weeks, (3) with different feeding levels and frequencies of algal mixture, or with an addition of food to algal mixture, and (4) with different water renewal types (static renewal vs flow through). The results of this study are useful to refine test conditions to maximize control survival and growth of juvenile mussels in long-term exposures and to revise the ASTM (2017) methods for conducting chronic water-only toxicity tests with juvenile mussels.

MATERIALS AND METHODS

Brooding female fatmucket were collected in February 2014 from the Silver Fork of Perche Creek (Boone County, MO) and shipped to Missouri State University, Springfield, MO for production of juvenile mussels. Newly metamorphosed juveniles (<5 d old) were shipped overnight to the Columbia Environmental Research Center (CERC), Columbia, MO and cultured for 2 months in a recirculating mussel culture system (Barnhart 2006) before the feeding study. The mussels were fed once every hour automatically with an algal mixture to maintain a concentration of 2 nL cell volume/mL in the culture system (see the algal source, food preparation, and other details in main text of this manuscript). The culture water was the same water used in the feeding treatments and prepared by diluting the CERC well water (a hardness of ~300 mg/L as $CaCO_3$) with deionized water to a hardness of 100 mg/L as $CaCO_3$. Water temperature of 23±1°C and ambient laboratory light of 500 lux with 16:8 h light:dark photoperiod were maintained during the mussel culture and feeding treatments.

The feeding study started in July 2014 with 13 treatments for a test duration of 4 weeks, and 6 treatments for 12 weeks (Session 1-Table 1). Specific details are provided below.

Effects of beaker replacement and sand substrate under flow-through conditions. A previous 12-week NaCl toxicity test conducted in our laboratory failed because the control survival in exposures were below 80% after 8 weeks (unpublished data). A difficulty during chronic toxicity testing with mussels was cleaning debris in water-only beakers. Unconsumed food, mussel excreta, and, sometimes, fungi or algae accumulated and stuck on test beakers and mussel shells. Recent studies in our laboratory (unpublished data) have shown that periodic beaker replacement or placing a thin layer of silica sand in the beaker appeared to enhance hygiene (less fungi or algae growing on mussel shells). Four treatments were designed to confirm the effects of beaker replacement and sand substrate on survival and growth of juvenile fatmucket under the flow-through condition with a routine feeding level of 2 ml of algal mixture, which was prepared daily before morning feeding by adding 1 mL of Nanno 3600® and 2 mL of Shellfish Diet 1800® into 1.8 L of test water (algal concentration ~510 nl cell volume/ml; Wang et al. 2007):

> Treatment 1: No substrate and no beaker replacement: This treatment was the same as that used in our previous chronic toxicity tests with juvenile mussels (e.g., Wang et al. 2007, 2010, 2011; Besser et al. 2013) and was considered as a control to determine the amount of improvement of mussel survival and growth in other treatments. Test duration was 4 weeks.
>
> Treatment 2: No substrate but beaker replacement: Test beakers were replaced every 2 weeks. For the beaker replacement, the mussels in each replicate beaker were rinsed into a 200-mL glass dish with test water for survival determination. Surviving mussels (see details below) were transferred into new beakers. Test durations were 4 and 12 weeks.
>
> Treatment 3: Sand substrate addition but no beaker replacement: 5 mL of silica sand (~100 to 400-μm particles; Granusil #5010, Unimin Corporation, New Canaan, CT, USA), which had been held in control water for 24 h prior to the treatment, was added to each replicate beaker at the beginning of the treatment. Test duration was 4 weeks.

Treatment 4: Beaker and sand replacement: Sand was placed in beakers at the beginning of the treatments. The sand and beakers were replaced every 2 weeks. Test durations were 4 and 12 weeks.

Effects of feeding under flow-through condition. The routine feeding rate of 2 ml of algal mixture twice daily used in our previous studies (Wang et al. 2007, 2010, 2011) was based on a mussel feeding study in water-only (no substrate) beakers at 20°C (Wang et al. 2007). Recent studies have indicated that juvenile fatmucket grew faster at 23°C than 20°C in the culture and testing (J.M. Kunz; C.M. Barnhart of Missouri State University, unpublished data). In addition to the routine feeding (2 mL of algal mixture twice daily in Treatment 4), 4 treatments were designed to determine the optimum feeding levels of algal mixtures for the growth of juvenile fatmucket at 23°C in test beakers containing sand substrate and with beaker and sand replacement once every other week:

Treatment 5: 3 mL of algal mixture twice daily manually in the early morning and late afternoon. Test durations were 4 and 12 weeks.

Treatment 6: 2 mL of algal mixture 3 times daily manually in the early morning, noon, and late afternoon. Test durations were 4 and 12 weeks.

Treatment 7: 1 mL of algal mixture 6 times daily. The food was delivered by a Hamilton syringe pump automatically every 4 hours. Test durations were 4 and 12 weeks.

Treatment 8: 2 mL of algal mixture 6 times daily. The food was delivered by a Hamilton syringe pumps automatically every 4 hours. Test durations were 4 and 12 weeks.

Effects of the addition of other food to algal mixture under flow-through condition. Klaine et al (1997) reported that a diet with alga *Selenastrum capricornutum* and combined yeast-cerophyl-trout chow (YCT) was the best food among several potential diets for the growth of juvenile mussels (*Utterbackia imbecillis*). However, yeast globules formed on mussel shells and could not be rinsed or removed without potentially damaging the juvenile shell (Klaine et al. 1997). Our design with sand substrate under flow-through conditions might help reduce globule formation. In addition, Eybe et al. (2013) found that including a protein-containing additive (crushed red bloodworms) with the algae improved survival and growth of juvenile freshwater pearl mussel

(*Margaritifera margaritifera*). A commercially available food Oyster-feast® (Reed Mariculture, Campbell, CA) was used for the present study. Oyster-feast® is a food mixture of oyster eggs and ovarian tissue for the culture of corals, other invertebrates, and fish, and contains high protein and omega-3 fatty acids (http://reefnutrition.com/oyster_feast.php). Three treatments were designed to evaluate the effects of the addition of YCT or Oyster-feast® to the algal mixture on survival and growth of juvenile fatmucket at 23°C in test beakers containing sand substrate and with beaker and sand replacement once every other week:

> Treatment 9: 2 mL of algal mixture twice daily + 0.25 mL of YCT (1,800 mg/L stock; USEPA 2000) once daily. Test duration was 4 weeks.
>
> Treatment 10: 2 mL of algal mixture twice daily + 0.5 mL of YCT once daily. Test duration was 4 weeks.
>
> Treatment 11: 2 mL of algal mixture twice daily + 5 µL concentrate of Oyster-Feast® once daily (1/2 of the maximum amount recommended by the food provider). Test duration was 4 weeks.

Effects of feeding under static-renewal conditions. Limited chronic toxicity tests with juvenile mussels have been conducted under static-renewal conditions in our laboratory unpublished data, James Kunz) and previous studies (Bringolf et al. 2007). A static-renewal test may be useful when a flow-through system (e.g. diluter) is not available or the volume of test solution is limited for a flow-through test (e.g., effluent). A recent 28-d static-renewal effluent test with juvenile fatmucket indicated frequent beaker replacement (once every week) improved survival and growth (unpublished data, James Kunz). The present study further determined the optimum feeding rate under static-renewal conditions. All test conditions were the same as Treatment 4 in flow-through conditions, except that the treatments were conducted for 4 weeks and about 70% of the water was renewed on Mondays, Wednesdays, and Fridays, and beakers and sand were replaced once every week. Two feeding levels of algal mixture were used:

> Treatment 12: 2 mL of algal mixture twice daily in the early morning and late afternoon. Test duration was 4 weeks.

Treatment 13: 3 mL of algal mixture twice daily in the early morning and late afternoon. Test duration was 4 weeks.

All experiments were conducted concurrently in 3 intermittent flow-through proportional diluter systems modified from Mount and Brungs (1967, Wang et al. 2007). For the treatments with a 4-week test duration, 4 replicate glass beakers per treatment were placed in a water bath of the diluter. For the treatments with the 4- and 12-week test durations, 8 replicate beakers were placed in the bath (4 replicates for sampling survival and growth data at week 4, and 4 replicates for sampling at week 12). Test water was maintained at 23±1°C. Each beaker (300 mL) had a 2.5-cm hole in the side covered with 50-mesh (279-µm width opening) stainless-steel screen and held 200 ml of water. For the experiments under flow-through condition, the diluter provided 125 ml of water to each replicate beaker every 4 h (3.6 water volume additions per day). At the start of the experiments, 10 juvenile mussels (starting age ~60 d old) exhibiting foot movement were impartially transferred into each beaker. Additionally, 4 replicates of 10 mussels per replicate were preserved in 70% ethanol for the measurements of initial length and dry weight. Algal mixture was prepared daily before morning feeding by adding 1 mL of Nanno 3600® and 2 mL of Shellfish Diet 1800® (Reed Mariculture, Campbell, CA) into 1.8 L of test water (algal concentration ~510 nl cell volume/ml; Wang et al. 2007) and kept in a refrigerator at <4ºC for the feeding in the rest of the day. For the auto-feeding treatments, a stock of the algal mixture was maintained in an aerated cooler with ice packs and changed daily, and 3 syringe pumps (Hamilton, Reno, NV, USA) automatically delivered the targeted amount of algal mixture to exposure mixing chambers of the diluter right before each water cycling (Wang et al. 2011). In the treatments with a longer-term duration of 12 weeks, feeding levels were increased 50% every 4 weeks.

Water quality (dissolved oxygen, pH, conductivity, hardness, alkalinity, and ammonia) were determined using standard methods (Eaton et al. 2005) on composite water samples collected from the replicates at least every 2 weeks. At the end of the 4- or 12-week study, mussels in each replicate beaker was examined for survival under a dissecting microscope. Mussels with an empty shell or with a gaped shell containing decomposed tissue were classified as dead. Surviving mussels in each replicate were counted and preserved in 70% ethanol for subsequent dry weight measurements. Dry weight of pooled mussels per replicate was determined by drying the mussels at 60°C for 48 h. The differences in mean weight increases (calculated as final weight/starting weight) among each of the 4 4-week treatment groups and among the 6 12-week treatments were determined using ANOVA and Duncan's multiple range test using SAS/STAT version 9.4, SAS Institute). The level of statistical significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Water quality

Measured water quality characteristics are summarized in Session S1-Table 2. Mean measured concentrations of dissolved oxygen were ≥ 7.6 mg/L. Mean measured concentrations of total ammonia nitrogen ranged from 0.04 to 0.08 mg N/L in Treatments 1 to 11 under flow-through conditions and were 0.10 and 0.16 mg N/L in Treatments 12 and 13 under static-renewal conditions. Mean measured conductivity, pH, hardness, and alkalinity were similar within a treatment and among different treatments (conductivity 253 to 265 µS/cm, pH 8.0 to 8.3, alkalinity 91 to 102 mg/L as $CaCO_3$, hardness 101 to 106 mg/L as $CaCO_3$; Session S1-Table 2).

4-week treatments

Mean survival was similar among the 13 treatments, ranging from 95 to 100% (Session S1-Table 1). Under flow-through conditions, the mean weight increase in Treatment 1 conducted under traditional conditions (i.e., no sand and no beaker replacement, fed 2 mL algal mixture 2 times daily; ASTM 2017) was about 3 fold of the starting weight, whereas the other 10 treatments exhibited about 5- to 13-fold weight increase (Session S1-Figure 1ABC). Specifically, under the same feeding of 2 mL algal mixture 2 times daily, Treatments 2 (with beaker replacement every 2 weeks), Treatment 3 (with sand addition but no beaker and sand replacement), and Treatment 4 (with sand addition plus sand and beaker replacement every 2 weeks) had significantly greater weight increase than Treatment 1(Session S1-Figure 1A). The results indicate that replacing beakers every 2 weeks or adding sand substrate improved mussel growth.

Among Treatments 4 to 8, all with sand and beaker replacement but at different feeding rates and frequencies of algal mixture, Treatment 8 with the auto-feeding 6 times 2 mL algal mixture had significantly greater weight increase than the other 4 treatments (Session S1-Figure 1B). The weight increases among the 3 treatments with manual feeding (Treatments 4, 5, and 6) were not significantly different although Treatment 6 with the 3 times 2 mL algal mixture showed a slightly higher weight increase with less variability than Treatment 4 (2 times 2 mL) and Treatment 5 (2 times 3 mL; Session S1-Figure 1B). The weight increase in Treatment 7 with the auto-feeding 6 times 1 mL algal mixture was relatively low although the daily total amount of food was designed to be equal to or more than the 3 manual feeding treatments. It is likely that a portion of food had been lost when the diluter delivered the water (mixed with the food) to replicate beakers and the excess water, and potentially food, flowed out through the side screen. Therefore, the increased feeding rate and frequency of the auto-feeding 6 times 2 mL algal mixture supports the best growth.

The mean weight increases of mussels fed algae plus the 2 levels of YCT (Treatments 9 and 10) and fed algae plus Oyster-feast® were similar; however, the variation among replicates in Treatment 10 (algae plus high YCT treatment was much lower (Session S1-Figure 1C). The growth of mussels in these 3 treatments with additional food to the algal mixture were not significantly different from the growth of mussels only fed algal mixture 2 times daily in Treatment 4 (Session S1-Figure 1C). Notably, the mean weight increase in Treatment 10 with the additional 0.5 mL YCT was 27% greater than the weight increase in the algal feeding-only treatment, indicating the mussels may have benefited some from the YCT. Further studies need to confirm the effect of YCT on mussel growth. However, increasing YCT amount may likely cause elevated ammonia (e.g., Wang et al. 2013, to which juvenile mussels are highly sensitive (Wang et al. 2007, 2011).

Under static-renewal conditions, the weight increases in Treatments 12 and 13 with 2 different feeding rates were not significantly different (Session S1-Figure 1D). Surprisingly, the mussels under static-renewal conditions grew much faster (>70%) than the mussels fed similarly under flow-through conditions (Treatments 4 and 5). A possible reason was higher food availability in the static-renewal treatments, where the water in test chambers was renewed once Mondays, Wednesdays, and Fridays, compared to the flow-through condition, where each water addition every 4 h flushed away some remaining food in test chambers. This speculation appeared to be supported by the fact that increasing food availability by auto-feeding with each water addition in the flow-through Treatment 8 (Session S1-Figure 1B) substantially improved the growth of mussels, and the mussels grew as fast as those under static-renewal conditions (Session S1-

Figure 1D). The high survival and growth of mussels in the static-renewal treatments indicated that chronic toxicity tests with juvenile mussels can be successfully completed under static-renewal conditions, with a minimum water renewal on Mondays, Wednesdays, and Fridays, and sand and beaker replacement every week. The static-renewal test is useful when a flow-through system is not available or there is limited solution (e.g., effluent) for testing. However, mean concentrations of ammonia of 0.10 and 0.16 mg N/L in the 2 static-renewal treatments (Treatments 12 and 13) were 2- or 3-fold higher than those with similar feeding rates but under flow-through conditions (Treatments 4 and 5; Table Session S1-Table 2), indicating the potential problem of water quality. In a previous 4-week ammonia toxicity test with test water and pH (8.2) similar to those in the present study, the lowest effect concentration to juvenile fatmucket was as low as 0.26 mg N/L (Wang et al. 2011). Increasing water renewal (e.g., once daily, rather than once on Mondays, Wednesdays, and Fridays in the present study) may keep the ammonia below 0.1 mg N/L at a feeding rate that results in good growth of the mussels.

12-week treatments

Mean survival ranged from 78 to 98% at the end of the 6 12-week treatments (Session S1-Table 1). In Treatments 5, 6, and 7, one replicate had much lower survival (40 or 50%) compared to the other 3 replicates (typically 90 or 100%). This replicate effect has been found in previous chronic 4-week toxicity testing (e.g., Wang et al. 2016) and more often in our longer-term (e.g., 2 months) mussel culture (unpublished data). The mean weight increase ranged from 31 to 60 fold among the 6 12-week treatments, and, as observed in the 4-week study, the growth in Treatment 8 with the auto-feeding 6 times 2 mL algal mixture was significantly greater than the growth in the other 5 treatments (Session S1-Figure 2). Although the growth rates among the 5 treatments were not significantly different, the mussels in Treatment 6 with the 3 times 2 mL algal mixture had the highest weight increase (Session S1-Figure 2), which was consistent with the results obtained in the 4-week study (Session S1-Figure 1B). The results indicate that a long-term 12-week toxicity test can be successfully completed starting with 2-month-old juvenile mussels under flow-through condition with beaker replacement every 2 weeks. Adding a sand substrate may have kept food more dispersed and limited the growth of fungi or algae on the mussel shell. Increasing feeding frequency improved the mussel growth. The results of the overall feeding study can be useful for the revision of the ASTM standard for conducting chronic toxicity test with juvenile mussels (ASTM 2017).

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Session S1-Table 1. Summary of 13 treatments with different feeding and holding conditions and mean survival (n=4; standard deviation in parentheses) in the 4- or 12-week study conducted at 23°C in 2014

Study goal	Treat.[a]	Description[b]	Survival (%)	
			4 weeks	12 weeks
Effects of sand presence or sand and beaker replacement under flow-through condition, with a routine feeding of algal mixture (Wang et al. 2007)	1	2X2 mL algal mixture, no substrate, and no beaker replacement as recommended in ASTM (2017). The treatment served as a control to evaluate the improvement of mussel growth in other treatments	98 (5.0)	NA
	2*	2X2 mL algal mixture, no substrate, with beaker replacement after 2 weeks	100 (0)	95 (10)
	3	2X2 mL algal mixture, no beaker replacement, with 5 mL of sand	98 (5.0)	NA
	4*	2X2 mL algal mixture, with sand and beaker replacement after 2 weeks	98 (5.0)	88 (5.0)
Effects of feeding levels under flow-through condition, with sand and beaker replacement	5*	2X3 mL algal mixture (one in the morning and another in the afternoon)	100 (0)	83 (29)
	6*	3X2 mL algal mixture (morning, noon, and afternoon)	100 (0)	78 (22)
	7*	6X1 mL algal mixture (once every 4 h automatically; see text)	100 (0)	88 (25)
	8*	6X2 mL algal mixture (once every 4 h automatically; see text)	100 (0)	98 (5.0)
Effects of a food addition to algae under flow-through condition, with sand and beaker replacement	9	2X2 mL algal mixture + 1X0.25 mL of yeast-cerophyl-trout chow	100 (0)	NA
	10	2X2 mL algal mixture + 1X0.50 mL of yeast-cerophyl-trout chow	95 (5.8)	NA
	11	2X2 mL algal mixture + 1X5 µL concentrate of Oyster-Feast® (1/2 of the maximum amount recommended by the food provider)	98 (5.0)	NA
Effects of feeding under static-renewal condition	12	2X2 mL algal mixture, sand and beaker replacement once per week	98 (5.0)	NA
	13	2X3 mL algal mixture, sand and beaker replacement once per week	100 (0)	NA

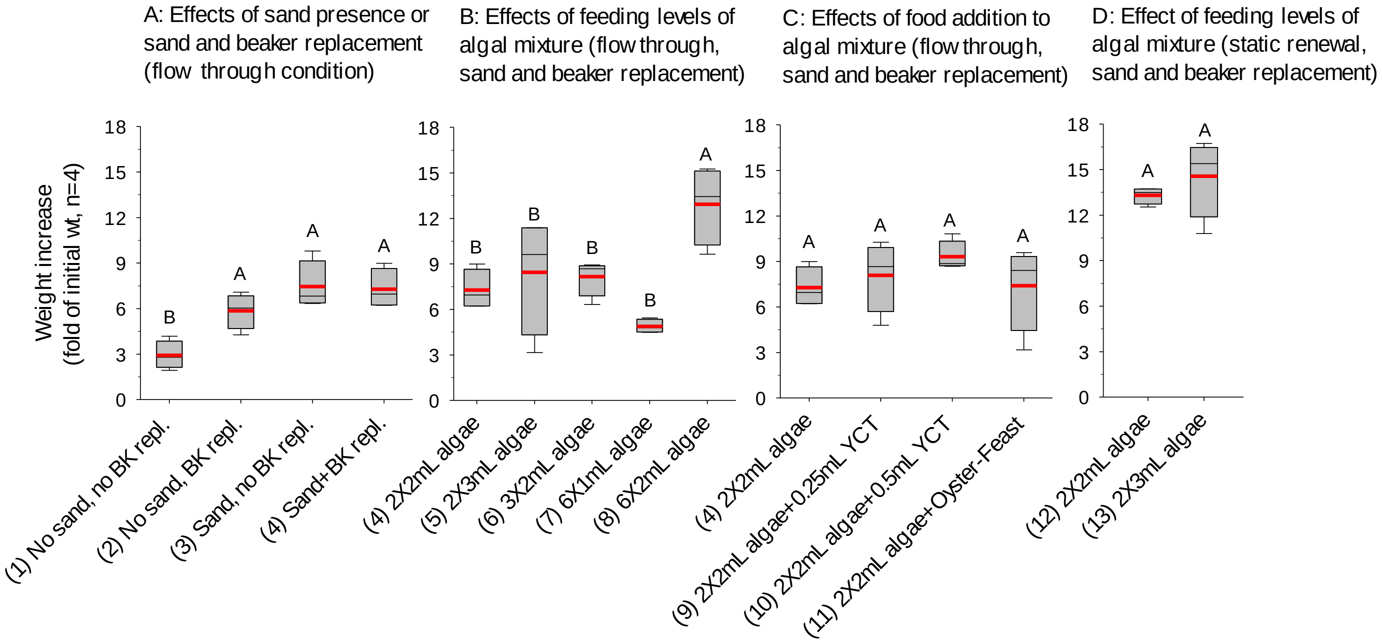
[a] All 13 treatments were conducted for 4 weeks, of which 6 treatments indicated with an asterisk (*) had additional replicates for an extended study period to 12 weeks.

[b] 2X, 3X, or 6X indicates 2, 3, or 6 time feeding per day, respectively.

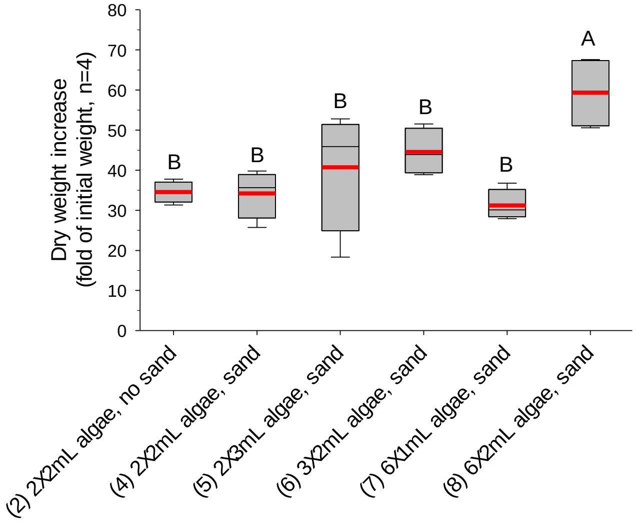
NA = Not applicable.

Session S1-Table 2. Mean measured water quality characteristics in the feeding study with juvenile fatmucket (*Lampsilis siliquoidea*) in 2014. See Session S1-Table 1 for the description of treatments

	Dissolved oxygen (mg/L)		Conductivity (µS/cm, 25 °C)		pH		Hardness (mg/L as $CaCO_3$)		Alkalinity (mg/L as $CaCO_3$)		Total ammonia (mg TN/L)	
Treatment	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
4-week study (n=3-6)												
1	8.0	0.6	254	8.3	8.2	0.2	101	1.2	91	6.1	0.05	0.02
2	7.9	0.6	254	7.9	8.2	0.1	104	4.0	92	3.5	0.05	0.02
3	8.0	0.6	253	8.5	8.2	0.1	103	2.3	93	3.1	0.05	0.02
4	8.2	0.5	255	5.5	8.2	0.1	103	2.3	93	2.3	0.04	0.01
5	8.0	0.6	255	7.2	8.1	0.1	101	2.3	92	3.5	0.05	0.02
6	8.0	0.6	256	7.0	8.1	0.1	102	3.5	102	16	0.05	0.01
7	8.0	0.6	259	9.4	8.0	0.1	101	3.1	95	6.1	0.05	0.01
8	7.9	0.4	258	4.4	8.1	0.1	103	4.6	92	3.5	0.06	0.01
9	8.0	0.5	254	6.5	8.3	0.3	103	2.3	93	3.1	0.06	0.02
10	8.0	0.5	255	5.9	8.2	0.1	101	2.3	91	4.2	0.07	0.04
11	8.1	0.7	254	7.7	8.2	0.2	101	1.2	92	3.5	0.04	0.02
12	7.9	0.4	265	7.5	8.1	0.1	102	2.0	99	11	0.10	0.05
13	7.6	0.4	264	5.9	8.1	0.1	101	1.2	94	3.5	0.16	0.12
12-week study (n=6-14)												
2	7.8	0.6	258	6.7	8.2	0.1	106	5.2	92	2.8	0.07	0.04
4	7.9	0.7	258	5.8	8.2	0.1	105	2.8	93	3.0	0.05	0.03
5	7.8	0.7	258	5.8	8.1	0.1	105	3.6	92	2.7	0.06	0.02
6	7.8	0.7	259	6.4	8.2	0.1	103	2.8	96	11	0.06	0.03
7	7.8	0.7	262	8.1	8.1	0.1	103	4.1	93	4.3	0.05	0.02
8	7.7	0.6	264	8.5	8.1	0.1	105	3.9	93	2.7	0.08	0.03



Session S1-Figure 1. The fold increases in dry weight of juvenile fatmucket (*Lampsilis siliquoidea*) in 13 treatments with different feeding and holding conditions over a 4-week period. The boundary of the box indicates the 25th and 75th percentiles, and the error bars indicate 10th and 90th percentiles (n=4). A thin black line within the box marks the median and a thick red line shows a mean. Means with a same letter above the bars within each of 4 treatment groups (panel A, B, C, and D) are not significantly different (Duncan’s multiple range test, p>0.05).



