Equilibrium Environmental Inc.

FRESH WATER AQUATIC LIFE WORKING GROUP

(FWAL)

DEVELOPMENT OF A CHLORIDE WATER QUALITY GUIDELINE INCORPORATING HARDNESS MODIFYING FACTORS

Prepared for:

Petroleum Technology Alliance Canada
Suite 400, Chevron Plaza
500 – Fifth Avenue SW
Calgary, AB
T2P 3L5

Prepared by:

Equilibrium Environmental Inc.
3004 Ogden Road SE
Calgary, Alberta
T2G 4N5

Tel: (403) 286-7706
Cell: (403) 862-7758
Fax: (403) 286-8173
Email: tknafla@eqm.ca
imcivor@eqm.ca

Date of Submission:
December 2014
Equilibrium File Number: PT-12

TABLE OF CONTENTS

Page

EXECUTIVE SUMMARY ... iv
1 INTRODUCTION AND SCOPE ... 1
2 METHODOLOGY ... 3
2.1 TOXICOLOGICAL DATABASE COMPILATION ... 3
2.2 SHORT-TERM AND LONG-TERM DATA QUALITY REQUIREMENTS ... 4
2.3 SPECIES SENSITIVITY DISTRIBUTION (SSD) – GUIDELINE DERIVATION ... 5
2.4 HARDNESS ADJUSTMENT – GUIDELINE DERIVATION ... 7
3 ACUTE TOXICOLOGICAL GUIDELINE EXCLUDING HARDNESS ... 10
3.1 COMPARATIVE TOXICITY OF CHLORIDE SALTS (NaCl, KCl, $CaCl_2$, $MgCl_2$) ... 10
3.2 SHORT-TERM SPECIES SENSITIVITY DISTRIBUTION (SSD) ... 13
3.2.1 Evaluation of Short-Term Toxicological Data ... 18
3.2.2 Derivation of the Short-Term SSD and Benchmark Concentration ... 20
4 CHRONIC TOXICOLOGICAL EFFECTS EXCLUDING HARDNESS ... 22
4.1 LONG-TERM SPECIES SENSITIVITY DISTRIBUTION (SSD) ... 22
4.1.1 Evaluation of Long-Term Toxicological Data ... 24
4.1.2 Derivation of the Long-Term SSD and Water Quality Guideline ... 27
5 HARDNESS MODIFICATION ... 29
5.1 BACKGROUND AND THEORETICAL CONSIDERATIONS ... 29
5.1.1 Effects of Water Hardness on the Toxicity of Chloride to Aquatic Life ... 34
5.1.2 Studies Showing no Ameliorating Effect of Hardness on Chloride Toxicity ... 42
5.2 HARDNESS ADJUSTED SHORT-TERM SSD ... 44
5.2.1 Short-Term Hardness Adjustment Equation ... 44
5.2.2 Short-Term Hardness Adjusted SSD and Benchmark Concentration ... 48
5.2.3 Hardness Equation for the Short-Term Benchmark ... 50
5.3 HARDNESS ADJUSTED CHRONIC SSD ... 53
5.3.1 Long-Term Hardness Adjustment Equation ... 53
5.3.2 Long-Term SSD and Water Quality Guideline ... 56
5.3.3 Hardness Equation for Calculating Long-Term Water Quality Guidelines ... 58
6 ASSESSMENT OF GUIDELINE PROTECTIVENESS ... 61
6.1 PROTECTIVENESS OF THE SHORT-TERM BENCHMARK ... 61
6.2 PROTECTIVENESS OF THE LONG-TERM WATER QUALITY GUIDELINE ... 64
7 GUIDELINE SUMMARY ... 67
8 KNOWLEDGE GAPS AND RESEARCH RECOMMENDATIONS ... 70
8.1 SUMMARY OF HARDNESS-TOXICITY DATA LIMITATIONS ... 70
8.2 SUMMARY OF TAXA SPECIFIC RESEARCH RECOMMNDATIONS ... 72
8.2.1 Bivalvia ... 73
8.2.2 Branchipoda ... 77
8.2.3 Algae ... 78
8.2.4 Gastropoda ... 79
8.2.5 Amphibia ... 79
8.2.6 Actinopterygii ... 80