Session S1-Figure 2. The fold increases in dry weight of juvenile fatmucket (*Lampsilis siliquoidea*) in 6 treatments with different feeding over a 12-week period. The boundary of the box indicates the 25th and 75th percentiles, and the error bars indicate 10th and 90th percentiles (n=4). A thin black line within the box marks the median and a thick red line shows a mean. Means with a same letter above the bars are not significantly different (Duncan’s multiple range test, p>0.05). Note that feeding rates were increased 50% every 4 weeks.

ATTACHMENT 2

CHLORIDE IMPAIRED STREAMS IN ILLINOIS

Order	Priority	Hydrologic Unit Code	Water Name	Assessment ID	Water Size	Designated Use	Cause
1299	Medium	714010604	Akin Creek	IL_NHG-02	3.24	Aquatic Life	Chloride
1363	Medium	714010603	Casey Fork	IL_NJ-07	17.88	Aquatic Life	Chloride
802	Medium	712000403	Des Plaines River	IL_G-07	10.78	Aquatic Life	Chloride
150	Medium	712000405	Des Plaines River	IL_G-15	3.52	Aquatic Life	Chloride
158	Medium	712000405	Des Plaines River	IL_G-22	4.31	Aquatic Life	Chloride
168	Medium	712000405	Des Plaines River	IL_G-28	9.02	Aquatic Life	Chloride
174	Medium	712000405	Des Plaines River	IL_G-30	5.19	Aquatic Life	Chloride
181	Medium	712000405	Des Plaines River	IL_G-32	6.18	Aquatic Life	Chloride
323	Medium	712000407	Des Plaines River	IL_G-03	8.41	Aquatic Life	Chloride
331	Medium	712000407	Des Plaines River	IL_G-11	9.05	Aquatic Life	Chloride
343	Medium	712000407	Des Plaines River	IL_G-39	11.25	Aquatic Life	Chloride
2781	Low	713001003	Drowning Fork	IL_DGLC-01	18.83	Aquatic Life	Chloride
2665	Low	712000408	Du Page River	IL_GB-11	10.07	Aquatic Life	Chloride
556	Medium	712000611	Fiddle Creek	IL_DTRA-W-C1	2.04	Aquatic Life	Chloride
547	Medium	712000611	Fox River	IL_DT-22	7.86	Aquatic Life	Chloride
931	Medium	712000406	Hickory Creek	IL_GG-04	8.11	Aquatic Life	Chloride
935	Medium	712000406	Hickory Creek	IL_GG-06	12.63	Aquatic Life	Chloride
938	Medium	712000406	Hickory Creek	IL_GG-22	2.25	Aquatic Life	Chloride
1000	Medium	709000602	Huntley Ditch	IL_PQIB-H-C1	0.6	Aquatic Life	Chloride
679	Medium	712000701	Indian Creek	IL_DTZK	7.86	Aquatic Life	Chloride
2121	Medium	712000410	Lacey Creek	IL_GBLC	3.69	Aquatic Life	Chloride
2768	Low	714020101	Lake Fork	IL_OW-01	9.72	Aquatic Life	Chloride
2770	Low	714020101	Lake Fork	IL_OW-02	4.91	Aquatic Life	Chloride
748	Medium	712000304	Little Calumet River South	IL_HB-01	8.68	Aquatic Life	Chloride
2740	Low	713000103	Little Vermilion River	IL_DR-01	3.79	Aquatic Life	Chloride
1089	Medium	712000409	Manhattan Creek	IL_GCA-M-A1	2.53	Aquatic Life	Chloride
1290	Medium	714010604	Middle Fork Big Muddy River	IL_NH-06	12.49	Aquatic Life	Chloride
1293	Medium	714010604	Middle Fork Big Muddy River	IL_NH-07	19.74	Aquatic Life	Chloride
2653	Low	712000301	Middle Fork North Branch Chicago River	IL_HCCC-02	18.57	Aquatic Life	Chloride
2656	Low	712000301	Middle Fork North Branch Chicago River	IL_HCCC-04	3.51	Aquatic Life	Chloride
2647	Low	712000301	North Branch Chicago River	IL_HCC-07	11.9	Aquatic Life	Chloride
1320	Medium	514020404	North Fork Saline River	IL_ATF-05	7.95	Aquatic Life	Chloride
1323	Medium	514020404	North Fork Saline River	IL_ATF-07	5.62	Aquatic Life	Chloride
2717	Low	714010605	Pond Creek	IL_NG-02	23.53	Aquatic Life	Chloride
1117	Medium	712000612	Poplar Creek	IL_DTG-02	15.01	Aquatic Life	Chloride
1175	Medium	512011114	Robinson Creek	IL_BFC-11	0.92	Aquatic Life	Chloride
1783	Medium	512010903	Salt Fork Vermilion River	IL_BPJ-07	3.12	Aquatic Life	Chloride
1589	Medium	713000608	Sangamon River	IL_E-05	13.58	Aquatic Life	Chloride
112	Medium	712000301	Skokie River	IL_HCCD-01	13.47	Aquatic Life	Chloride
117	Medium	712000301	Skokie River	IL_HCCD-09	1.76	Aquatic Life	Chloride
1007	Medium	709000602	South Branch Kishwaukee River-East	IL_PQI-H-C5	4.29	Aquatic Life	Chloride
278	Medium	712000408	Spring Brook	IL_GBKA	1.74	Aquatic Life	Chloride
1168	Medium	512011114	Sugar Creek	IL_BF-01	4.84	Aquatic Life	Chloride
2630	Low	712000302	Thorn Creek	IL_HBD-04	4.32	Aquatic Life	Chloride
859	Medium	712000302	Thorn Creek	IL_HBD-06	2.21	Aquatic Life	Chloride
951	Medium	712000406	Union Ditch	IL_GGC-FN-C1	1.23	Aquatic Life	Chloride
2650	Low	712000301	West Fork North Branch Chicago River	IL_HCCB-05	14.48	Aquatic Life	Chloride

ATTACHMENT 3

LIST OF ORGANIZATIONS CONTACTED

ATTACHMENT 4-LIST OF ORGANIZATIONS CONTACTED REGARDING THE COLD TEMPERATURE COLD RESEARCH

Abbott Laboratories
AbbVie
Akzo Nobel Surface Chemistry LLC
American Commercial Lines
Citgo Petroleum Corporation
City of Aurora
City of Batavia
City of Chicago
City of Chicago Dept. of Law
City of Crystal Lake
City of Elmhurst
City of Geneva
City of Joliet
City of St. Charles
ComEd
Cook County Dept of Env. Control
DeKalb Sanitary District
Downers Grove Public Works
Downers Grove Sanitary District
DuPage County DOT
DuPage County Public Works
Elk Grove Village
ExxonMobil Joliet Refinery
Fairmount Santrol
Fox Metro Water Reclamation District
Fox River Water Reclamation District
Illinois Department of Transportation
Illinois State Toll Highway Authority
IMTT Illinois
Ingredion
Ingrediron
Kane County Dept. of Environmental Mgmt.
Kane County DOT
KASteel Chemicals Inc.
Lake County Division of Transportation
McHenry County Highway Dept.
MWRDGC
Noramco Chicago
NRG Energy
Salt Institute
Seaways, Inc.

Thompson Coburn LLP
Village of Antioch
Village of Barrington
Village of Bartlett
Village of Bensenville
Village of Carol Stream
Village of Cary
Village of Deerfield
Village of Glen Ellyn
Village of Glenview
Village of Grayslake
Village of Hinsdale
Village of Homer Glen
Village of LaGrange
Village of Lake Barrington
Village of Lake in the Hills
Village of Libertyville
Village of Lisle
Village of Lombard
Village of Maywood
Village of Mokena
Village of Montgomery
Village of Morton Grove
Village of New Lenox
Village of Northbrook
Village of Oak Brook
Village of Orland Park
Village of Romeoville
Village of Round Lake Beach
Village of Skokie
Village of Tinley Park
Village of Villa Park
Village of Wauconda
Village of Westchester
Village of Western Springs
Village of Wheeling
Village of Willowbrook
Village of Wilmette
Village of Winnetka
Village of Woodridge
Will County Division of Transportation

ATTACHMENT 4

SORSI ARTICLE

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River chloride trends in snow-affected urban watersheds: increasing concentrations outpace urban growth rate and are common among all seasons

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HIGHLIGHTS

- Chloride trends in northern U.S. urban streams are computed.
- The rate of chloride concentration increase outpaced urbanization from 1990 to 2011.
- The greatest chloride concentration increase was during the winter.
- Increasing chloride concentration trends were observed in all seasons.
- Chronic water quality criteria for chloride were exceeded for extended durations.

GRAPHICAL ABSTRACT

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ABSTRACT

Chloride concentrations in northern U.S. included in this study have increased substantially over time with average concentrations approximately doubling from 1990 to 2011, outpacing the rate of urbanization in the northern U.S. Historical data were examined for 30 monitoring sites on 19 streams that had chloride concentration and flow records of 18 to 49 years. Chloride concentrations in most studied streams increased in all seasons (13 of 19 in all seasons; 16 of 19 during winter); maximum concentrations occurred during winter. Increasing concentrations during non-deicing periods suggest that chloride was stored in hydrologic reservoirs, such as the shallow groundwater system, during the winter and slowly released in baseflow throughout the year. Streamflow dependency was also observed with chloride concentrations increasing as streamflow decreased, a result of dilution during rainfall- and snowmelt-induced high-flow periods. The influence of chloride on aquatic life increased with time; 29% of sites studied exceeded the concentration for the USEPA chronic water quality criteria of 230 mg/L by an average of more than 100 individual days per year during 2006–2011. The rapid rate of chloride concentration increase in these streams is likely due to a combination of possible increased road salt application rates, increased baseline concentrations, and greater snowfall in the Midwestern U.S. during the latter portion of the study period.

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1. Introduction

The impact of road salt on aquatic ecosystems continues to increase as urban development and subsequent road salt applications increase with time. Substantial application of road salt in the U.S. began in the 1940s increasing to an annual average of 9.6 million metric tons/yr of NaCl-based road salt in the 1980s and 19.5 million metric tons/yr in the last 5 years reported, ending in 2011 (Kelly and Matos, 2013). Increasing trends in chloride concentrations have been observed in water bodies of the U.S. and attributed, at least in part, to road salt influence. These trends have included rivers (Godwin et al., 2003; Interlandi and Crockett, 2003; Thunqvist, 2004; Kaushal et al., 2005; Kelly et al., 2012a), groundwater (Reisch and Toran, 2013; Kelly, 2008; Perera et al., 2009; Cassanelli and Robbins, 2013), inland lakes (Ramstack et al., 2004; Novotny and Stefan, 2010; Müller and Gächter, 2012), and even water bodies as large as the Laurentian Great Lakes (Chapra et al., 2009, 2012).

Elevated salt concentrations in surface waters can exert an adverse effect on aquatic organisms (Cañedo-Argüelles et al., 2013). The U.S. Environmental Protection Agency (USEPA) ambient water quality criteria for chloride (when associated with sodium) defines the chronic criterion as a 4-day average concentration exceeding 230 mg/L and the acute criterion as a 1-h average concentration exceeding 860 mg/L (U.S. Environmental Protection Agency, 1988). Given the sensitivity of freshwater organisms to chloride, exceedances of these criteria have the potential to affect a substantial number of species (U.S. Environmental Protection Agency, 1988). In a thorough assessment of the environmental impacts of road salt, Environment Canada estimated that 5% of aquatic species would be affected at chloride concentrations of 210 mg/L and 10% of aquatic species would be affected at chloride concentrations of 240 mg/L for chronic exposures (Environment Canada, 2001). Multiple studies have observed chloride concentrations greater than these benchmark concentrations in streams as a result of road salt runoff. These studies have included local (Ruth, 2003; Trowbridge et al., 2010; Allert et al., 2012; Morgan et al., 2012), regional (Kelly et al., 2012b), and national geographic scopes (Corsi et al., 2010).

Urban land cover in the U.S. has also increased over time from an estimated 61,000 km^2 in 1945 to 247,000 km^2 in 2007 (Nickerson et al., 2011). With urban land cover projected to continue increasing (Alig et al., 2004), applications of road salt for deicing impervious surfaces are also likely to increase. Adding to the current and past water quality issues resulting from the salinization of streams, including road salt runoff, an analysis of water quality in the northeastern U.S. predicted that many surface waters in that area of the country would not be potable for human consumption and would become toxic to freshwater life within the next century (Kaushal et al., 2005).

The primary objectives of this study were to define temporal trends in chloride concentrations in the context of chloride dependency on streamflow rates, compare temporal chloride trends among seasons, and compare these trends to changes in urban land cover, aquatic life criteria, and road salt sales patterns. Trend analysis was done using the modern water quality trend modeling technique that controlled for streamflow rate and season to help avoid confounding results due to natural variability (Hirsch et al., 2010).

2. Methods

2.1. Site selection

An initial focus for 14 sites on 3 streams in the Milwaukee metropolitan area was conducted. To assess the broader geographic impact, 11 additional streams in urban areas of the northern U.S. were studied, 4 streams in northern areas with little urban impact were studied, and one stream in an urban area of the southern U.S. was studied as a warm-climate reference.

Sites were initially chosen based on proximity to areas of urban influence in the northern U.S. (Fig. 1, Table 1). Three sites with a low degree of urbanization in northeast Wisconsin and one site in Oregon were included to evaluate non-urban influence, and the Trinity River in Texas was also examined as a non-deicing reference site in an urban area. Second, adequate data availability for modeling was necessary. Most sites had 200 or more chloride observations and 20 or more years of record with no significant gaps in data collection (i.e., larger than 5 years), and sample representation during all seasons throughout the water quality record (Table S1). The exceptions include five sites that had between 151 and 194 observations, and one site that had a 6-yr gap. These sites were included to maintain adequate geographic representation of sites (Table S1). Sites located within or just downstream from large lakes or impoundments were omitted. A continuous record of streamflow data concurrent with the chloride record was required at the selected site or at a nearby site on the same stream. Sites selected in the Milwaukee metropolitan area were chosen from a dense network of available sites in an effort to adequately represent changes in the Milwaukee, Menomonee, and Kinnickinnic Rivers.

2.2. Data sources

Chloride data were obtained from the Milwaukee Metropolitan Sewerage District (MMSD), the Wisconsin Department of Natural Resources (WDNR), and the Water Quality Portal (WQ portal; http://www.waterqualitydata.us/), which includes data from the USGS National Water Information System (NWIS) and EPA STOrage and RETrieval Data Warehouse (STORET). Coordinate bounding boxes were used to query the WQ Portal to locate streams in metropolitan areas of primarily the northern U.S. with sufficient data (Fig. 1). Where data from different sources overlapped at common sites, data were combined except for one site where data from one of the sources were not considered valuable due to many duplicate data points and data differences that called into question analytical results.

If available, streamflow data from the USGS National Water Information System (http://waterdata.usgs.gov/nwis) were retrieved from the same location where chloride samples were collected; otherwise, data from a nearby location(s) on the same stream were scaled by drainage area to estimate streamflow at the chloride sampling location. In two cases, there were data gaps in streamflow that were estimated using an ordinary least squares regression with streamflow data from a nearby site (Meno 70th, $R^2 = 0.65$; Milw Cedarburg, $R^2 = 0.95$).

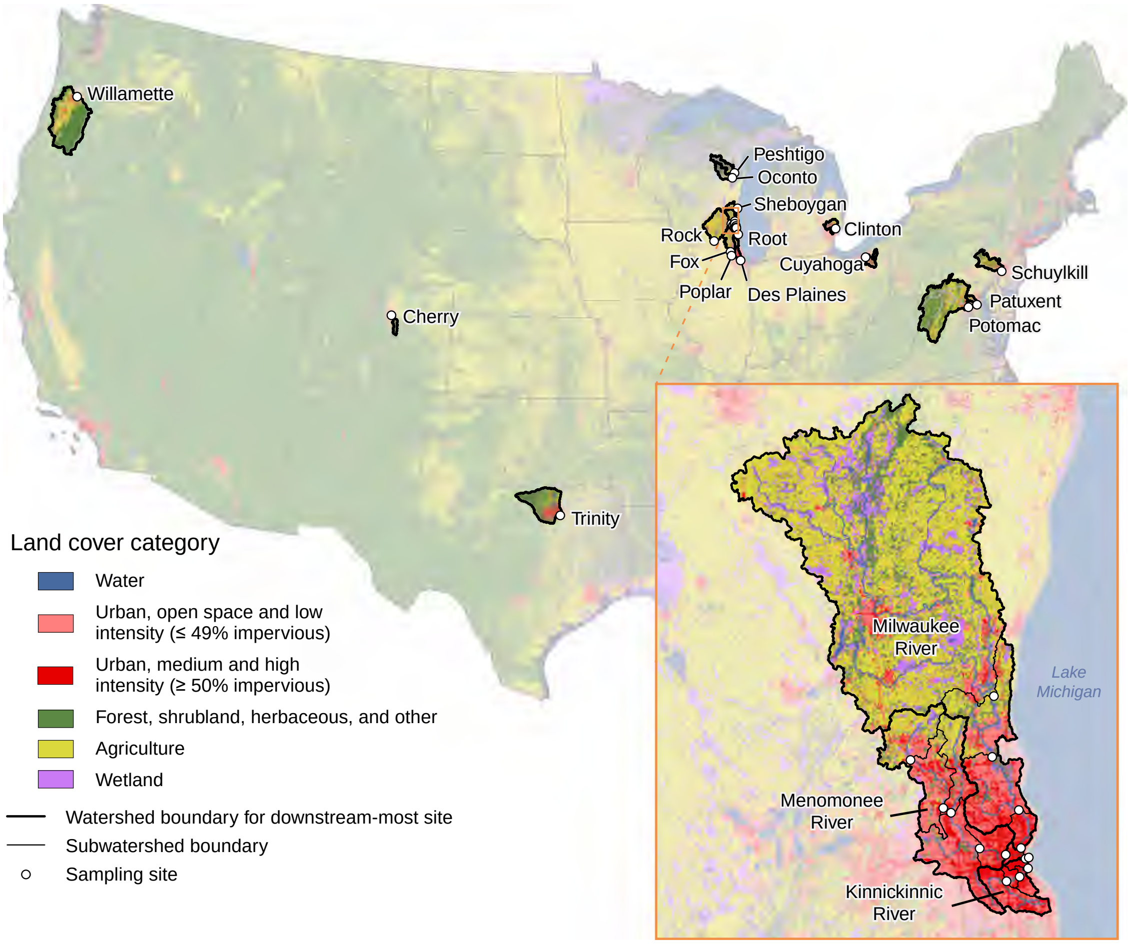
Road salt sales data were compiled from an annual reporting of Historical Statistics for Mineral and Material Commodities in the United States (Kelly and Matos, 2013) and used as a proxy for assessing overall road salt applications in the studied watersheds. Road salt sales were used in place of actual application numbers due to the complicated nature of gathering road salt application data from all municipalities and private applicators on the scale of this study.

Daily snowfall data for eight weather stations in the U.S. were retrieved from the National Climate Data Center (http://www.ncdc.noaa.gov/cdo-web/) for evaluation of snowfall changes throughout the study period (Table S2). Stations were chosen based on proximity of chloride study sites and availability of data during the study period.

Land cover composition and other watershed characteristics were determined from several published GIS datasets and provided in Table 1, and methods are described in Supplemental Information.

2.3. Data analysis

Data analysis included water quality trend modeling, graphical analysis of trends, and exploration of modeling results in comparison to land use, the USEPA water quality criterion, and road salt sales in the U.S.



Base composited from Instituto Nacional de Estadística Geografía e Informática, The Atlas of Canada, and U.S. Geological Survey North American Atlas - Political Boundaries, 1:10,000,000, 2006; U.S. Geological Survey National Land Cover Database 2006, 30-meter resolution, 2011; U.S. Environmental Protection Agency and U.S. Geological Survey National Hydrography Dataset Plus (modified), 1:100,000, 2005. Albers Equal Area Conic USGS CONUS Projection, referenced to North American Datum of 1983.

Fig. 1. Study site locations and watershed characteristics.

2.3.1. Rationale for water quality modeling technique

Chloride concentrations in streams have the potential to vary depending on several factors that all arise from the nature of the contamination sources, transport characteristics, and hydrologic characteristics of a given watershed. Considerations when attempting to understand these influential factors are very similar to those outlined previously describing potentially influential factors in stream nutrient concentrations and fluxes (Hirsch et al., 2010). The primary considerations are as follows: As urban development continues, sources of nonpoint pollution such as road salt application tend to increase as well, so it is logical to expect chloride concentrations in streams to change with time. Given that road salt is applied only during cold-weather periods, seasonal differences in chloride concentrations are also expected. The nature of road salt transport to surface waters causes chloride concentrations to change with streamflow. For example, when road salt melts ice and snow during periods of low streamflow, stream chloride concentrations can become very high, but when road salt runoff periods are coincident with high-streamflow periods resulting from snowmelt or rainfall events, chloride concentrations are likely to be lower due to the larger amounts of water available to dilute the road salt.

Long-term changes in chloride concentrations from road salt can follow a variety of temporal patterns responding to factors such as: the rate of urban development, changes in road salt application practices, long-term storage and release processes from large water bodies and groundwater, and changing climate conditions. Other major sources include treated wastewater as well as fertilizer and livestock, with other minor sources also contributing (Kelly et al., 2012b). These influences led to the use of WRTDS, a data analysis technique that can describe long-term change in a flexible manner (not simply as linear or quadratic time trends) and account for the seasonal- and streamflow-related dependencies which may, themselves, be changing over a period of many years or decades (Hirsch et al., 2010). The WRTDS analysis used here is implemented within the EGRET package (Hirsch and De Cicco, 2014) in the R statistical language (R Development Core Team, 2008).

2.3.2. Modeling water quality changes

The WRTDS method is based on weighted linear regression to estimate daily concentration throughout time, discharge (streamflow), and seasonal dimensions of the data used to calibrate the model. For any given estimation point in the data domain (where the point is defined by year, season and streamflow) the model gives increased weight to values similar in time, streamflow, and season to the estimation point. For example, concentrations of samples collected in year two of a sample period will have little influence on model estimates for year 10, concentrations of samples collected during low flow periods will have little influence on model estimates for high flow, and concentrations of samples collected during summer will have little influence on model estimates for winter periods. Weighting for proximity of the estimation point to the observed data by time (the time distance),

Table 1
Watershed characteristics of study sites.

	Metropolitan				Percent land cover in 2006				Percent land cover in 1992[c]		
Site name	State	Area	Short Name	Drainage area (km^2)	Urban[a]	Agricultural[a]	Forest/ other[a]	% Impervious[b]	Urban	Agricultural	Forest/ other
Milwaukee River at Pioneer Rd near Cedarburg	WI	Milwaukee	Milw Cedarburg	1555	11.0	56.2	32.8	2.9	9.6	58.5	31.9
Milwaukee River at Brown Deer Rd	WI	Milwaukee	Milw Brown Deer	1674	12.7	54.9	32.4	3.4	11.1	57.4	31.5
Milwaukee River at Estabrook Park	WI	Milwaukee	Milw	1785	17.5	51.4	31.0	5.6	16.0	53.9	30.1
Milwaukee River at Wells St	WI	Milwaukee	Milw Wells	1808	18.5	50.8	30.7	6.3	17.0	53.3	29.8
Milwaukee River at Jones Island at Mouth at Milwaukee	WI	Milwaukee	Milw Jones	2240	29.2	43.8	27.0	11.3	27.1	46.7	26.2
Menomonee River at County Line Road	WI	Milwaukee	Meno County	79	30.1	46.2	23.7	10.0	24.5	53.0	22.4
Menomonee River at 127th St	WI	Milwaukee	Meno 127th	153	52.0	28.5	19.4	17.8	43.0	37.7	19.3
Menomonee River at Hampton Ave	WI	Milwaukee	Meno Hampton	211	51.0	29.6	19.4	18.6	43.3	37.7	19.0
Menomonee River at 70th St Bridge at Wauwatosa	WI	Milwaukee	Meno 70th	318	65.1	20.0	14.9	24.7	59.4	26.1	14.5
Menomonee River at 25th St	WI	Milwaukee	Meno 25th	355	68.6	17.9	13.5	27.9	63.5	23.4	13.1
Kinnickinnic River at S 27th St	WI	Milwaukee	KK 27th	45	99.0	0.0	1.0	49.8	98.8	0.4	0.8
Kinnickinnic River at S 7th St	WI	Milwaukee	KK 7th	53	98.2	0.0	1.8	50.3	98.1	0.4	1.5
Kinnickinnic River at 1st St	WI	Milwaukee	KK 1st	63	98.5	0.0	1.5	50.5	98.4	0.3	1.3
Kinnickinnic River at Jones Island Ferry	WI	Milwaukee	KK Jones	69	98.0	0.0	2.0	51.6	97.8	0.3	1.9
Root River at Racine, WI	WI	Racine	Root	480	29.8	52.7	17.4	10.0	26.0	52.8	21.2
Peshtigo River at Peshtigo, WI	WI	Rural	Peshtigo	2872	4.3	15.5	80.3	0.4	4.6	14.3	81.1
Oconto River near Oconto, WI	WI	Rural	Oconto	2473	4.9	21.8	73.4	0.5	5.2	21.2	73.6
Sheboygan River at Sheboygan, WI	WI	Rural	Sheboygan	1103	8.1	64.7	27.2	2.2	7.7	66.6	25.7
Rock River at Afton, WI	WI	Janesville	Rock	8661	11.3	65.6	23.1	3.2	10.0	66.7	23.3
Willamette River at Portland, OR	OR	Rural	Willamette	28,967	7.3	20.4	72.2	2.6	6.9	20.6	72.4
Des Plaines River at Riverside, IL	IL	Chicago	Des Plaines	1643	63.8	18.8	17.4	27.5	60.0	22.0	18.0
Fox River at Algonquin, IL	IL	Chicago	Fox	3601	24.8	45.2	30.0	7.0	21.8	47.3	31.0
Poplar Creek at Elgin, IL	IL	Chicago	Poplar	92	67.9	7.5	24.6	26.5	62.0	15.0	22.9
Cherry Creek at Denver, CO	CO	Denver	Cherry	1063	21.7	0.6	77.7	6.9	15.9	0.7	83.4
Clinton River at Moravian Drive at Mt. Clemens, MI	MI	Detroit	Clinton	1937	52.3	19.9	27.8	20.0	49.7	23.0	27.3
Cuyahoga River at Independence, OH	OH	Cleveland	Cuyahoga	1836	39.8	17.4	42.8	10.9	34.1	20.7	45.3
Schuylkill River at Philadelphia, PA	PA	Philadelphia	Schuylkill	4888	24.2	29.7	46.1	6.4	18.6	39.1	42.3
Patuxent River near Bowie, MD	MD	Columbia	Patuxent	906	31.9	26.4	41.8	8.7	20.0	44.1	35.9
Potomac River at Chain Bridge, at Washington, DC	DC	Washington, DC	Potomac	29,967	10.1	29.6	60.3	2.1	8.3	32.1	59.6
Trinity River below Dallas, TX	TX	Dallas	Trinity	16,224	22.3	14.4	63.3	7.9	19.3	15.8	64.9

[a] Watershed urban, agriculture, and forest/other percentages for 2006 were determined from the National Land Cover Database 2006 Land Cover dataset (Fry et al., 2011).

[b] Watershed impervious percentages for 2006 were determined from the National Land Cover Database 2006 Percent Developed Imperviousness dataset (Fry et al., 2011).

[c] Watershed urban, agriculture, and forest/other percentages for 1992 were determined from the National Land Cover Database 1992/2001 Retrofit Land Cover Change dataset (Fry et al., 2009).

streamflow (the discharge distance), and season (the seasonal distance) are assigned using a tricube weight function with half-window widths of 10 years, 2 natural log units, and 0.5 years in the time, streamflow, and seasonal dimensions respectively. These values were chosen after experimentation of the smallest values that did not cause unrealistic oscillations. The overall weight on any given observation is the product of the three weights. Estimation by the WRTDS model was performed using measured chloride and corresponding daily streamflow values. This tool has not previously been used to examine chloride trends, but applications using WRTDS have primarily been used for examination of nutrients (Hirsch et al., 2010; Medalie et al., 2012; Green et al., 2014).

2.3.3. Examination of water quality changes

One type of output produced using WRTDS for each of the study locations was graphics showing estimated concentrations for three levels of streamflow (the 10, 50, and 90 percentile points on the daily streamflow frequency distribution), four seasons of the year (centered on February 15 [winter], May 15 [spring], August 15 [summer], and November 15 [fall]), and each year of the period of record. Another type of output is a contour plot of the differences in estimated chloride concentration between the years 1981 and 2010 as a function of time-of-year and streamflow. WRTDS was also used to compute flow-normalized annual chloride concentrations over the period of record. Flow-normalization is a technique that removes the effect of year-to-year variations in streamflow (but not seasonal variations) on average chloride concentrations (Hirsch et al., 2010).

Linear regression was used to explore the response of flow-normalized annual chloride concentrations as well as the number of individual days that these concentrations exceeded the USEPA water quality (exceedance days) to the percent of urban land cover in the watershed. Linear temporal regression was also used to compare the change in national road salt sales in the U.S. to the change in urban land cover in the northern U.S. to coincide with the location of most road salt sales in the U.S. The calculation of expected number of exceedance days was determined using logistic regression based on the output of the WRTDS model for the two periods 1990–1994 and 2006–2010. These dates were chosen based on the inclusion of at least 20 years, while minimizing the number of sites that had to be excluded due to missing data. The non-deicing reference site (Trinity River) and seiche-affected sites (Milw Jones, KK 1st, KK Jones) were not used in these analyses. The period of record did not have sufficient data between 1990 and 2011 for the Clinton, Cuyahoga, and Fox River, so these sites were not included in these analyses. Dates for the Schuylkill and Des Plaines Rivers did not match perfectly, but were near enough to provide an estimate for the later time period. The WRTDS method has been extended here to estimate the daily probability of exceedance of a threshold. Because the WRTDS model provides a conditional mean and conditional variance of concentration for each day as a function of streamflow, time of year, and year (trend) it is possible to compute a conditional probability of exceedance of the threshold under the assumption that the conditional distribution of concentration is log-normal. Using these results from all of the sites, logistic regressions were fit for each of the two 5-year time periods. These logistic regression models estimate the daily probability of exceedance at a site as a function the square root of the percent of the watershed that was urbanized during that time period. The number of predicted exceedances per year was then determined by summing these probabilities for the year. These calculations were implemented in R using the betareg function. Pseudo R-squared values for both models were approximately 0.83.

3. Results

Three major watersheds cover the bulk of the Milwaukee metropolitan area: the Milwaukee River, the Menomonee River, and the Kinnickinnic River. These three watersheds have all experienced increased chloride concentrations from 1980 to 2010 during winter, spring, summer, and fall (Fig. 2). The greatest increases in chloride concentrations were in watersheds with the greatest urban land cover percentage. Concentrations for the Milwaukee study sites were greatest in the Kinnickinnic River followed by the Menomonee River and then the Milwaukee River (drainage areas of 45, 355, and 1808 km^2, with 99, 68.6, and 18.5% urban land cover respectively).

In addition, chloride concentrations increased with decreasing streamflow for all three of these watersheds in each of the four seasons. The mean chloride concentration in the Milwaukee River exceeded 140 mg/L during winter low flow periods and was approaching 100 mg/L during summer low-flow periods toward the end of the study period. Mean chloride concentrations in the Menomonee and Kinnickinnic Rivers exceeded the USEPA chronic water quality criteria of 230 mg/L during the winter and spring at all three flow rates in the latter years of the study, and exceeded 100 mg/L during summer and fall periods at all three streamflow rates toward the end of the study duration.

Similar four-season graphs illustrating streamflow dependency for all remaining study sites except those impacted by backwater influences from Lake Michigan (hereafter referred to as seiche) are provided in the supporting information (Figs. S1–S25). Chloride concentrations also increased over all four seasons and decreased with streamflow at all of these additional sites except three with a few notable exceptions: Chloride concentrations at the Peshtigo River had increasing trends over the course of the study period, but concentrations did not increase with decreasing flow; concentrations were relatively constant in the Willamette River. Both of these sites have primarily forested land cover and little urban influence. With these watershed conditions, there is likely to be low road salt application and this is confirmed by very low chloride concentrations (less than 10 mg/L). Chloride concentrations in the Kinnickinnic River at 7th St. did not vary substantially with flow, but did have a slightly increasing relation with increasing streamflow during winter due to samples with high concentrations during high flow periods in the winter. Changes in concentrations with respect to streamflow at the southern urban reference site (Trinity River) were not consistent through the study period.

The change in concentration over time at three different locations within the Milwaukee River watershed is another illustration of increasing road salt effect with urban land cover. These three monitoring sites increased in downstream order from 11% to 17.5% to 18.5% urban land cover (Fig. 3, top to bottom panels, respectively; NLCD 2006 (Fry et al., 2011)). Even with these relatively minor differences in urban land cover, the effect on chloride concentration changes from 1981 to 2010 is apparent in the Milwaukee River with the Wells St. results indicating higher concentrations during winter than the two upstream sites. The greatest increase in chloride concentrations occurred during winter low-flow periods at all three sites, with greater changes as urban influence in the watershed increased. Although the greatest concentration increase was seen during these low flow winter events, substantial increases in chloride concentration also occurred during higher flow periods and extended throughout the year.

Flow-normalized concentrations estimated from 1980 to 2010 for 30 selected sites on 19 streams indicated that concentrations increased for the majority of the sites (Fig. 4). For the more urban watersheds, increases in concentration were greatest in winter periods, but summer periods also experienced increasing chloride concentrations. For the less urban watersheds, there were increasing trends over time (winter and summer slopes were similar) and the concentrations during winter were greater than or similar to concentrations during the summer. The magnitude of chloride concentrations as well as the slope of concentration change increased as the impervious land cover in the watershed increased. For example, the highest chloride concentrations and the greatest change (increase) in chloride concentration over this time period were sites with the highest degree of impervious area including those in Milwaukee, Chicago, Detroit, Cleveland, and Racine metropolitan areas (Fig. 4, top three rows, Table 1). Chloride concentrations generally decreased with decreasing impervious area in the bottom four rows of Fig. 4. The two exceptions are the sites in Portland (Willamette River) and Dallas (Trinity River) which show little or no trend in chloride concentration over the study period. Land cover in the Willamette River had 72% forest and natural area in the watershed, road salt was not commonly used in Oregon during the study period, and the climate

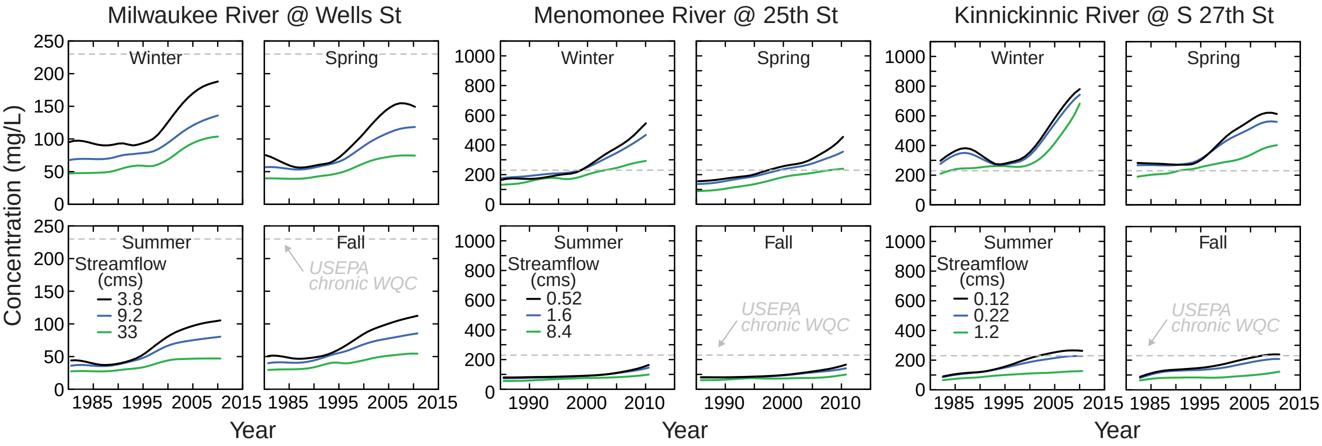


Fig. 2. Chloride concentration estimates at 10, 50, and 90 percentile flow rates from the WRTDS model over time and grouped by season for three Milwaukee streams. Graphs are presented in order of decreasing watershed size and increasing urban land cover from left to right. Streamflow is expressed in cubic meters per second (cms). Dashed line for USEPA chronic water quality criteria represents 230 mg/L.

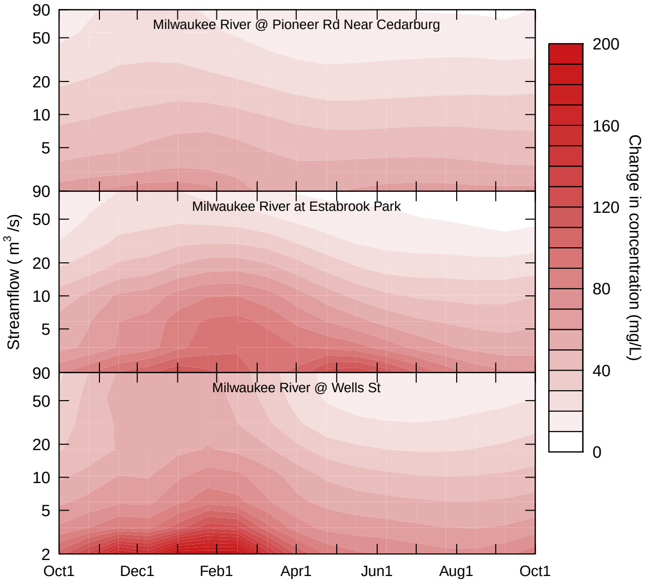


Fig. 3. Contour map of the change in chloride concentration by streamflow and time of year between 1981 and 2010 on the Milwaukee River. Sites are ordered from top to bottom with increasing urban land cover in the watershed. The color legend indicates change in concentration in mg/L.

in the Trinity River is warm enough that road salt application is not common.

Notable differences were observed among sites with low watershed imperviousness in the Midwest U.S. (Rock River; Milwaukee River at Cedarburg, which is upstream from the Milwaukee metropolitan area; and the Sheboygan River) versus those outside of the Midwest (Potomac and Willamette Rivers).

Sites with low imperviousness in the Midwest have higher chloride concentrations, when compared with sites in other areas of the country. Sites with low imperviousness in these different regions also show notable differences in non-urban land cover types; whereas the Midwest sites have large proportions of agricultural land, sites in other regions were dominated by forest and natural areas (Table 1). Concentrations at seiche-affected sites on the Milwaukee and Kinnickinnic Rivers also increased, but the magnitude of these trends was typically muted in comparison with upstream, non-seiche affected sites. Chloride concentrations were lower at study sites with the lowest percent impervious watersheds (less than 0.5%), but increasing trends were still apparent in winter and summer seasons.

Flow-normalized chloride concentration estimates from the WRTDS model were compared with urban land cover in the contributing watershed as 5-yr means for 1990–1994 and 2006–2010 (Fig. 5-A). There was a linear relation between mean concentration and percent urban land cover for both time periods, but regression slopes indicated a change in this relation over time with slopes for these regression equations indicating an increase in chloride concentration of 2.9 and 5.8 mg/L/% urban land cover for the 1990–94 and 2006–10 time periods respectively. Concentrations from 2006 to 2010 were approximately double the concentrations for 1990–1994 for the same percentage of urban land cover.

The logistic models for the probability of water quality criteria exceedance as a function of the square root of the percent of urban land cover in the watershed were significantly different ($p < 0.001$) between the two periods (1990–1994 and 2006–2010; Fig. 5-B). For a watershed with 25% urban area, the expected number of days exceeding 230 mg/L per year increased from 5 to 14, and for a watershed with 90% urban area, it increased from 95 to 231 days per year. An expected value of 17 days exceeding 230 mg/L per year decreased from 50% to 29% urban land cover, and an expected value of 95 exceedance days per year decreased from 95% to 63% urban land cover. About 29% of sites studied exceeded the concentration for the USEPA chronic water quality criteria of 230 mg/L by an average of more than 100 individual days per year during 2006–2011. All regression slopes in Fig. 5 were significant with $p < 0.001$, and R^2 values for all regressions ranged between 0.83 and 0.99. Slopes were significantly different in each of the analyses represented in Fig. 5 ($p < 0.001$ for panels A and B and $p < 0.05$ for panel C).

Beginning in 1987 and ending in 2010, road salt sales in the U.S. increased at an average rate of 3.9%/yr, and urban land cover in the northern part of the U.S. increased at a rate of 2.8%/yr (Fig. 5-C). These trends indicate that road salt usage increased at a rate 40% greater than the increase in urban land cover in the northern U.S. during this period.

4. Discussion

4.1. Temporal trends and relation with land use

Results of the present research indicate that chloride concentrations increased with time in most streams studied in the northern U.S. throughout the study period. While there were trends present in streams with watersheds dominated by urban, agriculture, and forest/natural areas alike, there was a clear increase in concentration as urban land cover (and impervious surfaces) in the watershed increased.

The concentration increase in watersheds with relatively small amounts of urban land use may be influenced by road salt, but may also be a result of other sources such as agricultural runoff which is another potentially important source of chlorides in rural watersheds (Mullaney et al., 2009). Still, the greater winter concentrations suggest that road salt was an important factor in observed trends in the rural watersheds. In contrast with the Midwest sites, which are more highly developed for agriculture, requiring a more extensive road network, the Potomac and Willamette River watersheds have larger percent forest and natural areas (60% and 72% respectively). A detailed watershed-specific investigation would be needed to better understand relative contributions in these rural sites.

The rate of chloride concentration increase outpaced that of urbanization for this study, so urban land cover information alone cannot account for these chloride trends. This changing relation of chloride with urban land cover over time (Fig. 5-A) may be attributed to several potential factors. First, it is possible that more salt was applied per unit urban area during the latter portion of the study period than during the early portion. This appears likely given that road salt sales in the northern U.S. outpaced the rate of increase in urban land cover by 40% during the study period (Fig. 5-C). More salt could be applied per unit area due to three primary reasons: 1) the application rate could have increased as an attempt to maintain more ice-free conditions; 2) the density of impervious area per unit urban area could have increased, thereby increasing the need for road salt, or 3) the difference in weather conditions between the early and latter portions of the study could have warranted different application rates.

Second, the baseline concentrations have been increasing over time due to continued road salt input to the shallow groundwater system and inability of the system to recover to true background concentrations before the next deicing season begins. The result is an increase in baseline concentrations from shallow groundwater discharge to the stream during low flow, as indicated by increasing summer concentrations. Since baseline concentrations increased with time over the course of the study, less additional road salt runoff was needed to reach concentrations of concern in the later years of the study than in the early years, effectively changing the slope of the chloride to urban land cover relation. With baseline concentrations governed by groundwater discharges in many instances, this finding is consistent with other research that has observed elevated chloride concentrations in groundwater which has caused elevated stream concentrations (Kelly, 2008; Eyles et al., 2013).

To explore the possibility of changing weather patterns as potential explanation of increased salt application, snowfall data were examined

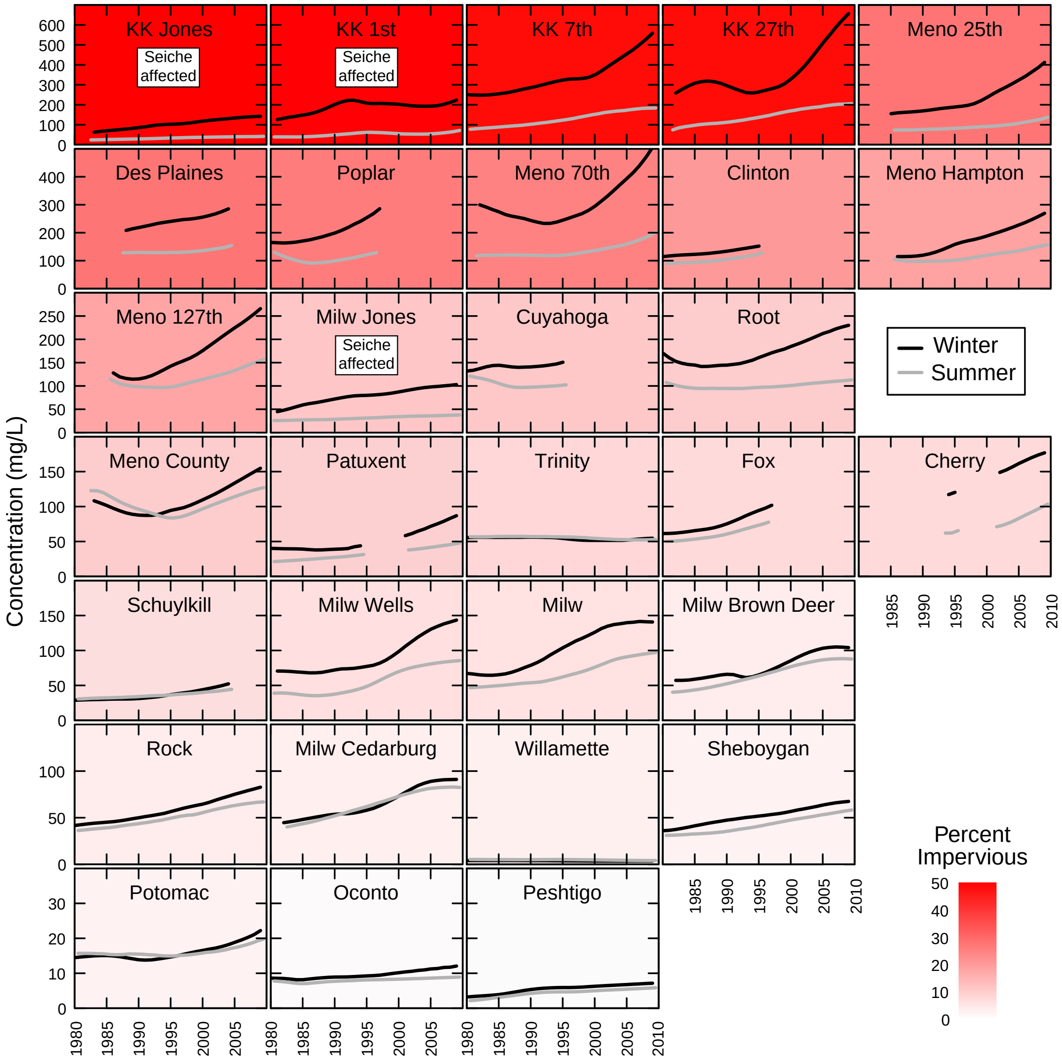


Fig. 4. Winter (black line) and summer (gray line) flow-normalized chloride concentration trends for 30 sites in 19 streams across the United States. The background color represents watershed percent imperviousness as determined using the National Land Cover Database from 2006 (Fry et al., 2011). Sites are ordered by percent imperviousness. Seiche affected is defined by backwater influence from Lake Michigan.

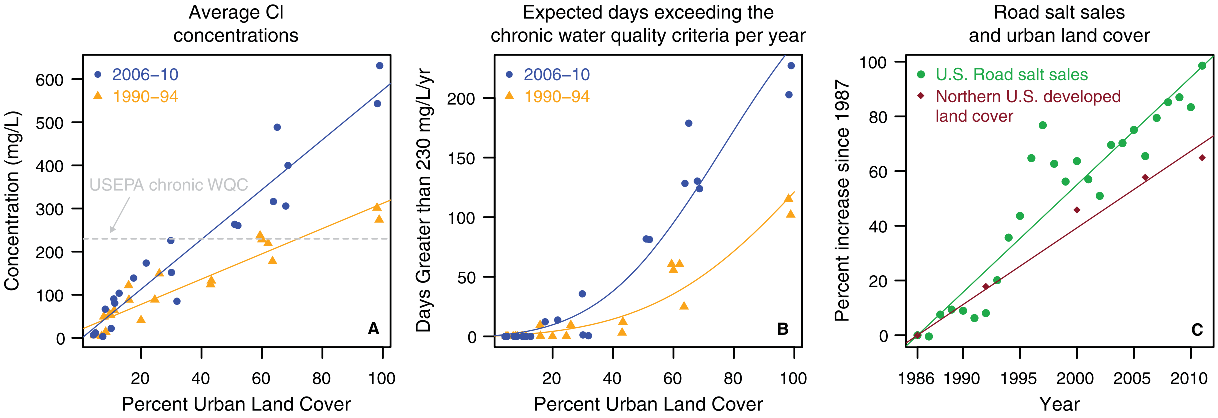


Fig. 5. Average chloride concentration (A) and expected number of individual days per year with concentration exceeding the USEPA chronic water quality criteria of 230 mg/L (B) from modeling results compared to urban land cover percentage in the contributing watershed, and the percent increase in U.S. annual road salt sales compared to the percent increase in urban land cover in the northern U.S. since 1987 (C). Lines (A and C) represent ordinary least squares regression models and curves (B) are based on results of logistic regressions of the probability of daily exceedance as a function of urban land cover. In panel C, U.S. road salt sales are presented as a 5-yr moving average. Dashed line for USEPA chronic water quality criteria represents 230 mg/L (A).

for nine National Weather Service monitoring stations ranging geographically from Washington, D.C., to Denver, Colorado (Table S2, supporting information). For each station, the average total annual snowfall and the average annual number of individual days with snow exceeding 20 mm were computed as a measure of potential for road salt application. An increase in the annual number of days with snow exceeding 20 mm was observed at five stations, and a decrease was observed at four stations. An increase in annual snowfall was observed at six stations, and a decrease was observed at three stations. These data indicated a potentially mixed influence of weather on road salt applications among chloride monitoring sites. Given that 23 of the 30 stream sites were located in the Midwest, the result that data from all four weather stations in the Midwest had increased annual snowfall (average increase of 16%) and days with greater than 20 mm of snow (average increase of 13%) indicated a potential for road salt application increase due to snowfall conditions. In contrast, two of four weather stations in the eastern part of the country had decreased annual snowfall (average overall decrease of 11%) and three of four weather stations had decreased days with greater than 20 mm of snow (average overall decrease of 23%) indicating a potential for road salt application decrease due to snowfall conditions. Snowfall (annual depth and days with greater than 20 mm) in Denver, Colorado decreased by more than 40%. Despite the mixed trends in snow records, stream chloride concentrations increased in each of these three areas of the country, suggesting that increasing baseline concentrations and possible increasing application rates due to factors other than snow cover contribute to the changing relation of chloride with urban land cover over time. Since weather patterns and road salt application methods are locally variable, it would be valuable to extend this study in future research by examining the overall concentrations and the baseline concentrations with respect to precipitation and total salt application on an individual watershed basis.