8.2.7 Vascular Plants 80
8.3 SUMMARY OF CHLORIDE SALT RESEARCH RECOMMENDATIONS 81
8.3.1 Calcium Chloride – Data Limitations and Research Recommendations 81
8.3.2 Potassium Chloride – Data Limitations and Research Recommendations 81
8.3.3 Magnesium Chloride – Data Limitations and Research Recommendations 82
8.4 TOXICITY TESTING WORK 84
8.4.1 Target Chloride Concentrations for Testing 84
8.4.2 Target Hardness Levels for Testing 84
9 CLOSURE 85
10 REFERENCES 86
Appendix A 104
Appendix B 246
Appendix C 254

LIST OF TABLES

Table 2.1. Minimum short and long-term SSD requirements

Table 3.1. Derived BMDs for various chloride salts – severe endpoints (EC/LC50)
Table 3.2. EC/LC50 (severe endpoints) used in the derivation of the short-term SSD
Table 3.3. Raw data used in the derivation of short-term SSD averaged data points

Table 4.1. No and low effect concentrations used in the derivation of the long-term SSD
Table 4.2. Raw data used in the derivation of long-term SSD averaged data points

Table 5.1. Summary of chronic hardness dependent WQGs for metals
Table 5.2. Studies investigating the effect of water hardness on chloride toxicity to aquatic life
Table 5.3. Short-term hardness-toxicity regression slopes
Table 5.5. Long-term hardness-toxicity regression slopes
Table 5.6. Long-term hardness-adjusted WQG concentrations

Table 6.1. Studies reporting endpoints near or below the long-term hardness adjusted WQG

Table 7.1. Summary of chloride guidelines for the protection of aquatic life
Table 7.2. Water hardness summary for major surface waters across Canada

Table 8.1. Targeted species and toxicity assays for future research into chronic endpoints
Table 8.2. Summary of short- and long-term SSD requirements not met by calcium, potassium, and magnesium chloride salts

LIST OF FIGURES

Figure 3.1. Comparative toxicity of the common salts of chloride – severe endpoints (EC/LC50)
Figure 3.2. Short-term SSD – severe endpoints (EC/LC50) for NaCl

Figure 4.1. Long-term SSD – no and low effect endpoint toxicity data for NaCl

Figure 5.1. Hardness-toxicity relationship established for Lampsilis siliquoidea (Gillis 2011)
Figure 5.2. Regional water hardness trends in Canada
Figure 5.3. Short-term hardness-toxicity relationships
Figure 5.4. Hardness adjusted short-term SSD – severe endpoints (EC/LC50) for NaCl
Figure 5.5. Adjusted short-term SSD at various water hardness levels
Figure 5.6. Long-term hardness-toxicity relationships
Figure 5.7. Hardness adjusted long-term SSD – no and low effect concentrations for NaCl
Figure 5.8. Adjusted long-term SSD at various water hardness levels

Figure 6.1. Protectiveness of the short-term benchmark
Figure 6.2. Protectiveness of the long-term WQG

Figure 8.1. Short- and long-term SSDs with associated hardness-toxicity relationships

EXECUTIVE SUMMARY

The Canadian Council of Ministers of the Environment (CCME) has recently revised the Water Quality Guideline for chloride (from 230 to 120 mg/L for chronic exposure), applicable to freshwater aquatic life receptors where COSEWIC (Committee on the Status of Endangered Wildlife in Canada) Endangered or Special Concern Pelecypoda (clams, mussels) are not present (CCME 2011). In their analysis, toxicity testing data demonstrates that Pelecypoda at the glochidia lifestage can be particularly sensitive to the adverse effects associated with chloride exposure, which was a partial determinant of the guideline revision.