Given the increasing road salt sales per unit area of urban land cover in the U.S., the increasing baseline chloride concentrations during summer periods, and the difference in snow conditions, it appears multiple factors could plausibly be contributors to the changing relation between average chloride concentrations and urban land cover within the watershed.

4.2. Seasonality

Increasing chloride trends were present all year, including seasons that do not require deicer application; however, the highest concentrations occurred during winter periods. A similar year-round influence has been noted multiple times in previous research (Williams et al., 2000; Kelly, 2008; Perera et al., 2013). This non-deicing season effect has been attributed to salt infiltrating into the shallow groundwater system thereby serving as a “reservoir” of salt that is slowly discharged into streams as baseflow. Relatively slow travel times in the shallow groundwater system could account for the time lag between deicer applications and eventual discharge into the stream.

4.3. Streamflow dependency

Chloride concentrations commonly increased with decreasing streamflow throughout all seasons of the year in most streams studied. The same relation has previously been observed in streams of Illinois (Kelly et al., 2010) and Toronto (Meriano et al., 2009). This behavior can be explained primarily by the factors that govern hydrology throughout the year. During cold-weather months, road salt applications occur during many types of precipitation events. These include a gradient of precipitation forms ranging from purely snowfall events to mixed rainfall and snowfall events to purely rainfall events when freezing temperatures are expected. Precipitation with very little or no liquid precipitation provide little dilution of road salt as it melts snow and ice and eventually drains to nearby streams. These are also low-flow periods, so the stream itself provides little dilution. The combination of these two factors results in high chloride concentrations in the streams. Conversely, during deicing events with greater quantities of liquid precipitation, more dilution of the road salt is provided directly from the precipitation. In addition, when snow is present on the ground, melting is enhanced by contact with rainfall, streamflow is elevated, and dilution potential in the stream is greater. These high-dilution events still have elevated chloride concentrations, but not as high as the low-dilution events.

During non-deicing months, chloride concentrations also decreased with increasing streamflow. Precipitation events again serve to dilute chloride concentrations more than those during low-flow periods that are dominated by groundwater discharge, which is a substantial source of chloride during the non-deicing months.

4.4. Comparison to aquatic toxicity benchmarks

Elevated chloride concentrations resulting from road salt application and runoff in watersheds have potential impacts on aquatic organisms (U.S. Environmental Protection Agency, 1988; Environment Canada, 2001). Increasing trends over time have resulted in increasing exceedances of concentrations that are likely to be harmful to aquatic life. The current research indicates that the relation between urban land cover and the number of daily exceedances of the USEPA chronic water quality criteria concentration of 230 mg/L has changed during the study period (Fig. 5). The number of exceedances for a particular percent of urban land cover was greater during the latter portion of the study as compared to the early portion of the study. As described above for increasing concentrations, an increase in road salt application rates over time, an increase in the baseline concentrations as indicated by summer chloride trends, and changes in snowfall are likely causes of the increased water quality criterion exceedance rate.

Previous research has indicated that degradation of biological integrity is evident beginning below 1% impervious area (Stepenuck et al., 2002; Cuffney et al., 2010; King et al., 2010). Results from the present research are consistent with these findings as chloride concentrations began to increase as soon as urban land cover was present, and concentrations exceeded the chronic water quality criterion beginning at approximately 10% impervious area (approximately 25% urban land cover; Table 1, Fig. 5). A review of road salt effects conducted by Environment Canada concluded that high concentrations of chloride may have immediate or long-term effects on ecosystem populations and that lower concentrations may have adverse effects on community structure, diversity, and productivity (Environment Canada, 2001). Studies reviewed for this Environment Canada effort found that some of the biological components affected included densities of bacteria and algae, drift of stream benthic invertebrates, as well as diversity and community structure of aquatic invertebrates (Evans and Frick, 2001). Other work has determined that elevated chloride concentrations can also influence reproduction of aquatic organisms (Beggel and Geist, 2015). All of this information is primarily based on direct influence from chloride exposures, but indirect exposures caused by mobilization of heavy metals may also have impacts on aquatic organisms (Amrhein et al., 1992; Bäckström et al., 2004; Nelson et al., 2009). These chloride influences are yet another stressor in addition to those commonly thought to impact biological integrity of urban streams such as hydraulic and hydrologic factors, degraded water quality from point and non-point source runoff, and altered habitat and stream channels (Walsh et al., 2005; Steuer et al., 2010).

The multi-season impacts presented in this research suggest the possibility of extended-duration, high-concentration exposure to chloride in urban streams of the northern U.S. This possibility appears credible given that extended-duration (multiple months), high-concentration exposures to chlorides have previously been documented in urban streams receiving road salt runoff (Corsi et al., 2010; Baldwin et al., 2012; Kelly et al., 2012b). Further work to define concentration–duration relations is warranted given that the current USEPA chronic water quality criterion is designed for a 4-day exposure period, and it appears that exposures have potential to be much longer than 4 days. Longer-duration exposures may result in additional impacts on the full life-cycle of aquatic organisms that may not be evident with common evaluation methods.

4.5. Salt management and alternatives

The nature of salt presence in environmental waters makes this issue very difficult to address with common stormwater management practices that rely most commonly on settling or filtration of particulate matter (Waschbusch, 1999; Greb et al., 2000; Horwatich et al., 2011). Since salt dissolves readily in water, these types of management practices will not remove salt from runoff. The only reliable way to reduce the impact of road salt on receiving streams is to reduce applications. There are a host of techniques that have been identified and documented for reduction of road salt application. For example, many municipalities have salt management plans that include a strategy for minimizing road salt usage. Some of these practices include training programs for most effective use, pre-wetting of granular salt to maximize salt retention on paved surfaces, applicators that are calibrated and vary by ground-speed, anti-icing that reduces bonding between snow and pavement and makes plowing more effective, and more efficient predictions of icing conditions to inform deicing activities (Kramberger and Zerovnik, 2008; Fay et al., 2013). In addition, there are a number of alternative chemicals that have been used. These alternative chemicals commonly include other chloride-containing salts such as magnesium chloride or calcium chloride, organic salts such as calcium magnesium acetate, potassium acetate, or sodium acetate, different variations of salt brines, and organic deicers such as glycols. Unfortunately, none of these options are without potential environmental impact as well. All of these alternative deicers have varying degrees of associated aquatic toxicity (Environment Canada, 2001). In addition, organic chemicals used as deicers have an additional impact from increased biochemical oxygen demand (Corsi et al., 2012) and excessive biofilm growth (Mericas et al., 2014). Still, road salt is more common than the alternatives due to the performance effectiveness and relatively low cost compared to alternatives.

5. Conclusions

The U.S. is an urbanizing nation, and with increasing development, previous data and results from this research indicate that road salt applications, chloride concentrations, and resulting adverse impacts on aquatic organisms in streams are likely to increase along with urban development. This research indicates that chloride concentrations in urban streams of the northern U.S. and resulting water quality criteria exceedances have increased at a greater rate than the rate of urban development. In addition, elevated chloride concentrations in these streams through all seasons have implications on long-term exposures to chloride for aquatic organisms. Results of this research provide verification that chloride concentrations in urban streams continue to increase, influencing the potential for aquatic life in affected streams.

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Appendix A. Supplementary data

Methods for the determination of land cover and other watershed characteristics, characteristics of water quality and associated streamflow sites (Table S1), snowfall information at nine National Weather Service Stations (Table S2), WRTDS modeling results during the periods from 1990 to 1994 and 2006 to 2010 for winter and summer periods (Table S3). This material is available free of charge via the Internet at STOTEN TO http://dx.doi.org/10.1016/j.scitotenv.2014.12.012.

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ATTACHMENT 5

TOLLWAY OFFSET PROGRAM

Wood Dale Chloride Offset

INTERGOVERNMENTAL AGREEMENT BETWEEN THE ILLINOIS STATE TOLL HIGHWAY AUTHORITY AND THE CITY OF WOOD DALE FOR CHLORIDE OFFSET PROGRAM

This INTERGOVERNMENTAL AGREEMENT ("AGREEMENT") is entered into this 13TH day of JUNE, 2017, by and between The Illinois State Toll Highway Authority, an instrumentality and administrative agency of the State of Illinois, hereinafter called the "ILLINOIS TOLLWAY", and The City of Wood Dale, a municipal corporation of the State of Illinois, hereinafter called the "CITY", individually referred to as "PARTY", and collectively referred to as "PARTIES".

WITNESSETH:

WHEREAS, the ILLINOIS TOLLWAY in order to facilitate the free flow of traffic and ensure safety to the motoring public, intends to improve the existing Elgin O'Hare Expressway, extend the expressway from its eastern terminus at Rohlwing Road (Illinois Route 53) to O'Hare International Airport (ORD) to be known entirely as Illinois Route 390, and construct the Western Access connecting the Jane Addams Memorial Tollway (I-90) with the Tri-State Tollway (I-294) (hereinafter sometimes referred to as the Elgin O'Hare Western Access "EOWA"), and included in multiple ILLINOIS TOLLWAY construction contracts. The ILLINOIS TOLLWAY will implement, operate and maintain the mainline improvements as tolled facilities (hereinafter sometimes referred to as "Toll Highway"); and

WHEREAS, highway de-icing practices during winter months commonly use de-icing salts to provide for safe vehicular travel and winter maintenance for the EOWA will require the use of chlorides; and

WHEREAS, studies acknowledged the potential for the addition of chloride concentrations in area streams and as the EOWA project is advancing in implementation, permits for Section 404 and Section 401 of the Clean Water Act (CWA) have been secured from the United States Army Corp of Engineers (USACE) and the Illinois Environmental Protection Agency (IEPA); and

WHEREAS, conditions established within the ILLINOIS TOLLWAY's CWA Section 401 permit require the implementation of a "Chloride Offset Program" (hereinafter called the "PROGRAM") to mitigate for increased chloride loading in the Total Maximum Daily Loading (TMDL) watersheds throughout the EOWA project area; and

WHEREAS, a Memorandum of Understanding (MOU) between the ILLINOIS TOLLWAY and DuPage River Salt Creek Workgroup (DRSCW) was executed October 31, 2013 to outline the PROGRAM, which is attached hereto as "Exhibit A" and incorporated by reference; and

WHEREAS, per the MOU, it was agreed that the entirety of the offset will occur within the impacted areas and be tailored to individual receiving stream segments to the maximum extent possible. As such, local watershed communities and agencies participating in the PROGRAM will be responsible for winter operations on the land surfaces that drain to those segments and are herein referred to as "TIER 1 COMMUNITY" or "TIER 1 COMMUNITIES"; and

WHEREAS, the CITY has been identified as a TIER 1 COMMUNITY and has, in coordination with the DRSCW, conducted a review of their snow and ice operations in an effort to identify areas of operational improvements relating to efficiency gains in winter chloride usage, and per the CITY'S review, it is believed that an effective reduction in chloride usage can be attained, as part of the PROGRAM through the upgrading of equipment utilized for winter operations by the CITY; and

WHEREAS, the PARTIES mutually agree to review winter operation policies and procedures and work in coordination with the DRSCW to monitor the administration and progress of the PROGRAM; and

WHEREAS, the ILLINOIS TOLLWAY by virtue of its powers as set forth in the "Toll Highway Act," 605 ILCS 10/1 *et seq.* is authorized to enter into this AGREEMENT; and

WHEREAS, the CITY by virtue of its powers as set forth in the "Illinois Municipal Code," 65 ILCS 5/1-1-1 *et seq.* is authorized to enter into this AGREEMENT; and

WHEREAS, a cooperative Intergovernmental Agreement is appropriate and such an Agreement is authorized by Article VII, Section 10 of the Illinois Constitution and the "Intergovernmental Cooperation Act", 5 ILCS 220/1 *et seq.*

WHEREAS for recording purposes this AGREEMENT shall be known as #004685.

NOW, THEREFORE, in consideration of the aforementioned recitals and the mutual covenants contained herein, the PARTIES hereto agree to the following summary of the responsibilities and participation of each PARTY in the implementation of the PROGRAM.

I. PURPOSE and SCOPE

A. The PROGRAM established a projected increase over existing conditions or baseline for the EOWA of 3,888 tons of salt per year, per a baseline 39.7 tons/lane mile/year application rate. The goal of the offset program will be to reduce salt usage in roadway applications in the project related watersheds by the amount of the increase (3,888 tons per year) plus a margin of safety of 25 percent or a total reduction of 4,860 tons per year.

B. The ILLINOIS TOLLWAY, as part of the PROGRAM, has established a rate reduction goal of 20% from the EOWA's established baseline salt application rate of 39.7 tons/lane mile/year, thereby accounting for 972 tons per year of the 4,860 tons per year of salt required to be reduced, as part of the PROGRAM.

C. The CITY, as part of the PROGRAM and per this AGREEMENT, has established a rate reduction goal of 17.5% from its established baseline application salt application rate of 12.3 tons/lane mile/year, thereby accounting for 204 tons per year of the 4,860 tons per year of salt required to be reduced, as part of the PROGRAM.

D. Both structural and non-structural practices will comprise the PROGRAM to provide the needed improvements in water quality but it is understood that offset reductions can only be accounted for through non-structural practices.

II. STRUCTURAL BEST MANAGEMENT PRACTICES

A. Grass swales, bio-swales, infiltration basins, and other designs will be incorporated by the ILLINOIS TOLLWAY into the EOWA projects in order to minimize the effects of roadway runoff and improve the quality of roadway runoff discharged to receiving waters and/or nearby wetlands.

III. NON-STRUCTURAL BEST MANAGEMENT PRACTICES

A. A salt reduction goal has been established by the ILLINOIS TOLLWAY and DRSCW for the PROGRAM through the analysis of existing conditions, existing practices in the affected watersheds, and planned highway improvements. Offsets will include both the adoption of salt reduction strategies implemented by the ILLINOIS TOLLWAY and providing salt application data to DRSCW, including but not limited to their application rates for each lane mile, as well as financial and other support to other mutually agreed upon partners.

B. The ILLINOIS TOLLWAY recognizes that meeting the objectives of the Total Maximum Daily Loads ("TMDL's") will require reductions in area chloride loading above those set out in the PROGRAM and agrees to review its practices at an agency wide scale and to actively partner with the DRSCW, its members or successors, in working for PROGRAM area chloride reductions beyond the life of the PROGRAM with the goal of meeting the applicable water quality standard.

IV. RESPONSIBILITIES

A. Pursuant to EXHIBIT A, a guideline for monitoring and reporting chloride offsets has been established. Both PARTIES agree that the requirements embodied in Clean Water Act Section 401 certification(s) or Section 404 or NPDES permit(s), are the sole responsibility of the ILLINOIS TOLLWAY, and that the CITY is not responsible in any way for the ILLINOIS TOLLWAY's failure to comply with such requirements.

B. The CITY shall provide the ILLINOIS TOLLWAY a summary raw data report that includes the following data for the most recent winter season for a minimum of five consecutive winter seasons, post equipment purchase and installation; 1) total lane miles maintained, 2) actual road salt usage in tons per lane mile, 3) baseline road salt usage in tons per lane mile, 4) target road salt usage in tons per lane mile, 5) total winter season precipitation events requiring deicing efforts, 6) average precipitation in inches of snow, ice and liquid form of winter season precipitation per precipitation event, 7) duration of each operator deicing efficiency training session and number of operators attending, 8) new equipment installation and new practices implemented and identification and practices planned and 9) the completed DRSCW's Winter Public Agency Deicing Questionnaire.

C. The PARTIES agree that the PROGRAM may require several years of monitoring and reporting from PROGRAM partners.

D. The PARTIES will develop and maintain a guidance document for the PROGRAM which will at minimum detail the methods for calculating the build scenario non-PROGRAM increase, the needed offset, BMP's the monitoring PROGRAM and reporting baseline requirements. The document will be updated by agreement between the PARTIES as the PROGRAM advances.

V. DELIVERABLES

A. The deliverables will be transmitted to the ILLINOIS TOLLWAY, by the CITY and include written reports documenting, as defined in Article IV, Paragraph B. of this AGREEMENT by June 1st pertaining to the preceding winter season.

B. The ILLINOIS TOLLWAY maintains its responsibility to remain compliant with the "Clean Water Act" as determined and administered by the IEPA.

VI. FINANCIAL

A. The ILLINOIS TOLLWAY as sponsor of the PROGRAM will compensate the CITY as outlined in this AGREEMENT and included in the CITY's Request for Equipment and Training "PROPOSAL".

B. The purchase of equipment identified within the CITY's PROPOSAL, as part of the PROGRAM shall be reimbursed by the ILLINOIS TOLLWAY to the CITY.

C. The CITY will be paid based upon its invoice(s) which shall include detailed receipts of purchased items in substantial conformance with the Budget included in the CITY's PROPOSAL.

D. The CITY shall certify in writing, upon presentation of each invoice hereunder, that items as invoiced have been actually purchased and installed and that the CITY is in fact complying with all other provisions of this AGREEMENT. Invoicing shall be sufficiently itemized to permit the ILLINOIS TOLLWAY or its consultant(s) or cooperating governmental unit(s) to verify performance of the work so invoiced.

E. It is mutually agreed that the estimated cost to the ILLINOIS TOLLWAY shall not exceed $139,500 for the term of this AGREEMENT through five consecutive winter seasons, beginning with the first winter season in which purchased equipment has been utilized and use data has been provided to the ILLINOIS TOLLWAY.

VII. GENERAL PROVISIONS

A. It is understood and agreed that this is an AGREEMENT between the City of Wood Dale and the Illinois State Toll Highway Authority.

B. All equipment proposed for purchase and installation, shall be done so prior to the 2018/2019 winter season but efforts will be made for purchases and installations to occur prior to the 2017/2018 winter season.

C. The term of this AGREEMENT shall extend through five consecutive winter seasons in which purchased equipment has been utilized and use data has been provided to the ILLINOIS TOLLWAY.

D. PARTIES agree to collaborate in effort to fulfill applicable goals of the PROGRAM as established in Section IV. of EXHIBIT A. Should the PARTIES mutually agree that the CITY did not operate in a manner demonstrating intent achieve the goal reduction of 17.5% in a PROGRAM year, the ILLINOIS TOLLWAY shall provide written notice to the CITY identifying delinquencies of agreed upon practices. Should the PARTIES mutually agree that the CITY did not operate in a manner in which to achieve the goal reduction of 17.5% for a second year during the term of this AGREEMENT, the ILLINOIS TOLLWAY and shall provide written notice to the CITY identifying delinquencies of agreed upon practices and the CITY shall provide reimbursement to the ILLINOIS TOLLWAY for all payments made by the ILLINOIS TOLLWAY associated with this agreement within 90 days of CITY's receipt of the notice.

E. Equipment purchased under this AGREEMENT by the ILLINOIS TOLLWAY on behalf of the CITY becomes the property and responsibility of the CITY and the CITY shall indemnify, hold harmless and defend the ILLINOIS TOLLWAY, its officials, directors, officers, employees, and agents from and against all liability, claims, suits, demands, proceedings and action, including costs, fees and expense of defense, arising from, growing out of, or related to, any loss, damage, injury, death, or loss or damage to property resulting from, or connected with, the CITY's negligent or willful acts, errors or omissions in its performance under this AGREEMENT, including, but not limited to, use of the equipment described herein. The CITY expressly acknowledges that the ILLINOIS TOLLWAY, through provision of funding, training, and certification under this AGREEMENT, is not intended to be a joint employer of the CITY's employees and agents and does not excerpt control over such persons in their use of the equipment that is the subject of this AGREEMENT.

F. It is understood and agreed that this AGREEMENT constitutes the complete and exclusive statement of the agreement of the PARTIES relative to the subject matter hereof and supersedes all previous oral and written proposals, negotiations, representations or understandings concerning such subject matter.

G. This AGREEMENT may be executed in two (2) or more counterparts, each of which shall be deemed an original and all of which shall be deemed one and the same instrument.

H. Under penalties of perjury, the CITY certifies that its correct Federal Tax Identification number is 36-6008547 and it is doing business as a governmental entity, whose mailing address is The City of Wood Dale, 404 North Wood Dale Road, Wood Dale, Illinois 60143.

I. The PARTIES agree to maintain books and records related to the performance of this AGREEMENT and necessary to support amounts charged to the ILLINOIS TOLLWAY and/or any of the PARTIES under the AGREEMENT for a minimum of five (5) years from the last action on the AGREEMENT. The PARTIES further agree to cooperate fully with any audit and to make its books and records, and books and records within its custody or control available to the Illinois Attorney General, the Illinois Auditor General, the ILLINOIS TOLLWAY Inspector General, the ILLINOIS TOLLWAY Department of Internal Audit, the ILLINOIS TOLLWAY or any other governmental agency or agent thereof that is authorized to audit or inspect such books and records.

J. The introductory recitals included at the beginning of this AGREEMENT are agreed to and incorporated into this AGREEMENT.

(This section intentionally left blank.)

IN WITNESS WHEREOF, the PARTIES have caused this AGREEMENT to be executed on the dates indicated.

THE CITY OF WOOD DALE

By: ______________________ Attest: ______________________
Annunziato Pulice Shirley J. Siebert
Mayor City Clerk

Date: ______________________

THE ILLINOIS STATE TOLL HIGHWAY AUTHORITY

By: ______________________ Date: 2/1/17
Greg M. Bedalov
Executive Director

Approved as to Form and Constitutionality:

______________________ ______________________
Tiffany B. [illegible]
Senior Assistant Attorney General, State of Illinois

MEMORANDUM OF UNDERSTANDING
BETWEEN
THE ILLINOIS STATE TOLL HIGHWAY AUTHORITY
AND
THE DUPAGE RIVER SALT CREEK WORKGROUP
FOR
CHLORIDE OFFSET PROGRAM

This MEMORANDUM OF UNDERSTANDING (hereinafter referred to as the "MOU") is entered into this 31st day of October AD, 2013, by and between THE ILLINOIS STATE TOLL HIGHWAY AUTHORITY, an instrumentality and administrative agency of the State of Illinois, hereinafter called the "TOLLWAY", and THE DUPAGE RIVER SALT CREEK WORKGROUP, a group of local watershed stakeholders which include sanitary districts, municipalities, counties, forest preserve districts, state and federal agencies, and private environmental organizations of the State of Illinois, hereinafter called the "DRSCW", individually referred to as "PARTY", and collectively referred to as "PARTIES".

WITNESSETH:

WHEREAS, the TOLLWAY recently approved a 15 year Capitol Program, "Move Illinois; *The Illinois Tollway Driving the Future,*" which includes improving the Jane Addams Memorial Tollway (I-90) (hereinafter sometimes referred to as the "Toll Highway"), by reconstructing and widening from the John F. Kennedy Expressway to Interstate Route 39. The contemplated improvements are substantially included in multiple TOLLWAY construction contracts; and

WHEREAS, the TOLLWAY intends to improve the Elgin O'Hare Expressway, extending the expressway from its eastern terminus at Rohlwing Road (Illinois Route 53) to O'Hare International Airport (ORD) and constructing the Western Access connecting the Jane Addams Memorial Tollway (I-90) with the Tri-State Tollway (I-294) (hereinafter sometimes referred to as the Elgin O'Hare Western Access "EOWA"), and included in multiple TOLLWAY construction contract(s). The TOLLWAY will implement, operate and maintain the mainline improvements as tolled facilities (hereinafter sometimes referred to as the "Toll Highway"); and

WHEREAS, because the projects are in such close proximity, strategies for some aspects of environmental mitigation (i.e., water quality enhancements) are being considered collectively by the PARTIES, where feasible and practicable; and

WHEREAS, highway de-icing practices during winter months commonly use de-icing salts to provide for safe vehicular travel and winter maintenance for I-90 and the EOWA will require the use of salts; and

WHEREAS, studies acknowledged the potential for the addition of chloride concentrations in area streams and as the I-90 and EOWA projects are advancing to implementation, applications for Section 404 and Section 401 permits have been submitted to the United States Army Corp of Engineers (USACE) and the Illinois Environmental Protection Agency (IEPA); and

WHEREAS, the enhancement of water quality has been the focus of the DRSCW for many years, and the TOLLWAY in an effort to have the "Cleanest and Greenest" program possible is requesting that a partnership be developed between the PARTIES hereto, and a collaboration with permitting agencies, to achieve chloride offsets and reductions to enhance the water quality throughout the DRSCW's water-sheds affected by I-90 and the EOWA; and

WHEREAS, the waterways receiving storm water and snowmelt runoff from the I-90 and EOWA are on the IEPA's Section 303D List of impaired waters and thus require at a minimum no net increase in chlorides as defined in the "Clean Water Act"; and

WHEREAS, this MOU, for recording purposes shall be known as 002013-22, executed in duplicate, and has been prepared to outline the general understanding between the DRSCW and the TOLLWAY with regard to determine and establish their respective responsibilities toward a proposed "Chloride Offset Program" (hereinafter referred to as the "PROGRAM") and also serve as a basis for developing Intergovernmental Agreements with local watershed communities and agencies in the impacted area; and

WHEREAS the PARTIES agree that the entirety of the offset will occur with the impacted areas and be tailored to individual receiving stream segments to the maximum extent possible. As such local watershed communities and agencies participating in the PROGRAM will be responsible for winter operations on the land surfaces that drain to those segments and are herein referred to as "Tier 1 Communities";

NOW, THEREFORE, in consideration of the aforementioned recitals and the mutual covenants contained herein, the PARTIES hereto agree to the following summary of the responsibilities and participation of each PARTY in the implementation of the PROGRAM.

I. GOAL

A. The goal of the PROGRAM is to offset the increased chloride loadings from I-90 and the EOWA by affecting reductions in the use of winter de-icing salts from existing conditions. The TOLLWAY will reduce chloride applications in a quantifiable manner in support of the 401 Water Quality Certification process for I-90 and the EOWA projects and of local municipalities National Pollutant Discharge Elimination System Municipal Separate Storm Sewer System ("NPDES MS4") permit requirements.

B. The intent is to establish a partnership between the TOLLWAY and governmental bodies to achieve the chloride loading offset.

C. The partners will endeavor to achieve the offsets in communities straddling and or upstream of the I-90 and EOWA, but the TOLLWAY will expand beyond that area if needed to achieve the offset amounts.

D. Both structural and non-structural practices will comprise the PROGRAM to provide the needed improvements in water quality.

II. STRUCTURAL BEST MANAGEMENT PRACTICES

A. Grass swales, bio-swales, infiltration basins, etc. will be incorporated by TOLLWAY into the I-90 and EOWA projects in order to minimize the effects of roadway runoff and improve the quality of roadway runoff discharged to receiving waters and/or nearby wetlands.

III. NON-STRUCTURAL BEST MANAGEMENT PRACTICES

A. A salt reduction goal will be established by the TOLLWAY and DRSCW for the PROGRAM through further analysis of existing conditions, existing practices in the affected watersheds, and planned highway improvements. Offsets will include the adoption of salt reduction strategies (enhanced training, improved materials use, equipment upgrades), implemented by both the TOLLWAY and the Tier 1 communities. All participants will provide documentation on their salt application rates per lane mile, application totals, calibration logs and details on financial and other support to other mutually agreed upon partners.

B. The TOLLWAY recognizes that meeting the objectives of the Total Maximum Daily Loads ("TMDL's") will require reductions in area chloride loading above those set out in the PROGRAM and agrees to review its practices at an agency wide scale and to actively partner with the DRSCW, its members or successors, in working for PROGRAM area chloride reductions beyond the life of the PROGRAM with the goal of meeting the applicable water quality standard.

IV. TOLLWAY AND DRSCW RESPONSIBILITIES

A. Both PARTIES agree that the requirements embodied in Clean Water Act Section 401 certification(s) or Section 404 or NPDES permit(s), are the sole responsibility of the TOLLWAY, and that the DRSCW or participating agencies cannot be held liable in any way for failure to comply with such requirements.

B. The DRSCW will endeavor to unify stakeholders in the project areas with the common goal of improving chloride water quality. It is recognized that participating communities are voluntary agents and neither they nor the DRSCW can be held liable in any way for failure to collaborate in the plan.

C. The DRSCW will determine the baseline conditions through appropriate studies with stakeholders and other environmental evaluation which shall include sampling and analyses, as well as flow evaluation.

D. The DRSCW will determine the opportunities for improving de-icing practices through surveys and interviews with stakeholders.

E. The DRSCW and the TOLLWAY will set priorities, tracking offset progress, and the timeframe for achievement with concurrence from the IEPA.

F. The TOLLWAY will provide ongoing financial assistance supporting capital investments of alternative de-icing methods potentially for municipalities and agencies that are participating in the PROGRAM until such time as the PROGRAM objectives are met.

G. The TOLLWAY with the DRSCW's technical assistance will establish training and certifications for operators that would enhance awareness of best practices for snow and ice management operations.

H. The TOLLWAY with the DRSCW will establish data sites/sources for weather data and other information helpful in managing roadway de-icing.

I. The DRSCW with the support of the TOLLWAY will conduct long term monitoring that records salt usage and stream conditions. Evaluation of future operating conditions will be compared to baseline conditions.

J. The TOLLWAY and the DRSCW will report annually by July 1st to the IEPA the resulting efforts and success of the PROGRAM on an annual basis beginning in July of 2014. Success will be measured primarily by the application rate and totals reported with consideration given to the ambient monitoring system.

K. The TOLLWAY and the DRSCW will collaborate to put in place an ambient monitoring system that will be part of the PROGRAM monitoring and evaluation, and will document pre and post PROGRAM chloride conditions in the receiving streams. A monitoring plan will be developed through input from both PARTIES with the intent of conducting stream monitoring on Addison Creek, Salt Creek mainstem, Spring Brook, Meacham Creek and West Branch mainstem. The pre and post conditions will be synthesized in a report and submitted to IEPA on an annual basis along with other reporting data. The system would assist in evaluating the success of the PROGRAM in meeting the TMLD's goals and will be funded wholly by the TOLLWAY.

L. The PARTIES agree that the PROGRAM may require several years of monitoring and reporting from PROGRAM partners.

M. The PARTIES will develop and maintain a guidance document for the PROGRAM which will at minimum detail the methods for calculating the build scenario non-PROGRAM increase, the needed offset, BMP's the monitoring PROGRAM and reporting baseline requirements. The document will be updated by agreement between the PARTIES as the PROGRAM advances.

V. INTERGOVERNMENTAL AGREEMENTS

A. The TOLLWAY shall use its best efforts to enter into Intergovernmental Agreements prepared by the TOLLWAY and Tier 1 communities based upon this MOU to further determine and establish respective responsibilities toward financial partnerships, information sharing, and training.

B. These Intergovernmental Agreements shall not relieve the TOLLWAY of their responsibility to comply with the "Clean Water Act" as determined and enforced by the IEPA.

VI. FINANCIAL

A. The TOLLWAY will support financial partnerships through an Intergovernmental Agreement requiring cost sharing with a local partner as defined by the PROGRAM.

B. Project requests to the TOLLWAY will be subject to DRSCW and TOLLWAY approvals, to cost effectively promote salt usage reduction.

VII. TERMS OF THE MOU

A. The term of this MOU shall extend until such time that the PROGRAM offset has been achieved reductions have been demonstrated for a minimum of three (3) years. Either PARTY shall have the right to terminate this MOU at any time by providing at least ninety (90) days written notice to the other party in the event either PARTY breaches the terms and conditions of this MOU. At the end of the agreement period this document may be renewed by the mutual consent of the PARTIES.

(This space intentionally left blank)

IN WITNESS THEREOF, the PARTIES have entered into this MOU as of the date written below.

THE DUPAGE RIVER SALT CREEK WORKGROUP

By: ______________________
David Gorman, President

Date: 10 - 30 - 13

Attest: Stephen McCracken
10-30-13.
(Please Print Name)

THE ILLINOIS STATE TOLL HIGHWAY AUTHORITY

By: ______________________
Kristi Lafleur, Executive Director

Date: 10/31/13

Approved as to Form and Constitutionality

Tiffany I Bohn, AAG. 10/31/13
Tiffany I. Bohn, Assistant Attorney General, State of Illinois

Illinois Tollway

The Illinois Tollway
2700 Ogden Avenue
Downers Grove, Illinois 60515-1703
Phone: 630/241-6800
Fax: 630/241-6100
TTY: 630/241-6898

November 4, 2013

Mr. Stephen McCracken
DuPage River Salt Creek Workgroup
The Conservation Foundation
10 S. 404 Knoch Knolls
Naperville, IL 60565

Re: Memorandum of Understanding between the The Illinois State Toll Highway Authority and the DuPage River Salt Creek Workgroup for Chloride Offset Program.

Dear Mr. McCracken:

Enclosed please find one (1) fully executed Memorandum of Understanding between the Illinois State Toll Highway Authority and The DuPage River Salt Creek Workgroup for Chloride Offset Program on I-90 and the Elgin O'Hare Western Access.

Very truly yours,

Tiffany I Bohn

Tiffany I. Bohn
Assistant Attorney General

TIB:mw
Enclosure

Illinois Tollway

THE ILLINOIS STATE TOLL HIGHWAY AUTHORITY

TO: Eileen Cosgriff, CIS

FROM: Tiffany I. Bohn, Assistant Attorney General

DATE: November 4, 2013

SUBJECT: ***Memorandum of Understanding between The DuPage River Salt Creek Workgroup and The Illinois State Toll Highway Authority for Chloride Offset Program.***

Attached please find one (1) fully executed original Memorandum of Understanding between the DuPage River Salt Creek Workgroup and the Illinois State Toll Highway Authority Chloride Offset Program.

This document is transmitted to your attention for the Department's records.

This Memorandum of Understanding does not require a Board Resolution.

TIB:mw
Attachment

cc:
- V. Avila
- T. Bohn
- K. Kell
- P. Kovacs
- D. Manetti
- M. Molliconi
- P. Pearn
- J. Romano
- S. Talaber
- B. Wagner
- V. Yee
- G. Zimmer
- R. Zucchero

Activity	Description	Municipal Estimated Grant Cost	Wood Dale Match	Estimated chloride reduction	Schedule
Staff Training	Concentrated small group training on interpreting weather forecasts, developing site specific storm management plans, and reconfiguring operations plans from updated forecasts.		$8,000.00	Cumulative 17.5%	Budgeted Item: Would be implemented this winter.
Purchase of 2.5 Ton Dump with sling host for v-box and anti-icing	This will allow the City to expand it's anti-icing program to cover the entire City and the V-Box would serve the industrial thus decreasing current number of passes required for salting creating less waste.	$139,500.00	170,500.00	Cumulative 17.5%	Budgeted Item for FY 18: Truck would be ordered as soon as confirmation of grant is received.
Total		$139,500.00	$178,500	Cumulative 17.5%	

This process is inter-dependent; one missing link will stop the effectiveness that the organizations involved are looking to accomplish.

Request $139,500.00; City investment $178,500.00

In the event that costs for equipment and training exceed the funds granted the City of Wood Dale, the City of Wood Dale will offset costs to the extent that City funds have available.

Average total salt use (2013-2014 to 2015-2016) 1,166 tons.
Average annual use per street mile (1,166 /95 = 12.3 tons per lane mile annual
Estimated Average Total Reduction is 17.5% or 204 tons

Basin	Lane Miles	Annual Load	17.5% reduction	Basin reduction Target
Salt Creek Main Stem	52.2	642.06	112.3605	667.2
Addison Creek	9.4	115.62	20.2335	207.2
Bensenville Ditch	18.2	223.86	39.1755	228.8
Willow Creek	15.2	186.96	32.718	980

05/25/17 6.3/2

RESOLUTION NO. 21286

Background

It is in the best interest of the Illinois State Toll Highway Authority (the "Tollway") to enter into an Intergovernmental Agreement with the City of Wood Dale in connection with the construction of Illinois Route 390. In order to remain compliant with guidelines established by the Illinois Environmental Protection Agency, the Tollway and the DuPage River Salt Creek Workgroup (DRSCW) established a Chloride Offset Program to minimize chloride usage and enhance water quality throughout the DRSCW's water-sheds affected by Illinois Route 390. The Program is being implemented through partnerships with municipalities within the impacted areas by providing training to identify potential operational improvements, and the upgrading of equipment utilized for winter operations. The City of Wood Dale is an impacted municipality and requests the Illinois Tollway participate in the cost of providing training and purchasing upgraded equipment. The total estimated cost to the Tollway is not to exceed $139,500.

Resolution

The Chief Engineering Officer and the Acting General Counsel are authorized to negotiate and prepare an Intergovernmental Agreement between the Illinois State Tollway Highway Authority and the City of Wood Dale in substantially the form attached to this Resolution. The Chairman or the Executive Director is authorized to execute said agreement and the Chief Financial Officer is authorized to issue warrants in payment thereof.

Approved by: ______________________________
Chairman

Bensenville Chloride Offset

INTERGOVERNMENTAL AGREEMENT BETWEEN
THE ILLINOIS STATE TOLL HIGHWAY AUTHORITY
AND
THE VILLAGE OF BENSENVILLE
FOR
CHLORIDE OFFSET PROGRAM

This INTERGOVERNMENTAL AGREEMENT ("AGREEMENT") is entered into this 26TH day of JUNE, 2017, by and between The Illinois State Toll Highway Authority, an instrumentality and administrative agency of the State of Illinois, hereinafter called the "ILLINOIS TOLLWAY", and The Village of Bensenville, a municipal corporation of the State of Illinois, hereinafter called the "VILLAGE", individually referred to as "PARTY", and collectively referred to as "PARTIES".

WITNESSETH:

WHEREAS, the ILLINOIS TOLLWAY in order to facilitate the free flow of traffic and ensure safety to the motoring public, intends to improve the existing Elgin O'Hare Expressway, extend the expressway from its eastern terminus at Rohlwing Road (Illinois Route 53) to O'Hare International Airport (ORD) to be known entirely as Illinois Route 390, and construct the Western Access connecting the Jane Addams Memorial Tollway (I-90) with the Tri-State Tollway (I-294) (hereinafter sometimes referred to as the Elgin O'Hare Western Access "EOWA"), and included in multiple ILLINOIS TOLLWAY construction contracts. The ILLINOIS TOLLWAY will implement, operate and maintain the mainline improvements as tolled facilities (hereinafter sometimes referred to as "Toll Highway"); and

WHEREAS, highway de-icing practices during winter months commonly use de-icing salts to provide for safe vehicular travel and winter maintenance for the EOWA will require the use of chlorides; and

WHEREAS, studies acknowledged the potential for the addition of chloride concentrations in area streams and as the EOWA project is advancing in implementation, permits for Section 404 and Section 401 of the Clean Water Act (CWA) have been secured from the United States Army Corp of Engineers (USACE) and the Illinois Environmental Protection Agency (IEPA); and

WHEREAS, conditions established within the ILLINOIS TOLLWAY's CWA Section 401 permit require the implementation of a "Chloride Offset Program" (hereinafter called the "PROGRAM") to mitigate for increased chloride loading in the Total Maximum Daily Loading (TMDL) watersheds throughout the EOWA project area; and

WHEREAS, a Memorandum of Understanding (MOU) between the ILLINOIS TOLLWAY and DuPage River Salt Creek Workgroup (DRSCW) was executed October 31, 2013 to outline the PROGRAM, which is attached hereto as "Exhibit A" and incorporated by reference; and

WHEREAS, per the MOU, it was agreed that the entirety of the offset will occur within the impacted areas and be tailored to individual receiving stream segments to the maximum extent possible. As such, local watershed communities and agencies participating in the PROGRAM will be responsible for winter operations on the land surfaces that drain to those segments and are herein referred to as "TIER 1 COMMUNITY" or "TIER 1 COMMUNITIES"; and

WHEREAS, the VILLAGE has been identified as a TIER 1 COMMUNITY and has, in coordination with the DRSCW, conducted a review of their snow and ice operations in an effort to identify areas of operational improvements relating to efficiency gains in winter chloride usage, and per the VILLAGE'S review, it is believed that an effective reduction in chloride usage can be attained, as part of the PROGRAM through the upgrading of equipment utilized for winter operations by the VILLAGE; and

WHEREAS, the PARTIES mutually agree to review winter operation policies and procedures and work in coordination with the DRSCW to monitor the administration and progress of the PROGRAM; and

WHEREAS, the ILLINOIS TOLLWAY by virtue of its powers as set forth in the "Toll Highway Act," 605 ILCS 10/1 *et seq.* is authorized to enter into this AGREEMENT; and

WHEREAS, the VILLAGE by virtue of its powers as set forth in the "Illinois Municipal Code," 65 ILCS 5/1-1-1 *et seq.* is authorized to enter into this AGREEMENT; and

WHEREAS, a cooperative Intergovernmental Agreement is appropriate and such an Agreement is authorized by Article VII, Section 10 of the Illinois Constitution and the "Intergovernmental Cooperation Act", 5 ILCS 220/1 *et seq.*

WHEREAS for recording purposes this AGREEMENT shall be known as #004686.

NOW, THEREFORE, in consideration of the aforementioned recitals and the mutual covenants contained herein, the PARTIES hereto agree to the following summary of the responsibilities and participation of each PARTY in the implementation of the PROGRAM.

I. PURPOSE and SCOPE

A. The PROGRAM established a projected increase over existing conditions or baseline for the EOWA of 3,888 tons of salt per year, per a baseline 39.7 tons/lane mile/year application rate. The goal of the offset program will be to reduce salt usage in roadway applications in the project related watersheds by the amount of the increase (3,888 tons per year) plus a margin of safety of 25 percent or a total reduction of 4,860 tons per year.

B. The ILLINOIS TOLLWAY, as part of the PROGRAM, has established a rate reduction goal of 20% from the EOWA's established baseline salt application rate of 39.7 tons/lane mile/year, thereby accounting for 972 tons per year of the 4,860 tons per year of salt required to be reduced, as part of the PROGRAM.

C. The VILLAGE, as part of the PROGRAM and per this AGREEMENT, has established a rate reduction goal of 17.5% from its established baseline application salt application rate of 12.3 tons/lane mile/year, thereby accounting for 204 tons per year of the 4,860 tons per year of salt required to be reduced, as part of the PROGRAM.

D. Both structural and non-structural practices will comprise the PROGRAM to provide the needed improvements in water quality but it is understood that offset reductions can only be accounted for through non-structural practices.

II. STRUCTURAL BEST MANAGEMENT PRACTICES

A. Grass swales, bio-swales, infiltration basins, and other designs will be incorporated by the ILLINOIS TOLLWAY into the EOWA projects in order to minimize the effects of roadway runoff and improve the quality of roadway runoff discharged to receiving waters and/or nearby wetlands.

III. NON-STRUCTURAL BEST MANAGEMENT PRACTICES

A. A salt reduction goal has been established by the ILLINOIS TOLLWAY and DRSCW for the PROGRAM through the analysis of existing conditions, existing practices in the affected watersheds, and planned highway improvements. Offsets will include both the adoption of salt reduction strategies implemented by the ILLINOIS TOLLWAY and providing salt application data to DRSCW, including but not limited to their application rates for each lane mile, as well as financial and other support to other mutually agreed upon partners.

B. The ILLINOIS TOLLWAY recognizes that meeting the objectives of the Total Maximum Daily Loads ("TMDL's") will require reductions in area chloride loading above those set out in the PROGRAM and agrees to review its practices at an agency wide scale and to actively partner with the DRSCW, its members or successors, in working for PROGRAM area chloride reductions beyond the life of the PROGRAM with the goal of meeting the applicable water quality standard.

IV. RESPONSIBILITIES

A. Pursuant to EXHIBIT A, a guideline for monitoring and reporting chloride offsets has been established. Both PARTIES agree that the requirements embodied in Clean Water Act Section 401 certification(s) or Section 404 or NPDES permit(s), are the sole responsibility of the ILLINOIS TOLLWAY, and that the VILLAGE is not responsible in any way for the ILLINOIS TOLLWAY's failure to comply with such requirements.

B. The VILLAGE shall provide the ILLINOIS TOLLWAY a summary raw data report that includes the following data for the most recent winter season for a minimum of five consecutive winter seasons, post equipment purchase and installation; 1) total lane miles maintained, 2) actual road salt usage in tons per lane mile, 3) baseline road salt usage in tons per lane mile, 4) target road salt usage in tons per lane mile, 5) total winter season precipitation events requiring deicing efforts, 6) average precipitation in inches of snow, ice and liquid form of winter season precipitation per precipitation event, 7) duration of each operator deicing efficiency training session and number of operators attending, 8) new equipment installation and new practices implemented and identification and practices planned and 9) the completed DRSCW's Winter Public Agency Deicing Questionnaire.

C. The PARTIES agree that the PROGRAM may require several years of monitoring and reporting from PROGRAM partners.

D. The PARTIES will develop and maintain a guidance document for the PROGRAM which will at minimum detail the methods for calculating the build scenario non-PROGRAM increase, the needed offset, BMP's the monitoring PROGRAM and reporting baseline requirements. The document will be updated by agreement between the PARTIES as the PROGRAM advances.

V. DELIVERABLES

A. The deliverables will be transmitted to the ILLINOIS TOLLWAY, by the VILLAGE and include written reports documenting, as defined in Article IV, Paragraph B. of this AGREEMENT by June 1st pertaining to the preceding winter season.

B. The ILLINOIS TOLLWAY maintains its responsibility to remain compliant with the "Clean Water Act" as determined and administered by the IEPA.

VI. FINANCIAL

A. The ILLINOIS TOLLWAY as sponsor of the PROGRAM will compensate the VILLAGE as outlined in this AGREEMENT and included in the VILLAGE's Request for Equipment and Training "PROPOSAL".

B. The purchase of equipment identified within the VILLAGE's PROPOSAL, as part of the PROGRAM shall be reimbursed by the ILLINOIS TOLLWAY to the VILLAGE.

C. The VILLAGE will be paid based upon its invoice(s) which shall include detailed receipts of purchased items in substantial conformance with the Budget included in the VILLAGE's PROPOSAL.

D. The VILLAGE shall certify in writing, upon presentation of each invoice hereunder, that items as invoiced have been actually purchased and installed and that the VILLAGE is in fact complying with all other provisions of this AGREEMENT. Invoicing shall be sufficiently itemized to permit the ILLINOIS TOLLWAY or its consultant(s) or cooperating governmental unit(s) to verify performance of the work so invoiced.

E. It is mutually agreed that the estimated cost to the ILLINOIS TOLLWAY shall not exceed $367,000 for the term of this AGREEMENT through five consecutive winter seasons, beginning with the first winter season in which purchased equipment has been utilized and use data has been provided to the ILLINOIS TOLLWAY.

VII. GENERAL PROVISIONS

A. It is understood and agreed that this is an AGREEMENT between the VILLAGE of Bensenville and the Illinois State Toll Highway Authority.

B. All equipment proposed for purchase and installation, shall be done so prior to the 2018/2019 winter season but efforts will be made for purchases and installations to occur prior to the 2017/2018 winter season.

C. The term of this AGREEMENT shall extend through five consecutive winter seasons in which purchased equipment has been utilized and use data has been provided to the ILLINOIS TOLLWAY.

D. PARTIES agree to collaborate in effort to fulfill applicable goals of the PROGRAM as established in Section IV. of EXHIBIT A. Should the PARTIES mutually agree that the VILLAGE did not operate in a manner demonstrating intent achieve the goal reduction of 17.5% in a PROGRAM year, the ILLINOIS TOLLWAY shall provide written notice to the VILLAGE identifying delinquencies of agreed upon practices. Should the PARTIES mutually agree that the VILLAGE did not operate in a manner in which to achieve the goal reduction of 17.5% for a second year during the term of this AGREEMENT, the ILLINOIS TOLLWAY and shall provide written notice to the VILLAGE identifying delinquencies of agreed upon practices and the VILLAGE shall provide reimbursement to the ILLINOIS TOLLWAY for all payments made by the ILLINOIS TOLLWAY associated with this agreement within 90 days of VILLAGE's receipt of the notice.

E. Equipment purchased under this AGREEMENT by the ILLINOIS TOLLWAY on behalf of the VILLAGE becomes the property and responsibility of the VILLAGE and the VILLAGE shall indemnify, hold harmless and defend the ILLINOIS TOLLWAY, its officials, directors, officers, employees, and agents from and against all liability, claims, suits, demands, proceedings and action, including costs, fees and expense of defense, arising from, growing out of, or related to, any loss, damage, injury, death, or loss or damage to property resulting from, or connected with, the VILLAGE's negligent or willful acts, errors or omissions in its performance under this AGREEMENT, including, but not limited to, use of the equipment described herein. The VILLAGE expressly acknowledges that the ILLINOIS TOLLWAY, through provision of funding, training, and certification under this AGREEMENT, is not intended to be a joint employer of the VILLAGE's employees and agents and does not excerpt control over such persons in their use of the equipment that is the subject of this AGREEMENT.

F. It is understood and agreed that this AGREEMENT constitutes the complete and exclusive statement of the agreement of the PARTIES relative to the subject matter hereof and supersedes all previous oral and written proposals, negotiations, representations or understandings concerning such subject matter.

G. This AGREEMENT may be executed in two (2) or more counterparts, each of which shall be deemed an original and all of which shall be deemed one and the same instrument.

H. Under penalties of perjury, the VILLAGE certifies that its correct Federal Tax Identification number is 36-6005794 and it is doing business as a governmental entity, whose mailing address is The Village of Bensenville, 12 S. Center Street, Bensenville, Illinois 60106.