The CCME also identified water hardness as an important factor that modifies the toxicity of chloride towards aquatic life. This phenomenon has been demonstrated in the literature over a range of species, resulting in various other regulatory agencies adopting hardness adjusted guidelines for chloride and other major ions. The state of Iowa has developed acute (287.8 * $(\text{hardness})^{0.205797}(\text{sulphate})^{-0.07452}$) and chronic (177.87 * $(\text{hardness})^{0.205797}(\text{sulphate})^{-0.07452}$) algorithms for the adjustment of a chloride water quality guidelines based on hardness (IDNR 2009a). Within Canada, British Columbia has recently established a hardness adjusted guideline for the sulphate ion varying from 128, 218, 309, and 429 mg SO4 /L for very soft (0-30 mg/L), soft / moderately soft (31-75 mg/L), moderately soft / hard (76-180 mg/L), and very hard (181-250 mg/L) water respectively (BCME 2013). Water hardness has also been identified as an ameliorating factor in aquatic guideline policy such as the Australian and New Zealand government's National Water Quality Management Strategy (ANZECC, 2000). Finally, the effects of hardness amelioration have been incorporated into site specific guidelines for chloride in Canada. Examples include the EKATI diamond mine (guideline calculated as: 124 * ln (hardness) -128) (Rescan 2007) and the Snap Lake diamond mine (De Beers 2013). Given the growing application of hardness derived guidelines to major ion toxicity, an important component of any future research on the toxicity of chloride towards sensitive aquatic organisms is to incorporate varying hardness levels and produce information that can be used to improve the accuracy of guidelines developed on a provincial or national scale.

Although recognized by CCME, as a result of limitations to the long-term dataset, a hardness derived guideline was not established in 2011. Long-term hardness-toxicity data was either not available or did not meet the minimum data requirements for hardness adjusted water quality guidelines at the time that CCME developed their limit in 2011.

The objectives of the present study were to:

1. conduct a detailed review of the literature to establish an aquatic toxicological database for chloride made up of data utilized in the CCME 2011 or any newly available data;
2. examine the relative toxicity of the four common chloride salts: NaCl, KCl, $CaCl_2$, and $MgCl_2$;
3. derive acute and chronic Species Sensitivity Distributions (SSDs) to establish a short-term benchmark and a long-term Water Quality Guideline (WQG) for comparison to the CCME 2011 guidelines for chloride;
4. re-examine hardness-toxicity relationships within the database and determine whether the requirements for a hardness-adjusted short-term benchmark and long-term WQG are met for chloride;

5. if applicable, derive a hardness-adjusted short-term benchmark and long-term WQG based on the SSD approach utilized by the CCME; and,
6. recommend additional aquatic toxicological research for sensitive species or species where hardness-toxicity relationships are not well established in order to further develop hardness as a toxicity modifying factor and continue to advance a scientifically defensible WQG for chloride.

An aquatic toxicological database for chloride was established using data from 189 studies, of which 69 represented additional studies not included in the previous CCME (2011) dataset. The dataset developed herein was comprehensive and captured various data, including: effect concentrations; multiple toxicity endpoints; exposure durations; information on other ions in solution (water hardness, major ion concentrations); organism data (taxonomic, life history, geographic distribution); and, environmental conditions (temperature, pH, dissolved oxygen, and light exposure).

Sufficient information was available to construct short-term SSDs to compare the relative toxicities of the four common chloride salts (NaCl, KCl, $CaCl_2$, and $MgCl_2$). Results agreed with the established literature and showed that the relative toxicity of the salt pairings was: KCl > $MgCl_2$ > NaCl ≈ $CaCl_2$, where the toxicity of potassium and magnesium chloride can be attributed to the associated cation rather than chloride. Based on these findings, the remainder of the guideline derivation was based on NaCl and $CaCl_2$ as was conducted by the CCME (2011). Unlike the CCME, $CaCl_2$ data was not pooled with NaCl data, and the protectiveness of the derived guidelines to $CaCl_2$ was assessed separately, which is anticipated to be a conservative procedure in terms of deriving a limit for NaCl as $CaCl_2$ appeared to be slightly less toxic.