I. The PARTIES agree to maintain books and records related to the performance of this AGREEMENT and necessary to support amounts charged to the ILLINOIS TOLLWAY and/or any of the PARTIES under the AGREEMENT for a minimum of five (5) years from the last action on the AGREEMENT. The PARTIES further agree to cooperate fully with any audit and to make its books and records, and books and records within its custody or control available to the Illinois Attorney General, the Illinois Auditor General, the ILLINOIS TOLLWAY Inspector General, the ILLINOIS TOLLWAY Department of Internal Audit, the ILLINOIS TOLLWAY or any other governmental agency or agent thereof that is authorized to audit or inspect such books and records.

J. The introductory recitals included at the beginning of this AGREEMENT are agreed to and incorporated into this AGREEMENT.

(This section intentionally left blank)

IN WITNESS WHEREOF, the PARTIES have executed this AGREEMENT on the dates indicated.

THE VILLAGE OF BENSENVILLE

By: ______________________
Frank DeSimone
Village President

Attest: ______________________
Nancy Quinn
Village Clerk

Date: ______________________

THE ILLINOIS STATE TOLL HIGHWAY AUTHORITY

By: ______________________
Greg M. Bedalov
Executive Director

Date: 6/16/17

By: ______________________
Michael Colsch
Chief Financial Officer

Date: [illegible]

By: ______________________
Elizabeth A. Oplawski
Acting General Counsel

Date: ______________________

Approved as to Form and Constitutionality

____
Hilary D. Hale
Senior Assistant Attorney General, State of Illinois

05/25/17 6.3/3

RESOLUTION NO. 21287

Background

It is in the best interest of the Illinois State Toll Highway Authority (the "Tollway") to enter into an Intergovernmental Agreement with the Village of Bensenville in connection with the construction of Illinois Route 390. In order to remain compliant with guidelines established by the Illinois Environmental Protection Agency, the Tollway and the DuPage River Salt Creek Workgroup (DRSCW) established a Chloride Offset Program to minimize chloride usage and enhance water quality throughout the DRSCW's water-sheds affected by Illinois Route 390. The Program is being implemented through partnerships with municipalities within the impacted areas by providing training to identify potential operational improvements, and the upgrading of equipment utilized for winter operations. The Village of Bensenville is an impacted municipality and requests the Illinois Tollway participate in the cost of providing training and purchasing upgraded equipment. The total estimated cost to the Tollway is not to exceed $367,000.

Resolution

The Chief Engineering Officer and the Acting General Counsel are authorized to negotiate and prepare an Intergovernmental Agreement between the Illinois State Tollway Highway Authority and the Village of Bensenville in substantially the form attached to this Resolution. The Chairman or the Executive Director is authorized to execute said agreement and the Chief Financial Officer is authorized to issue warrants in payment thereof.

Approved by: ______________________________
Chairman

Framework for Chloride Mitigation

MEMORANDUM

Elgin O'Hare Western Access and I-90 East Widening and Reconstruction: A Framework for Chloride Mitigation

PREPARED FOR:	Bryan Wagner/Illinois Tollway
PREPARED BY:	Larry Martin/CH2M HILL Mark Mittag/CH2M HILL Jim Huff/Huff and Huff
DATE:	March 10, 2014

The Elgin O'Hare Western Access (EOWA) and I-90 East Widening and Reconstruction projects (Projects) are major transportation investments in the Chicagoland area located generally west of O'Hare Airport. The projects span various watersheds (see Exhibit 1). Most all the watersheds have water quality impairments ranging from low dissolved oxygen to elevated levels of total suspended solids (TSS), heavy metals, and chlorides from winter road and parking lot deicing operations. Many streams do not meet the Illinois chloride water quality standard of 500 mg/L. Because the chloride standard is exceeded in several locations, the Illinois Environmental Protection Agency (IEPA) has established Total Maximum Daily Loads (TMDLs) for the West Branch DuPage River, Salt Creek, and Addison Creek. Higgins Creek has a TMDL study underway. The allowable chloride loads require that measures be implemented to achieve the load reductions, which will be realized with more efficient winter deicing practices.

In December 2012 and February 2013, the Illinois Tollway submitted applications for Section 404 and Section 401 permits to the U.S. Army Corps of Engineers (USACE) and IEPA for the Projects. During the planning phases of the Projects, and more recently during the permitting processes, water quality has been a topic repeatedly emphasized by regulatory agencies, environmental groups, and individuals. In recent meetings with the IEPA, the agency acknowledged that the planned stormwater best management practices (BMPs) for the Projects would adequately manage the quality of the roadway runoff for TSS and heavy metals. For streams impaired for chlorides, however, IEPA indicated that there will be no net increase in chlorides with the projects to ensure compliance with the applicable regulations.

Program Organization

In response to IEPA's directives, the Illinois Tollway has developed a program for salt reduction in the EOWA project area. The Illinois Tollway has resolved that for this program to be viable it requires collaboration and participation beyond the boundaries of the Illinois Tollway organization. The framework of this program involves a strategic alliance with the DuPage River Salt Creek Workgroup (DRSCW), other strategic watershed groups, and local units of government for advancing salt reduction in streams affected by the project. Moving forward, the Illinois Tollway will administer and manage the overall salt reduction program for the project with guidance and support from the DRSCW. The partnership with the DRSCW will be formalized with a Memorandum of Understanding (MOU). Working arrangements with local units of government will be formalized with the use of an Intergovernmental Agreement (IGA). IGA's will be established with communities that are directly adjacent to the EOWA project and would include Hanover Park, Schaumburg, Roselle, Itasca, Elk Grove Village, Wood Dale, Bensenville, Des Plaines, Mount Prospect, Franklin Park, City of Chicago, North Lake, and Elmhurst. IGA's would also be established with DuPage and Cook counties, and local townships.

It is anticipated that the program duration will be permanent and will be a condition of the 401 Water Quality Certification. The program will be implemented in steps. The initial step involves developing the overall program, which is currently underway with the Illinois Tollway and the DRSCW planning the framework of the program that includes the following elements:

- Establishing baseline conditions (the starting point),
- Developing salt reduction goals, enlisting the municipal partners,
- Aligning the salt reducing practices that best fit community needs,
- Establishing funding needs,
- Developing training workshops, and
- Developing a monitoring and reporting program to measure progress toward stated goals.

The MOU with the DRSCW is be finalized by summer 2013. The IGA process with municipal partners will commence in the fall of 2013 and be completed by mid-2014. Funding levels required to finance the program are currently being developed. Program funding will come from a combination of Illinois Tollway, local communities, and other sources. It is anticipated that the Illlinois Tollway would contribute the majority share. Funding put forth by the Illinois Tollway would be distributed to the local units of government for equipment upgrades and would be administered through the IGA's.

The major Investment in new salt reducing practices is expected in the early years of the program to maximize benefits to water quality. The goal is to have salt reducing practices in place in the east-west corridor (~10 mile section between Gary Avenue on the west to York Road on the east) prior to the opening of new pavement in late 2015. The completion of the east-west corridor is schedule for 2018, and the completion of the Western Access is scheduled for 2025.

Program reporting will occur on an annual basis. Information will be collected on an annual basis to compare usage under the program with the current baseline conditions. Salt usage will be expressed in tons of salt applied in a deicing season per lane-mile. An end-of-season meeting of the communities and the Illinois Tollway may be sponsored to share lessons learned and consider adjustments prior to the next deicing season. Results below expectation will be assessed and adjustment made to the program approach.

Program Work Scope

The salt reduction scope of work is comprised of work tasks that are designed to achieve a "no net increase" in chloride with an added buffer of no less than 25 percent. Based on an assessment of current practices and future requirements, the program objective will be to achieve an annual reduction of 4,860 tons in watersheds affected by the EOWA project. The program consists of two elements:

- Improving current Illinois Tollway deicing practices.
- Expanding salt reduction practices to local communities within the EOWA drainage areas.

Improving Current Illinois Tollway Deicing Practices

An internal review of the Illinois Tollway's deicing practices was initiated in early 2013. In addition to the Illinois Tollway's internal staff expertise, the agency retained the services of an internationally known deicing expert, Dr. Wilfred Nixon, a professor and researcher on highway maintenance and ice engineering at the University of Iowa.

In 2013, Illinois Tollway staff and Dr. Nixon initiated a review of current Tollway winter maintenance practices and policies with the purpose of identifying the potential for more efficient ice control chemicals usage (e.g., salt and others) in winter maintenance activities, while maintaining a very high level of service and safety.

The review of current practices and policies focused on six principle areas of winter maintenance, including:

- Levels of service

- Performance measurement and continuous improvement
- Materials usage
- Equipment selection and operations
- Strategic operations
- Tactical operations

In the review of these practice areas, current salt loadings were obtained, detailed interviews were conducted with Illinois Tollway staff, and salt yard operations and equipment were examined. The following draft recommendations were formulated from the data, interviews, and observations:

- Pre-wetting salt solids on the truck – yields up to a 25 percent reduction in salt use.
- Use salt brine for pre-wetting regularly with periodic strategic use of calcium chloride brine.
- Optimizing salt application rates by considering pavement temperature and weather types may be able to reduce application rates from 500, 300 and 200 pounds per lane-mile to 375, 225, and 150 pounds per lane-mile. Reduction in salt application may require pilot testing to confirm application rate efficiency.
- Use direct liquid application prior to a storm to prevent bonding between the snow/ice and the pavement and reduce total chemical required during a storm event (anti-icing) – yields up to a 75 percent reduction in salt use during these events.
- Other practices that produce effective, but smaller reductions in salt use are the application of tow plows, new plow cutting edges, and communicating reduction methods with other agencies.

The implementation of these practices would be preceded by a review, evaluation, and approval process within the Illinois Tollway organization. As a first step, practices such as pre-wetting salt, using salt brine, adjusting salt application rates, and using direct liquid applications would be evaluated and screened based on performance, compatibility with current practice, etc. Practices that advance from the screening phase would be pilot-tested. Pilot projects would be conducted for at least one winter season, and would be pilot-tested in the region of one maintenance yard. The effectiveness, cost-savings, and comparative performance of each method would be assessed for system-wide usage. Initial pilot-testing is planned for the winter 2013-2014. The practice(s) selected for implementation and their associated reduction of salt in the project area will count towards meeting the goal of not increasing chloride discharge with the project.

Expanding Salt Reducing Practices to Local Communities - Chloride Off-Set Program

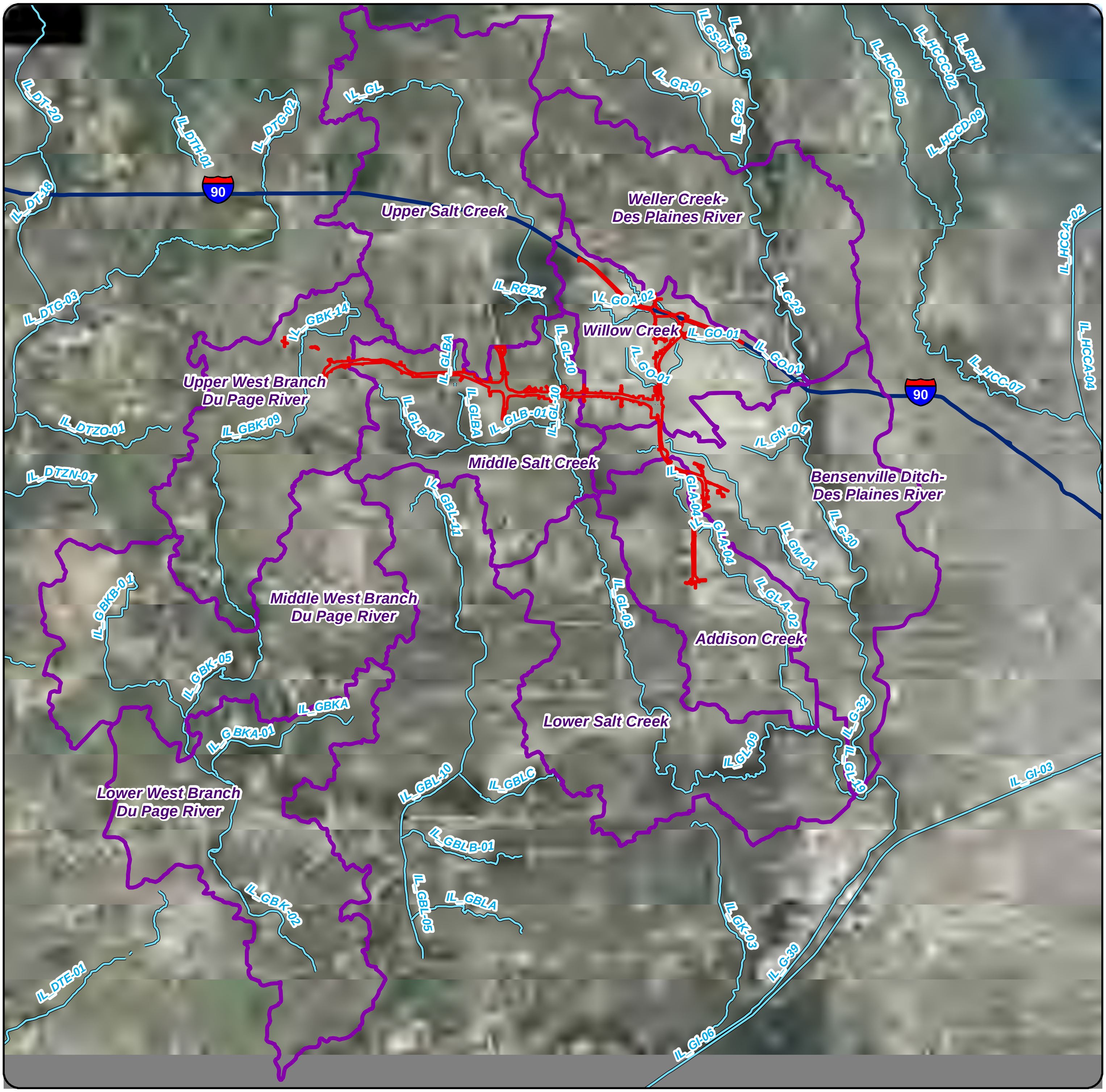
The Illinois Tollway and DRSCW have developed the Chloride Off-Set program to a concept level. It program concept provides the clarity necessary to obtain concurrence from their respective organizations to advance to program implementation. The scope of the program is described as follows.

- **Program facilitation and management.** The Illinois Tollway will manage the implementation of the salt reduction program, and be responsible to the regulatory agencies (i.e., IEPA and USACE) for compliance with the terms and conditions of the Section 404 and 401 permits. The use of IGAs would be used to formalize working arrangements with the DRSCW and local municipalities. The DRSCW will serve as a principal in the development and implementation of the program. Whereas, the DRSCW has long-term established relationships with the stakeholder communities and have been entrusted to provide guidance related to water quality issues, the DRSCW will facilitate the overall integration of the project area's salt reduction program into the water quality initiatives of the stakeholder community.

- **Program funding.** The funding for the program is currently under review. Funding would be directed to training, promotion of alternative deicing practices, and equipment upgrades that would provide greater efficiencies in salt usage. Funding will come from a combination of Illinois Tollway, local municipalities, and other sources. The use of IGAs will be the mechanism to enable partnership of the local communities and the funding arrangements that will affect salt reducing practices. Likely funding scenarios include the Illinois Tollway contributing the majority share, and the local communities and other sources (i.e., state and federal grant monies) comprising the remainder.
- **Assess existing practices.** Surveys and interviews are routinely conducted by the DRSCW with communities in the project area to determine current deicing practices. An additional survey will be conducted as part of this initiative to accurately define current community salt usage. This data will be used to tailor the types of salt reduction practices that would provide the best results for each community.
- **Establish baseline conditions.** The community surveys and interviews will also be used to establish baseline conditions for each potential partner. As an initial task, baseline estimates will be compiled from current practices and records, and in the absence of detailed records, a comparison to peer communities will be estimated. As a follow-on task, the collection of accurate application rates per lane-mile using existing practices will occur in Year One of the program. Standard procedures will be established to determine application rates for each community. Baseline conditions will be recorded as the rate of salt usage per lane-mile (pounds per lane-mile) under standard conditions, and total tons of salt used during the deicing season.
- **Finalize program goals and salt reduction targets.** Based on an assessment of current practices and future requirements, the program objective will be to achieve an annual reduction in tons of salt across the project area. Each partner community will be assigned a target goal wherein the combined targets of the individual communities will add to the program wide target. Individual community targets will be determined based on current practices and reasonably achievable reductions with the use of salt reducing practices.
- **Identify alternative practices best-suited to local communities.** The winter deicing practice surveys and interviews will be used to identify the types of deicing technology needed within each community for additional salt usage efficiency. The current practices will shape the menu of practices recommended for each community. Actual implementation of the new deicing practices would begin implementation in Year Two of the program.
- **Initiate training for more efficient deicing practices.** The DRSCW routinely provides training, workshops, and seminars concerning water quality practices. The organization in conjunction with the Illlinois Tollway will update training for achieving greater efficiencies in deicing practices. Appropriate training on best available deicing technologies and best practices will be shared across the partnering communities. The intent is to provide the best available information to the communities in the project area that result in salt reducing practices. Training would be initiated in Year One of the program.
- **Establish data sites (weather-related information) for managing roadway deicing.** The Illinois Tollway has a system of pavement temperature sensors and subscribes to instantaneous weather data. Data sharing with the municipal partner is contemplated and would aid local communities in making winter storm management decisions.
- **Monitor/report.** Salt use reporting will be a requirement of the program. The Tollway and each community will provide annual usage expressed as tons of salt applied per lane-mile annually. This usage rate combined with the miles of roads in the community will be used to compare progress in salt reduction and overall salt use efficiency. An end-of-season meeting will be hosted by the Illinois Tollway to share lessons learned and consider program adjustments for the next deicing season. Instreaming monitoring is planned to demonstrate that the reduction in salt use is reducing chloride

concentrations in receiving waters. The sampling locations and frequency of sampling is in the planning stages.

- **Reassess program objectives.** An annual assessment of the program will be conducted by the Illinois Tollway and the DRSCW. The program metrics will be evaluated to study trends, and salt reducing practices will be review to determine actual effectiveness compared to theoretical. The assessment will be documented as a report and submitted to regulatory agencies upon request. Results that are below expectations will be addressed with the necessary program revisions that produce the needed outcome



LEGEND

🟥 Full-Build 2040 Footprint
🟪 Watershed Boundary
River/Stream

0 1.5 3 6
Miles

Elgin O'Hare
Western Access

Exhibit 1
Watersheds

Liquid Deicing Options and Evaluation

MEMORANDUM

Liquid Deicing Options and Evaluation for the Elgin O'Hare Western Access Project

PREPARED FOR:	Bryan Wagner/Illinois Tollway	
PREPARED BY:	Steven Graziano/CH2M HILL Mark Mittag/CH2M HILL	
COPY TO:	Reed Panther/Illinois Tollway Steve McCracken/DRSCW Illinois Environmental Protection Agency	Jim Huff/Huff and Huff Larry Martin/CH2M HILL
DATE:	July 16, 2013	

As part of a chloride offset program for the Elgin O'Hare Western Access (EOWA) project, winter deicing efficiencies have been evaluated for Illinois Tollway and partnering communities. Technology experts have indicated a move towards using more liquid deicers provides efficiency in salt use. Improved efficiency results in less rock salt use. This memorandum summarizes the technology options most readily applicable to Illinois Tollway and local communities with the goal of estimating salt reduction with the implementation of more widespread use of liquid deicer.

The use of liquid brine through pre-wetting and anti-icing practices was investigated for the EOWA project. Pre-wetting involves applying salt brine directly to the surface of bulk salt solids prior to conventional deicing. The pre-wetting rapidly activates the salt melting process and reduces the degree of bounce-and-scatter of salt off the roadway surface. Anti-icing involves the application of brines or organic products to the roadway surface prior to a snow or ice event to prevent or weaken the bond between the snow or ice and the pavement surface. Preventing a bond to the pavement allows for easier and more efficient snow removal. Both practices provide considerable benefit to snow and ice management agencies.

Types of Brine

Pre-wetting and anti-icing liquid agents can be salt brines, organic agents or byproducts, or combinations of both. The focus of this general investigation involves only the use of salt brine created from bulk rock salt (sodium chloride) and water. It is assumes that brines are 23.3 percent salt by weight. Other common pre-wetting and anti-icing brines include those created from magnesium chloride and calcium chloride salts. Each salt has benefits and drawbacks and is typically appropriate for specific environmental and road conditions; however, rock salt brine is the most widely used and most affordable deicing agent. The Illinois Tollway or local communities may want to augment sodium chloride brines with calcium chloride or other liquids or mixtures based upon weather and travel conditions.

Advantages of Brine for Pre-wetting and Anti-Icing

The advantages of using brine for pre-wetting and anti-icing are listed below and are adapted from the Iowa Department of Transportation's Systems Operation Bureau, Office of Maintenance.

Pre-wetting

- Rock salt can be spread more uniformly and less is wasted on shoulders and ditches, reducing the impact to the environment.
- Materials adhere to the surface because the salt and brine mixture has a thicker consistency.
- Melting begins faster since a liquid has been introduced to the salt. It is important for salt to be in liquid form to prevent snow and ice bonding to pavement.
- Since the brine begins working and diluting as it hits the surface, it will dry much more rapidly, returning the roadway to normal winter driving conditions much sooner.

- Spreading speeds can be increased because more material stays on the roadway.
- Residual salt may remain on the road that will immediately begin working with the next storm.
- When dry materials are pre-wet with brine, the dry material application rate can actually be cut back 20 to 30 percent because more of the material remains on the roadway. In addition, the additional brine speeds the melting action.
- Can be used as a deicer on very thin layers of frost or black ice, but with caution. Salt brine is not recommended for snow pack unless it is treated regularly and frequently with a sufficient quantity of salt brine and or salt brine and rock salt. Brine alone will rapidly dilute and if an insufficient concentration is maintained and may refreeze before it can burrow through the snow or ice pack.

Anti-icing

- Application prior to a storm can help prevent snow and ice from bonding to the pavement and can help keep the roadway wet longer into a storm (or throughout the storm, if a very light precipitation event).
- Makes clean-up quicker and returns roads to normal driving conditions more rapidly.
- Reduces labor hours.
- Reduces wear on ice blades and underbody plows.

For additional information, the Iowa DOT winter maintenance website serves as a comprehensive resource and can be accessed at http://www.iowadot.gov/maintenance/materials.html.

Findings of the Nixon Report

The Illinois Tollway consulted with Dr. Nixon from the University of Iowa on a review of deicing procedures and efficiency opportunities. Dr. Nixon's report explained that approximately 30 percent of the salt spread on the road by conventional deicing methods can be expected to leave the road surface. The pre-wetting of road salt prior to spreading has shown to result in only a 4 percent loss of salt when spread on the center lane of a three lane road at vehicle speeds of 30 to 40 mph, application rates of 400 lb per lane mile, and at pre-wetting rates of 6 gallons per ton of salt applied.

As a result, a potential reduction in salt application by approximately 25 percent can be reasonably expected, meanwhile still maintaining the same level of service. Similar potential salt reductions between 20 and 30 percent have been supported by the Wisconsin Transportation Information Center, the Salt Institute's *Snowfighters Handbook*, the Iowa DOT, and the New Hampshire Department of Environmental Services. Anti-icing reductions were not explicitly stated in the Nixon Report.

Anti-icing efforts are typically more dependent on environmental conditions, road surface conditions, and on the timing of application prior to a storm or ice event. Therefore, salt savings when using anti-icing can vary but can be optimized with extensive operational experience. The *Chloride Reduction Implementation Plan for Dinsmore Brook Watershed, Windham, NH* (February 2011) from the New Hampshire Department of Environmental Services indicates that salt reductions between 10 and 30 percent can be expected when anti-icing.

Estimate of Current Salt Use and Planned Reductions with Pre-Wetting/Anti-Icing Strategies

Pre-wetting and anti-icing options were considered for communities that span the EOWA corridor. Tier 1 communities were designated as those located within the EOWA project footprint or within the USGS watersheds bordering the project footprint. Additional analysis has continued to evaluate the potential salt reduction in each community. These Tier 1 communities are located in watersheds of interest to the DuPage River Salt Creek Workgroup (DRSCW), as well as Des Plaines River watersheds, due to their proximity to the project footprint. Several communities span several watersheds. Consequently, the community salt reduction potential considers all reductions available as long as they are within the DRSCW or Des Plaines River watersheds.

The effectiveness of pre-wetting and anti-icing in the reduction of salt quantities were determined based on total lane miles present within the Tier 1 communities and typical per-lane-mile salt application rates. Total lane miles within Tier 1 communities are summarized in Table 1. Refer to Exhibits 1 and 2, which identify roadways within Tier 1 communities and watershed boundaries.

TABLE 1
Lane Miles per Jurisdiction Spanning EOWA Project Footprint

Jurisdiction[a]	DRSCW Watershed Lane Miles	Des Plaines Watershed Lane Miles	Total Lane Miles	Estimated Salt Usage, ton/yr[b]
Locally-Maintained				
Addison Township	25.4	7.2	32.6	457
Bensenville	57.6	49.2	106.7	1,494
Berkeley	21.7	0.0	21.7	304
Bloomingdale Township	7.2	0.0	7.2	101
Chicago	0.0	208.3	208.3	2,916
Elk Grove Township	14.6	6.5	21.0	295
Elk Grove Village	143.8	110.5	254.3	3,561
Elmhurst	289.1	0.0	289.1	4,047
Franklin Park	0.0	105.7	105.7	1,480
Hanover Park	137.0	0.0	137.0	1,918
Itasca	61.7	0.0	61.7	864
Leyden Township	1.6	44.9	46.6	652
Medinah (Unincorporated)	34.8	0.0	34.8	488
Northlake	57.7	12.3	69.9	979
Roselle	126.6	0.0	126.6	1,773
Schaumburg	246.4	0.0	246.4	3,450
Schaumburg Township	17.9	0.0	17.9	250
Wood Dale	46.9	37.1	84.0	1,176
Locally-Maintained Subtotal	1,289.9	581.6	1,871.5	26,202
DuPage County-Maintained	91.5	27.7	119.1	1,668
Cook County-Maintained	113.7	24.8	138.5	1,939
Total	**1,494**	**634**	**2,129**	**29,808**

[a] IDOT-maintained lane miles are excluded from these quantities. Includes locally-, DuPage Co.-, and Cook Co.-maintained lane miles.

[b] Assumed application rate: 14 tons/lane mile/year.

Note: IDOT-maintained lane miles are excluded from these quantities. Includes locally-, DuPage Co.-, and Cook Co.-maintained lane miles.

Roadway lane mile totals were based on a 2011 (latest available) Illinois Department of Transportation Roadway Data Layer GIS files (http://gis.dot.illinois.gov/gist2/), which also included jurisdictional information. The baseline road salt application rate assumed was 14 tons/lane mile/year, based on a 2007 survey conducted by the DRSCW on the average annual salt application reported by respondents located in within the Salt Creek watershed.

Table 2 indicates the lane miles and an estimate of added salt usage with the Initial Construction Plan (ICP) and the Full-Build phase of the Elgin O'Hare Western Access Project. Freeway/Toll road lane miles were assigned an average road salt application rate of 39.7 ton/lane mile/year based on IDOT and Illinois Tollway system-wide application rates during the 2000/2001 through 2010/2011 winter seasons. As shown in Table 2, the increase over existing conditions or baseline with the ICP is 3,888 tons per year. The goal of the offset program will be to reduce salt usage on roadway in the project related watersheds by the amount of the increase (3.888 tons per year) plus a margin of safety (MOS) of 25 percent or a total reduction of 4,860 tons per year.

TABLE 2

Salt Application Summary for EOWA Project Limits

Estimated Annual Salt Application Rates

Project Stage	Lane miles [a]	Salt Applied, ton/yr [b]	Increase from Baseline Condition, ton/yr
Existing (Baseline) Condition	159	3,959	N/A
Initial Construction Plan (ICP)	264	7,847	3,888
2040 Full Build	293	8,969	5,010

[a] Lane miles include arterial/collector and freeway lane miles located within the project limits.

[b] Considers two different salt application rates depending on the roadway class. Arterial/collector roads are loaded at 14 ton/lane mile/year. Freeways are loaded at 39.7 ton/lane mile/year.

Table 3 expands on the data provided in Table 1 and shows estimates of potential salt reductions with the use of pre-wetting or anti-icing management techniques. Overall, the use of pre-wetting and anti-icing has the potential to reduce salt use in the project watersheds by up to 40 percent or a reduction of approximately 11,900 tons per year. The combined potential of these practices to reduce salt use in the project related watersheds is far greater than the offset target of 4,860 tons per year. While unlikely, if additional offsets are needed, communities further upstream (Tier 2 communities) or downstream (Tier 3 communities) in these watersheds could be approached to achieve further reductions in salt application.

Table 4 is a breakdown of the salt reduction target (4,860 tons per year) distributed by watershed in the project area (see Exhibit 3). The breakdown is proportional to the additional miles of roadway added within each watershed.

TABLE 3

Salt Application Summary by USGS Watershed for Tier 1 Communities

Estimated Annual Salt Application Rates and Potential Reductions With Best Management Practices

USGS Watershed	Lane Miles	Salt Applied, ton/yr	Salt Reduction with Pre-Wetting, ton/yr [a]	Salt Reduction with Anti-Icing, ton/yr [b]	Potential Total Salt Reduction, ton/yr
Des Plaines River Watershed					
Addison Creek	319	4,462	1,116	669	1,785
Bensenville Ditch-Des Plaines River	435	6,085	1,521	913	2,434
Willow Creek	198	2,768	692	415	1,107
Des Plaines River Watershed Sub-total	*952*	*13,315*	*3,329*	*1,997*	*5,326*

TABLE 3

Salt Application Summary by USGS Watershed for Tier 1 Communities

Estimated Annual Salt Application Rates and Potential Reductions With Best Management Practices

USGS Watershed	Lane Miles	Salt Applied, ton/yr	Salt Reduction with Pre-Wetting, ton/yr [a]	Salt Reduction with Anti-Icing, ton/yr [b]	Potential Total Salt Reduction, ton/yr
DRSCW Watershed					
Middle Salt Creek	436	6,106	1,527	916	2,442
Upper West Branch DuPage River	329	4,603	1,151	690	1,841
DRSCW Watershed Sub-total	*756*	*10,709*	*2,678*	*1,606*	*4,283*
Grand Total	**1,717**	**24,024**	**6,007**	**3,603**	**9,609**

Note: IDOT-maintained lane miles are excluded from these quantities. Includes locally-, DuPage Co.-, and Cook Co.-maintained lane miles. Reductions were directly applied to the total annual salt usage based on an application rate of 14 tons salt/lane mile/year.

[a] Expected salt reduction attributed to pre-wetting is between 20 and 30 percent. Reduction assumed: 25 percent

[b] Expected salt reduction attributed to anti-icing is between 10 and 30 percent. Reduction assumed: 15 percent

TABLE 4

Summary by USGS Watershed for the Initial Construction Plan (ICP) EOWA Project

Estimated Annual Salt Application Rates and Increases from Existing Conditions

USGS Watershed	Reduction from Baseline Condition, ton/yr	Target Reduction with MOS, ton/yr [a]	Comparison to Local Offset Potential, ton/yr
Des Plaines River Watershed			
Addison Creek	259	324	1,785
Bensenville Ditch-Des Plaines River	645	806	2,434
Willow Creek	1,761	2,201	1,107
Des Plaines River Watershed Sub-total	*2,665*	*3,331*	*5,326*
DRSCW Watershed			
Middle Salt Creek	1,191	1,489	2,442
Upper West Branch DuPage River	32	40	1,841
DRSCW Watershed Sub-total	*1,223*	*1,529*	*4,283*
Grand Total	**3,888**	**4,860**	**9,609**

[a] Includes a margin of safety (MOS) to have 125 percent of the salt application increase for the Initial Construction Plan (ICP) condition.

Based on the estimate of current salt use in the watershed areas, the ability to offset the increased salt usage for the ICP is clearly achievable with the use of pre-wetting and anti-icing practices. With the project, the overall reduction in salt needed to achieve no net increase as well as the 25 percent margin of safety is 4,860 tons/year. The opportunity within local communities for pre-wetting and anti-icing practices to reduce salt use is far greater than the estimated offset requirement (approximately 10,000 tons/year).

Offsets will be made through a combination of Illinois Tollway reductions and partnership with local communities. For the Des Plaines River and DRSCW watersheds, the offsets available through local communities easily exceed the offset totals needed. If necessary, additional offsets can be achieved from further reductions upstream, from other Illinois Tollway operations not part of the EOWA project, other potential partners, or combinations of these options.

To track improvements in salt usage, both annual tons of salt applied and a typical storm application rate will be tracked to take into account variation in winter conditions from year to year. The implementation of these practices will be applicable to both the Illinois Tollway and local communities. The actual mix of these practices will be determined individually for each entity with the objective of tailoring salt reducing practices that best fit the community needs and yields a substantive reduction in salt use.

Major Equipment Requirements

Equipment options for pre-wetting and anti-icing operations using sodium chloride brine include:

Anti-icing

- Salt storage
- Brine production equipment (if produced onsite)
- Brine storage tank (whether purchased as bulk product or produced onsite)
- Truck-mounted brine storage, trailer brine storage, or combination storage
- Brine applicator

Pre-wetting

- Salt storage
- Brine production equipment (if produced onsite)
- Brine storage tank (whether purchased as bulk product or produced onsite)
- Truck-mounted brine and salt storage
- Brine applicator to salt

References such as the Iowa DOT Systems Operation Bureau, Office of Maintenance have additional information available on typical equipment needs. This source provides a comprehensive description of the equipment requirements and options available for anti-icing or pre-wetting programs.

Additional Ice/Snow Management Items

In addition to retrofitting fleet vehicles and constructing or purchasing brine production equipment to accommodate either anti-icing or pre-wetting practices, the following are recommendations that will further improve ice and snow removal efficiency.

- **Education and training of ice and snow management staff**. Education and training could include a one-day annual conference prior to the winter season to discuss proper operation and maintenance of ice and snow removal technologies, standards of practice, past observations, and potential solutions. This meeting would include regional ice and snow management staff.

- **Information sharing amongst members of the ice and snow management community.** This could include monthly meetings, webinars, or forums during the winter season for members to engage other municipalities or townships on the success of their deicing programs.

- **Equipment calibration program and training.** The offsets program will require that equipment is calibrated regularly and calibration documentation is provided to ensure proper and accurate salt application rates are reported annually.

References

DuPage River Salt Creek Workgroup and CDM. *Chloride Education and Reduction Program, 2010 Deicing Program Survey.* April 2011.

Environmental Impact Statement, Section 3.10: Water Resources and Aquatic Habitats.

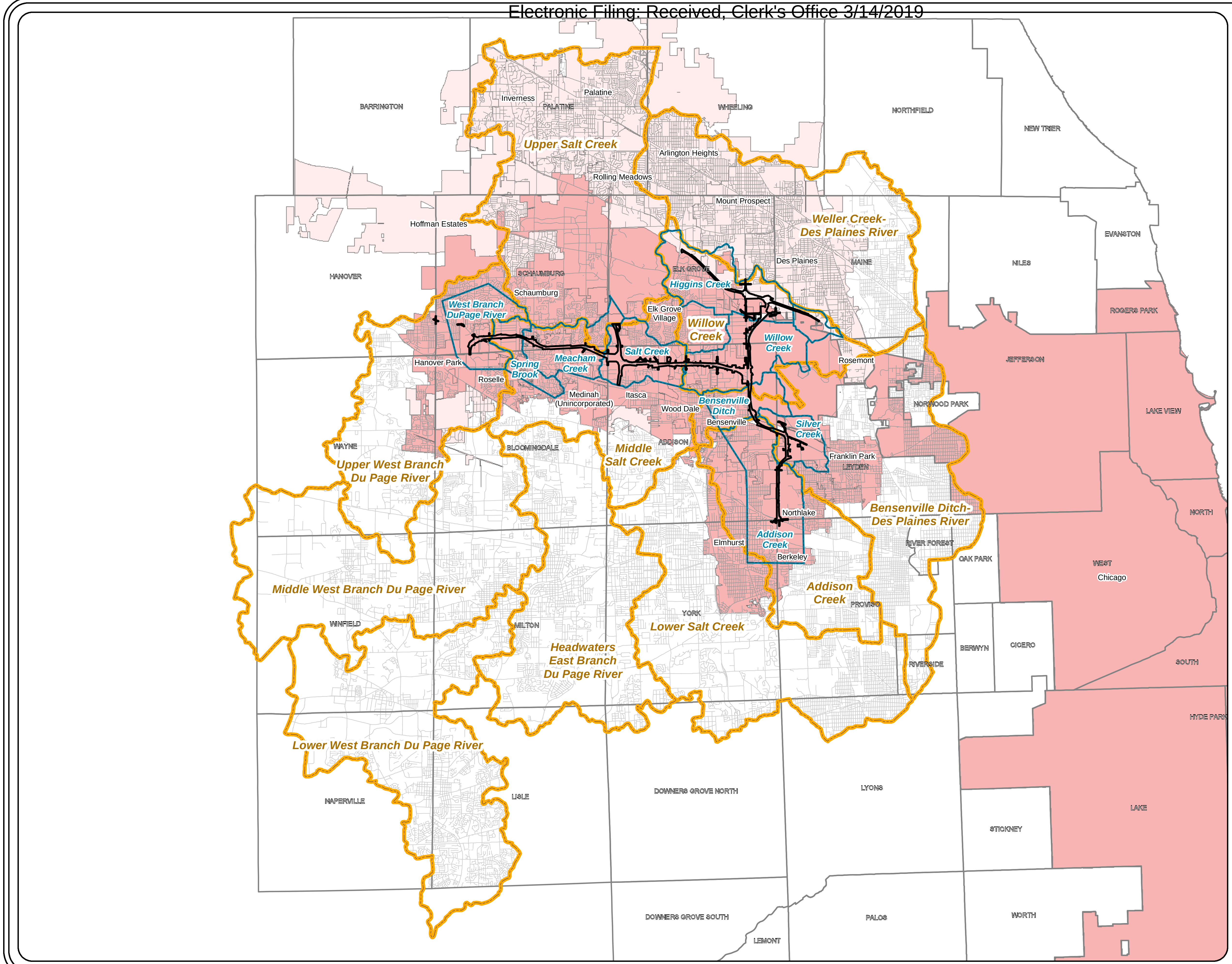
Iowa Department of Transportation Office of Maintenance. *Brine Production.* http://www.iowadot.gov/maintenance/materials.html

New Hampshire Department of Environmental Services, Watershed Management Bureau. *Chloride Reduction Implementation Plan for Dinsmore Brook Watershed.* Windham, NH. Feb. 2011.

Salt Institute. *The Snowfighters Handbook*, 40th Ed. Alexandria, VA. 2007.

Wisconsin Transportation Information Center. *Wisconsin Transportation Bulletin No. 22*. Madison, WI. Dec. 2005.

Elgin O'Hare Western Access



LEGEND

☐ Full-Build 2040 Footprint
☐ Township Boundary
☐ Sub-watershed Boundary
☐ Watershed Boundary

Municipality/Township:

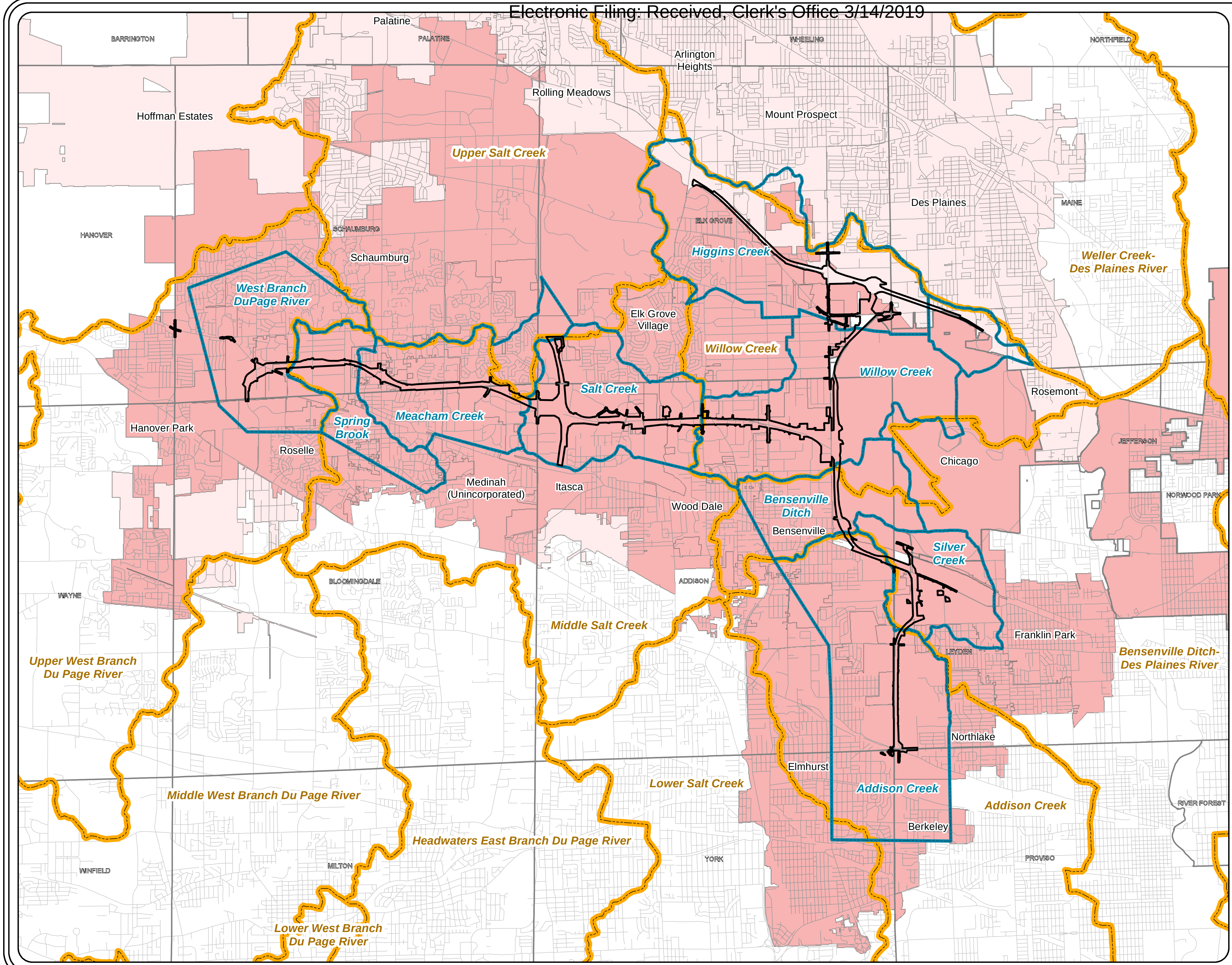
■ Tier 1
□ Tier 2

0 7,500 15,000 30,000
Feet

Exhibit 1

Roadways within
Watershed Boundaries

Elgin O'Hare Western Access



LEGEND

- Full-Build 2040 Footprint
- Township Boundary
- Sub-watershed Boundary
- Watershed Boundary

Municipality/Township:

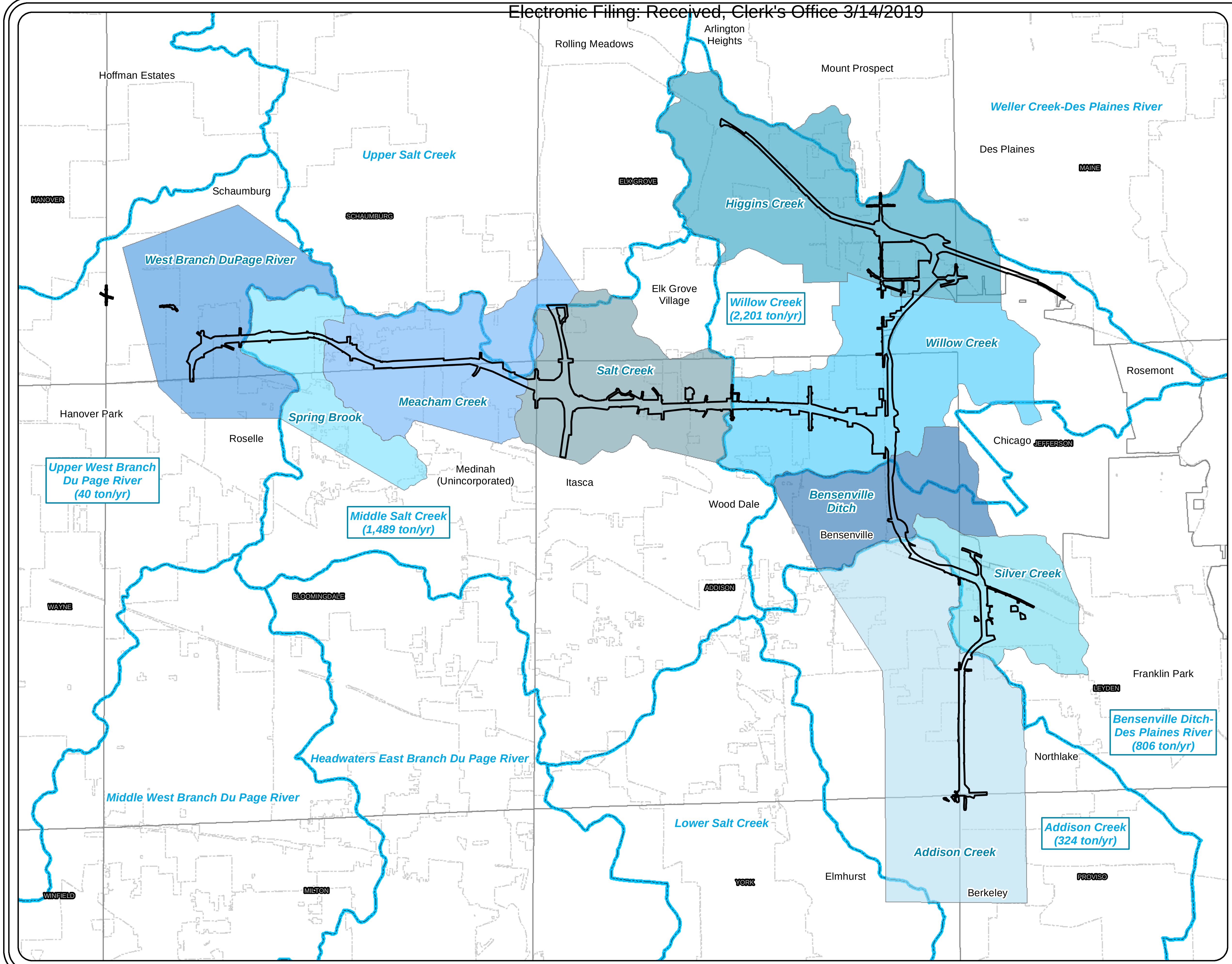
- 🟥 Tier 1
- ▢ Tier 2

0 3,000 6,000 12,000
Feet

Exhibit 2

Roadways within
Tier 1 Communities

Elgin O'Hare Western Access



LEGEND

- ▭ Full-Build 2040 Footprint
- ▭ Municipality/Township
- ▭ Watershed Boundary (Target Salt Reduction)

Sub-watershed Boundary

- 🟦 Addison Creek
- 🟦 Bensenville Ditch
- 🟦 Higgins Creek
- 🟦 Meacham Creek
- 🟦 Salt Creek
- 🟦 Silver Creek
- 🟦 Spring Brook
- 🟦 West Branch DuPage River
- 🟦 Willow Creek

0 3,000 6,000 12,000
Feet

Exhibit 3

Target Salt Reduction by Watersheds

DuPage River Salt Creek Workgroup
Chloride Offset Program

MEMORANDUM OF UNDERSTANDING BETWEEN THE ILLINOIS STATE TOLL HIGHWAY AUTHORITY AND THE DUPAGE RIVER SALT CREEK WORKGROUP FOR CHLORIDE OFFSET PROGRAM

This MEMORANDUM OF UNDERSTANDING (hereinafter referred to as the "MOU") is entered into this 31st day of October AD, 2013, by and between THE ILLINOIS STATE TOLL HIGHWAY AUTHORITY, an instrumentality and administrative agency of the State of Illinois, hereinafter called the "TOLLWAY", and THE DUPAGE RIVER SALT CREEK WORKGROUP, a group of local watershed stakeholders which include sanitary districts, municipalities, counties, forest preserve districts, state and federal agencies, and private environmental organizations of the State of Illinois, hereinafter called the "DRSCW", individually referred to as "PARTY", and collectively referred to as "PARTIES".

WITNESSETH:

WHEREAS, the TOLLWAY recently approved a 15 year Capitol Program, "Move Illinois; *The Illinois Tollway Driving the Future,*" which includes improving the Jane Addams Memorial Tollway (I-90) (hereinafter sometimes referred to as the "Toll Highway"), by reconstructing and widening from the John F. Kennedy Expressway to Interstate Route 39. The contemplated improvements are substantially included in multiple TOLLWAY construction contracts; and

WHEREAS, the TOLLWAY intends to improve the Elgin O'Hare Expressway, extending the expressway from its eastern terminus at Rohlwing Road (Illinois Route 53) to O'Hare International Airport (ORD) and constructing the Western Access connecting the Jane Addams Memorial Tollway (I-90) with the Tri-State Tollway (I-294) (hereinafter sometimes referred to as the Elgin O'Hare Western Access "EOWA"), and included in multiple TOLLWAY construction contract(s). The TOLLWAY will implement, operate and maintain the mainline improvements as tolled facilities (hereinafter sometimes referred to as the "Toll Highway"); and

WHEREAS, because the projects are in such close proximity, strategies for some aspects of environmental mitigation (i.e., water quality enhancements) are being considered collectively by the PARTIES, where feasible and practicable; and

WHEREAS, highway de-icing practices during winter months commonly use de-icing salts to provide for safe vehicular travel and winter maintenance for I-90 and the EOWA will require the use of salts; and

WHEREAS, studies acknowledged the potential for the addition of chloride concentrations in area streams and as the I-90 and EOWA projects are advancing to implementation, applications for Section 404 and Section 401 permits have been submitted to the United States Army Corp of Engineers (USACE) and the Illinois Environmental Protection Agency (IEPA); and

WHEREAS, the enhancement of water quality has been the focus of the DRSCW for many years, and the TOLLWAY in an effort to have the "Cleanest and Greenest" program possible is requesting that a partnership be developed between the PARTIES hereto, and a collaboration with permitting agencies, to achieve chloride offsets and reductions to enhance the water quality throughout the DRSCW's water-sheds affected by I-90 and the EOWA; and

WHEREAS, the waterways receiving storm water and snowmelt runoff from the I-90 and EOWA are on the IEPA's Section 303D List of impaired waters and thus require at a minimum no net increase in chlorides as defined in the "Clean Water Act"; and

WHEREAS, this MOU, for recording purposes shall be known as 002013-22, executed in duplicate, and has been prepared to outline the general understanding between the DRSCW and the TOLLWAY with regard to determine and establish their respective responsibilities toward a proposed "Chloride Offset Program" (hereinafter referred to as the "PROGRAM") and also serve as a basis for developing Intergovernmental Agreements with local watershed communities and agencies in the impacted area; and

WHEREAS the PARTIES agree that the entirety of the offset will occur with the impacted areas and be tailored to individual receiving stream segments to the maximum extent possible. As such local watershed communities and agencies participating in the PROGRAM will be responsible for winter operations on the land surfaces that drain to those segments and are herein referred to as "Tier 1 Communities";

NOW, THEREFORE, in consideration of the aforementioned recitals and the mutual covenants contained herein, the PARTIES hereto agree to the following summary of the responsibilities and participation of each PARTY in the implementation of the PROGRAM.

I. GOAL

A. The goal of the PROGRAM is to offset the increased chloride loadings from I-90 and the EOWA by affecting reductions in the use of winter de-icing salts from existing conditions. The TOLLWAY will reduce chloride applications in a quantifiable manner in support of the 401 Water Quality Certification process for I-90 and the EOWA projects and of local municipalities National Pollutant Discharge Elimination System Municipal Separate Storm Sewer System ("NPDES MS4") permit requirements.

B. The intent is to establish a partnership between the TOLLWAY and governmental bodies to achieve the chloride loading offset.

C. The partners will endeavor to achieve the offsets in communities straddling and or upstream of the I-90 and EOWA, but the TOLLWAY will expand beyond that area if needed to achieve the offset amounts.

D. Both structural and non-structural practices will comprise the PROGRAM to provide the needed improvements in water quality.

II. STRUCTURAL BEST MANAGEMENT PRACTICES

A. Grass swales, bio-swales, infiltration basins, etc. will be incorporated by TOLLWAY into the I-90 and EOWA projects in order to minimize the effects of roadway runoff and improve the quality of roadway runoff discharged to receiving waters and/or nearby wetlands.

III. NON-STRUCTURAL BEST MANAGEMENT PRACTICES

A. A salt reduction goal will be established by the TOLLWAY and DRSCW for the PROGRAM through further analysis of existing conditions, existing practices in the affected watersheds, and planned highway improvements. Offsets will include the adoption of salt reduction strategies (enhanced training, improved materials use, equipment upgrades), implemented by both the TOLLWAY and the Tier 1 communities. All participants will provide documentation on their salt application rates per lane mile, application totals, calibration logs and details on financial and other support to other mutually agreed upon partners.

B. The TOLLWAY recognizes that meeting the objectives of the Total Maximum Daily Loads ("TMDL's") will require reductions in area chloride loading above those set out in the PROGRAM and agrees to review its practices at an agency wide scale and to actively partner with the DRSCW, its members or successors, in working for PROGRAM area chloride reductions beyond the life of the PROGRAM with the goal of meeting the applicable water quality standard.

IV. TOLLWAY AND DRSCW RESPONSIBILITIES

A. Both PARTIES agree that the requirements embodied in Clean Water Act Section 401 certification(s) or Section 404 or NPDES permit(s), are the sole responsibility of the TOLLWAY, and that the DRSCW or participating agencies cannot be held liable in any way for failure to comply with such requirements.

B. The DRSCW will endeavor to unify stakeholders in the project areas with the common goal of improving chloride water quality. It is recognized that participating communities are voluntary agents and neither they nor the DRSCW can be held liable in any way for failure to collaborate in the plan.

C. The DRSCW will determine the baseline conditions through appropriate studies with stakeholders and other environmental evaluation which shall include sampling and analyses, as well as flow evaluation.

D. The DRSCW will determine the opportunities for improving de-icing practices through surveys and interviews with stakeholders.

E. The DRSCW and the TOLLWAY will set priorities, tracking offset progress, and the timeframe for achievement with concurrence from the IEPA.

F. The TOLLWAY will provide ongoing financial assistance supporting capital investments of alternative de-icing methods potentially for municipalities and agencies that are participating in the PROGRAM until such time as the PROGRAM objectives are met.

G. The TOLLWAY with the DRSCW's technical assistance will establish training and certifications for operators that would enhance awareness of best practices for snow and ice management operations.

H. The TOLLWAY with the DRSCW will establish data sites/sources for weather data and other information helpful in managing roadway de-icing.

I. The DRSCW with the support of the TOLLWAY will conduct long term monitoring that records salt usage and stream conditions. Evaluation of future operating conditions will be compared to baseline conditions.

J. The TOLLWAY and the DRSCW will report annually by July 1st to the IEPA the resulting efforts and success of the PROGRAM on an annual basis beginning in July of 2014. Success will be measured primarily by the application rate and totals reported with consideration given to the ambient monitoring system.

K. The TOLLWAY and the DRSCW will collaborate to put in place an ambient monitoring system that will be part of the PROGRAM monitoring and evaluation, and will document pre and post PROGRAM chloride conditions in the receiving streams. A monitoring plan will be developed through input from both PARTIES with the intent of conducting stream monitoring on Addison Creek, Salt Creek mainstem, Spring Brook, Meacham Creek and West Branch mainstem. The pre and post conditions will be synthesized in a report and submitted to IEPA on an annual basis along with other reporting data. The system would assist in evaluating the success of the PROGRAM in meeting the TMLD's goals and will be funded wholly by the TOLLWAY.

L. The PARTIES agree that the PROGRAM may require several years of monitoring and reporting from PROGRAM partners.

M. The PARTIES will develop and maintain a guidance document for the PROGRAM which will at minimum detail the methods for calculating the build scenario non-PROGRAM increase, the needed offset, BMP's the monitoring PROGRAM and reporting baseline requirements. The document will be updated by agreement between the PARTIES as the PROGRAM advances.

V. INTERGOVERNMENTAL AGREEMENTS

A. The TOLLWAY shall use its best efforts to enter into Intergovernmental Agreements prepared by the TOLLWAY and Tier 1 communities based upon this MOU to further determine and establish respective responsibilities toward financial partnerships, information sharing, and training.

B. These Intergovernmental Agreements shall not relieve the TOLLWAY of their responsibility to comply with the "Clean Water Act" as determined and enforced by the IEPA.

VI. FINANCIAL

A. The TOLLWAY will support financial partnerships through an Intergovernmental Agreement requiring cost sharing with a local partner as defined by the PROGRAM.

B. Project requests to the TOLLWAY will be subject to DRSCW and TOLLWAY approvals, to cost effectively promote salt usage reduction.

VII. TERMS OF THE MOU

A. The term of this MOU shall extend until such time that the PROGRAM offset has been achieved reductions have been demonstrated for a minimum of three (3) years. Either PARTY shall have the right to terminate this MOU at any time by providing at least ninety (90) days written notice to the other party in the event either PARTY breaches the terms and conditions of this MOU. At the end of the agreement period this document may be renewed by the mutual consent of the PARTIES.

(This space intentionally left blank)

IN WITNESS THEREOF, the PARTIES have entered into this MOU as of the date written below.

THE DUPAGE RIVER SALT CREEK WORKGROUP

By: ______________________
David Gorman, President

Date: 10 - 30 - 13

Attest: ______________________

10 - 30 - 13.
(Please Print Name)

THE ILLINOIS STATE TOLL HIGHWAY AUTHORITY

By: ______________________
Kristi Lafleur, Executive Director

Date: 10|31|13

Approved as to Form and Constitutionality

Tiffany I Bohn, AAG. 10/31/13
Tiffany I. Bohn, Assistant Attorney General, State of Illinois

Illinois
Tollway

The Illinois Tollway
2700 Ogden Avenue
Downers Grove, Illinois 60515-1703
Phone: 630/241-6800
Fax: 630/241-6100
TTY: 630/241-6898

November 4, 2013

Mr. Stephen McCracken
DuPage River Salt Creek Workgroup
The Conservation Foundation
10 S. 404 Knoch Knolls
Naperville, IL 60565

Re: Memorandum of Understanding between the The Illinois State Toll Highway Authority and the DuPage River Salt Creek Workgroup for Chloride Offset Program.

Dear Mr. McCracken:

Enclosed please find one (1) fully executed Memorandum of Understanding between the Illinois State Toll Highway Authority and The DuPage River Salt Creek Workgroup for Chloride Offset Program on I-90 and the Elgin O'Hare Western Access.

Very truly yours,

Tiffany I Bohn

Tiffany I. Bohn
Assistant Attorney General

TIB:mw
Enclosure

Illinois Tollway

THE ILLINOIS STATE TOLL HIGHWAY AUTHORITY

TO: Eileen Cosgriff, CIS

FROM: Tiffany I. Bohn, Assistant Attorney General

DATE: November 4, 2013

SUBJECT: ***Memorandum of Understanding between The DuPage River Salt Creek Workgroup and The Illinois State Toll Highway Authority for Chloride Offset Program.***

Attached please find one (1) fully executed original Memorandum of Understanding between the DuPage River Salt Creek Workgroup and the Illinois State Toll Highway Authority Chloride Offset Program.

This document is transmitted to your attention for the Department's records.

This Memorandum of Understanding does not require a Board Resolution.

TIB:mw
Attachment

cc:
V. Avila
T. Bohn
K. Kell
P. Kovacs
D. Manetti
M. Molliconi
P. Pearn
J. Romano
S. Talaber
B. Wagner
V. Yee
G. Zimmer
R. Zucchero

ATTACHMENT 6

MEAN HARDNESS / SULFATE CALCULATIONS

SampleGroup	StationCode	CollectionDate	CollectionTime	SampleMedium	Analyte	Result	ResultUnits	Qualifier	RESULT, MG/L	
								Mean Hardness = **N =**	**286.8** **23,208**	**mg/L**
B00097900	AD-02	1/19/2000	9:00	Water	Hardness, Ca, Mg	69	mg/l	C	69	
B00476100	AD-02	4/4/2000	9:00	Water	Hardness, Ca, Mg	90	mg/l	C	90	
B00711900	AD-02	5/11/2000	9:00	Water	Hardness, Ca, Mg	121	mg/l	C	121	
B00978200	AD-02	6/19/2000	9:00	Water	Hardness, Ca, Mg	52	mg/l	C	52	
B01243600	AD-02	7/25/2000	9:00	Water	Hardness, Ca, Mg	123	mg/l	C	123	
B01487400	AD-02	8/21/2000	9:00	Water	Hardness, Ca, Mg	113	mg/l	C	113	
B01768700	AD-02	10/2/2000	12:00	Water	Hardness, Ca, Mg	103	mg/l	C	103	
B02121100	AD-02	11/29/2000	9:00	Water	Hardness, Ca, Mg	80	mg/l	C	80	
B10163000	AD-02	1/31/2001	9:00	Water	Hardness, Ca, Mg	102	mg/l	C	102	
B10393500	AD-02	3/15/2001	10:30	Water	Hardness, Ca, Mg	89	mg/l	C	89	
B10595300	AD-02	4/16/2001	9:00	Water	Hardness, Ca, Mg	104	mg/l	C	104	
B10748700	AD-02	5/9/2001	9:00	Water	Hardness, Ca, Mg	135	mg/l	C	135	
B10963900	AD-02	6/6/2001	9:00	Water	Hardness, Ca, Mg	110	mg/l	C	110	
B11337700	AD-02	7/25/2001	9:00	Water	Hardness, Ca, Mg	142	mg/l	C	142	
B11592200	AD-02	8/27/2001	9:00	Water	Hardness, Ca, Mg	80	mg/l	C	80	
B11905800	AD-02	10/11/2001	9:00	Water	Hardness, Ca, Mg	112	mg/l	C	112	
B12179100	AD-02	11/20/2001	9:00	Water	Hardness, Ca, Mg	116	mg/l	C	116	
B12337700	AD-02	12/19/2001	10:00	Water	Hardness, Ca, Mg	71	mg/l	C	71	
B20254900	AD-02	2/6/2002	9:00	Water	Hardness, Ca, Mg	56	mg/l	C	56	
B20461800	AD-02	3/18/2002	9:00	Water	Hardness, Ca, Mg	58	mg/l	C	58	
B20715600	AD-02	4/23/2002	9:00	Water	Hardness, Ca, Mg	59	mg/l	C	59	
B20986900	AD-02	6/3/2002	10:00	Water	Hardness, Ca, Mg	102	mg/l	C	102	
B21273900	AD-02	7/11/2002	9:00	Water	Hardness, Ca, Mg	158	mg/l	C	158	
B21570800	AD-02	8/15/2002	9:00	Water	Hardness, Ca, Mg	143	mg/l	C	143	
B21872600	AD-02	10/1/2002	9:00	Water	Hardness, Ca, Mg	118	mg/l	C	118	
B22388800	AD-02	12/23/2002	11:00	Water	Hardness, Ca, Mg	57	mg/l	C	57	
B00054500	AK-02	1/10/2000	11:00	Water	Hardness, Ca, Mg	41	mg/l	C	41	
B00207600	AK-02	2/9/2000	11:00	Water	Hardness, Ca, Mg	55	mg/l	C	55	
B00651700	AK-02	5/2/2000	11:00	Water	Hardness, Ca, Mg	36	mg/l	C	36	
B01031100	AK-02	6/26/2000	10:00	Water	Hardness, Ca, Mg	37	mg/l	C	37	
B01433400	AK-02	8/17/2000	11:00	Water	Hardness, Ca, Mg	49	mg/l	C	49	
B02085600	AK-02	11/20/2000	11:00	Water	Hardness, Ca, Mg	46	mg/l	C	46	
B10124500	AK-02	1/23/2001	10:00	Water	Hardness, Ca, Mg	45	mg/l	C	45	
B10310200	AK-02	2/27/2001	12:00	Water	Hardness, Ca, Mg	30	mg/l	C	30	
B10493500	AK-02	4/3/2001	11:00	Water	Hardness, Ca, Mg	23	mg/l	C	23	
B10748400	AK-02	5/10/2001	11:00	Water	Hardness, Ca, Mg	51	mg/l	C	51	
B11068900	AK-02	6/20/2001	11:00	Water	Hardness, Ca, Mg	51	mg/l	C	51	
B11284600	AK-02	7/18/2001	11:00	Water	Hardness, Ca, Mg	55	mg/l	C	55	
B11496500	AK-02	8/15/2001	11:00	Water	Hardness, Ca, Mg	49	mg/l	C	49	
B11852800	AK-02	10/3/2001	11:00	Water	Hardness, Ca, Mg	48	mg/l	C	48	
B12113000	AK-02	11/7/2001	11:00	Water	Hardness, Ca, Mg	58	mg/l	C	58	
B20012000	AK-02	1/2/2002	11:00	Water	Hardness, Ca, Mg	43	mg/l	C	43	
B20227400	AK-02	2/4/2002	11:00	Water	Hardness, Ca, Mg	31	mg/l	C	31	
B20566600	AK-02	4/3/2002	11:00	Water	Hardness, Ca, Mg	37	mg/l	C	37	
B20884100	AK-02	5/15/2002	9:00	Water	Hardness, Ca, Mg	29	mg/l	C	29	
B21065400	AK-02	6/12/2002	11:00	Water	Hardness, Ca, Mg	43	mg/l	C	43	
B21345000	AK-02	7/17/2002	11:00	Water	Hardness, Ca, Mg	63	mg/l	C	63	
B21620000	AK-02	8/21/2002	11:00	Water	Hardness, Ca, Mg	63	mg/l	C	63	
B22160600	AK-02	11/7/2002	11:00	Water	Hardness, Ca, Mg	44	mg/l	C	44	
B22312300	AK-02	12/9/2002	11:00	Water	Hardness, Ca, Mg	49	mg/l	C	49	
B00054400	AT-06	1/10/2000	9:00	Water	Hardness, Ca, Mg	300	mg/l	C	300	
B00207500	AT-06	2/9/2000	9:00	Water	Hardness, Ca, Mg	768	mg/l	C	768	
B00651800	AT-06	5/2/2000	9:00	Water	Hardness, Ca, Mg	274	mg/l	C	274	
B01031400	AT-06	6/26/2000	8:00	Water	Hardness, Ca, Mg	92	mg/l	C	92	
B01434000	AT-06	8/17/2000	9:00	Water	Hardness, Ca, Mg	414	mg/l	C	414	
B01635600	AT-06	9/14/2000	9:00	Water	Hardness, Ca, Mg	700	mg/l	C	700	
B01768800	AT-06	10/3/2000	9:00	Water	Hardness, Ca, Mg	714	mg/l	C	714	
B02085400	AT-06	11/20/2000	9:00	Water	Hardness, Ca, Mg	441	mg/l	C	441	
B10124600	AT-06	1/23/2001	8:00	Water	Hardness, Ca, Mg	314	mg/l	C	314	
B10310500	AT-06	2/27/2001	10:00	Water	Hardness, Ca, Mg	185	mg/l	C	185	
B10310300	AT-06	2/27/2001	10:30	Water	Hardness, Ca, Mg	183	mg/l	C	183	
B10493400	AT-06	4/3/2001	9:00	Water	Hardness, Ca, Mg	192	mg/l	C	192	
B11068800	AT-06	6/20/2001	9:00	Water	Hardness, Ca, Mg	582	mg/l	C	582	
B11284700	AT-06	7/18/2001	9:00	Water	Hardness, Ca, Mg	341	mg/l	C	341	
B11852600	AT-06	10/3/2001	9:00	Water	Hardness, Ca, Mg	248	mg/l	C	248	
B12113400	AT-06	11/7/2001	9:00	Water	Hardness, Ca, Mg	579	mg/l	C	579	
B20011900	AT-06	1/2/2002	9:00	Water	Hardness, Ca, Mg	556	mg/l	C	556	
B20227500	AT-06	2/4/2002	9:00	Water	Hardness, Ca, Mg	143	mg/l	C	143	
B20566800	AT-06	4/3/2002	9:00	Water	Hardness, Ca, Mg	169	mg/l	C	169	
B20884200	AT-06	5/15/2002	11:00	Water	Hardness, Ca, Mg	73	mg/l	C	73	
B21065500	AT-06	6/12/2002	9:00	Water	Hardness, Ca, Mg	341	mg/l	C	341	
B21344900	AT-06	7/17/2002	9:00	Water	Hardness, Ca, Mg	1060	mg/l	C	1060	
B21619900	AT-06	8/21/2002	9:00	Water	Hardness, Ca, Mg	256	mg/l	C	256	
B22160200	AT-06	11/7/2002	9:00	Water	Hardness, Ca, Mg	402	mg/l	C	402	
B22311900	AT-06	12/9/2002	9:00	Water	Hardness, Ca, Mg	768	mg/l	C	768	

SampleGroup	StationCode	CollectionDate	CollectionTime	SampleMedium	Analyte	Result	ResultUnits	Qualifier	ReportingLimit
					Mean Sulfate =	**86.8**	**mg/L**		
					N =	**19,580**			
B00097900	AD-02	1/19/2000	9:00	Water	Sulfate	42.6	mg/l		
B01768700	AD-02	10/2/2000	12:00	Water	Sulfate	18.9	mg/l		
B21273900	AD-02	7/11/2002	9:00	Water	Sulfate	10.4	mg/l		
B00054500	AK-02	1/10/2000	11:00	Water	Sulfate	23.9	mg/l		
B00207600	AK-02	2/9/2000	11:00	Water	Sulfate	46.9	mg/l		
B00651700	AK-02	5/2/2000	11:00	Water	Sulfate	11.1	mg/l		
B01031100	AK-02	6/26/2000	10:00	Water	Sulfate	11.1	mg/l		
B01433400	AK-02	8/17/2000	11:00	Water	Sulfate	16.9	mg/l		
B02085600	AK-02	11/20/2000	11:00	Water	Sulfate	18.2	mg/l		
B10124500	AK-02	1/23/2001	10:00	Water	Sulfate	20.6	mg/l		
B10310200	AK-02	2/27/2001	12:00	Water	Sulfate	11.1	mg/l		
B10493500	AK-02	4/3/2001	11:00	Water	Sulfate		mg/l	ND	10
B10748400	AK-02	5/10/2001	11:00	Water	Sulfate	13.7	mg/l		
B11068900	AK-02	6/20/2001	11:00	Water	Sulfate		mg/l	ND	10
B11284600	AK-02	7/18/2001	11:00	Water	Sulfate		mg/l	ND	10
B11496500	AK-02	8/15/2001	11:00	Water	Sulfate		mg/l	ND	10
B11852800	AK-02	10/3/2001	11:00	Water	Sulfate		mg/l	ND	10
B12044900	AK-02	10/26/2001	12:20	Water	Sulfate	16.9	mg/l		
B12113000	AK-02	11/7/2001	11:00	Water	Sulfate	20.5	mg/l		
B20012000	AK-02	1/2/2002	11:00	Water	Sulfate	21.7	mg/l		
B20227400	AK-02	2/4/2002	11:00	Water	Sulfate	15.2	mg/l		
B20566600	AK-02	4/3/2002	11:00	Water	Sulfate	13.9	mg/l		
B20629400	AK-02	4/5/2002	16:30	Water	Sulfate	14	mg/l		
B20744800	AK-02	4/25/2002	15:30	Water	Sulfate	14.4	mg/l		
B20884100	AK-02	5/15/2002	9:00	Water	Sulfate	14.7	mg/l		
B20946000	AK-02	5/22/2002	14:30	Water	Sulfate	16.3	mg/l		
B21018600	AK-02	6/7/2002	13:20	Water	Sulfate	12.8	mg/l		
B21065400	AK-02	6/12/2002	11:00	Water	Sulfate	11.5	mg/l		
B21134500	AK-02	6/20/2002	14:40	Water	Sulfate	12.4	mg/l		
B21214400	AK-02	7/3/2002	12:00	Water	Sulfate	13.3	mg/l		
B21345000	AK-02	7/17/2002	11:00	Water	Sulfate		mg/l	ND	10
B21524200	AK-02	7/31/2002	17:00	Water	Sulfate	118	mg/l		
B21620000	AK-02	8/21/2002	11:00	Water	Sulfate	80.9	mg/l		
B21642900	AK-02	8/22/2002	16:30	Water	Sulfate	152	mg/l		
B21780800	AK-02	9/12/2002	18:20	Water	Sulfate		mg/l	ND	10
B22069000	AK-02	10/12/2002	14:30	Water	Sulfate	68.6	mg/l	J1,J3,J4	
B22205000	AK-02	11/1/2002	15:40	Water	Sulfate	20	mg/l	J1,J3,J4	
B22160600	AK-02	11/7/2002	11:00	Water	Sulfate	32.9	mg/l	J1,J3,J4	
B22204400	AK-02	11/15/2002	16:40	Water	Sulfate	17.3	mg/l	J1,J3,J4	
B22312300	AK-02	12/9/2002	11:00	Water	Sulfate	21	mg/l	J1,J3,J4	
B22385700	AK-02	12/12/2002	15:20	Water	Sulfate	23.1	mg/l	J1,J3,J4	
B00054400	AT-06	1/10/2000	9:00	Water	Sulfate	289	mg/l		
B00207500	AT-06	2/9/2000	9:00	Water	Sulfate	755	mg/l		
B00651800	AT-06	5/2/2000	9:00	Water	Sulfate	191	mg/l		
B01031400	AT-06	6/26/2000	8:00	Water	Sulfate	41.7	mg/l		
B01434000	AT-06	8/17/2000	9:00	Water	Sulfate	331	mg/l		
B01635600	AT-06	9/14/2000	9:00	Water	Sulfate	698	mg/l		
B01768800	AT-06	10/3/2000	9:00	Water	Sulfate	586	mg/l		
B02085400	AT-06	11/20/2000	9:00	Water	Sulfate	287	mg/l		
B10124600	AT-06	1/23/2001	8:00	Water	Sulfate	158	mg/l		
B10310500	AT-06	2/27/2001	10:00	Water	Sulfate	103	mg/l		
B10493400	AT-06	4/3/2001	9:00	Water	Sulfate	93.9	mg/l		
B11068800	AT-06	6/20/2001	9:00	Water	Sulfate		mg/l	ND	10
B11284700	AT-06	7/18/2001	9:00	Water	Sulfate	208	mg/l		
B11852600	AT-06	10/3/2001	9:00	Water	Sulfate	155	mg/l		
B12113400	AT-06	11/7/2001	9:00	Water	Sulfate	285	mg/l		
B20011900	AT-06	1/2/2002	9:00	Water	Sulfate	419	mg/l		
B20227500	AT-06	2/4/2002	9:00	Water	Sulfate	77.7	mg/l		
B20566800	AT-06	4/3/2002	9:00	Water	Sulfate	106	mg/l		