For comparative purposes, the initial assessment of the short- and long-term NaCl data involved creating SSDs following the general methods used by CCME (2011), without any adjustment for water hardness. The guideline derived from the unadjusted short-term SSD was 659 mg/L, close to the value of 640 mg/L established by the CCME (2011). This minor disparity might be explained by the different modeling approach (log-Normal model used by the CCME), the five additional species (*Hydra attenuate, sp., Pycnopsyche sp., Hyla chrysoscelis, Chironomus xanthus*) used in the current data set, or from the higher toxic effect concentration used for the fatmucket clam (*L. siliquoidea*). The collated effect concentration used for this species was increased from 709 mg Cl^- /L (CCME 2011) to 892 mg Cl^- /L, with the addition of data from Cope *et al.* (2008) that was not included by CCME. Because of its relatively low position in the SSD, this shift in effect concentration may have resulted in a higher modeled benchmark concentration. Other changes included limits for *C. dubia* (1080 to 1151 mg Cl^- /L), and *D. pulex* (1248 to 1597 mg Cl^- /L). Overall the 20 mg/L increase was not seen as a significant departure from the results obtained by the CCME (2011).

The guideline derived from the unadjusted long-term SSD was 112 mg/L, 8 mg/L lower than the 120 mg/L value established by the CCME (2011). In both derivations (CCME and herein), the Log-Logistic model was found to best fit the data. The more sensitive guideline calculated herein is the result of new additions to the SSD dataset, along with revised effect concentrations for a number of species located relatively low on the curve. Three data points in particular are important, and likely the primary factors driving the greater sensitivity of the current model. One is a 96h EC_{10} endpoint of 96 mg Cl^- /L for the green algae *P. subcapitata* (Simmons 2012). This data point represents a departure from the CCME derivation where algae species were only represented high on the curve with effect concentrations > 6,000 mg Cl^- /L (See Section 4.1.1 for further discussion). Two data points representing *C. dubia* and *L. minor* have also been reduced from 454 to 337 mg Cl^- /L and 1171 to 496 mg Cl^- /L, respectively, with the addition of data from Elphick *et al.* (2011a), Lasier and Harden (2010), and Simmons (2012).

In order for short- and long-term hardness adjusted guidelines to be established, chloride toxicity values from different studies must be compared by converting them to a standardized hardness value. Here, the standard hardness of 50 mg/L ($CaCO_3$ equivalents) was used, as has been done in previous guideline derivations for cadmium (CCME 2014, US EPA 2001). Using statistical analysis, empirical relationships were established for both short- and long-term data in order to convert toxicity data to the standard hardness value of 50 mg $CaCO_3$/L. This involved establishing hardness-toxicity slopes for organisms where effect concentrations were available over a wide range of water hardness concentrations. Individual hardness-toxicity slopes were then statistically pooled to determine an overall estimation of the hardness-toxicity relationship within the short- and long-term datasets. For a number of organisms, such as the bivalve *Lampsilis fasciola,* inverse hardness-toxicity relationships were established but could not be included in pooled slope calculations since hardness-toxicity relationships were not investigated over a wide enough range of hardness (*sensu* Stephan *et al.* 1985).

Short- and long-term hardness-adjusted (50 mg $CaCO_3$/L) datasets were then assessed using an SSD approach and the best fit cumulative distribution function was utilized for guideline derivation. The derived short-term benchmark concentration and long-term WQG are presented as exponential functions allowing the determination of guidelines based on site specific water hardness concentrations.

The chloride short-term benchmark concentration and long-term WQG for the protection of aquatic life were based on CCME protocols for Type A (statistical - SSD derivation) data (CCME 2007) and utilized endpoints for NaCl, which was the only chloride salt found to satisfy the requirements for Type A analysis. The short-term benchmark was based on the distribution of data from 46 species modeled using a log-logistic function. The long-term WQG was based on the distribution of data from 32 species also modeled using a log-logistic function. These guidelines are presented below, along with the results chloride concentrations at various water hardness concentrations.

Summary of chloride guidelines for the protection of aquatic life

	Short-term exposure (mg/L)	**Long-term exposure (mg/L)**
Hardness equation	***Benchmark =*** $10^{[0.294 (\log(\text{hardness})) + 2.179]}$	***WQG =*** $10^{[0.473 (\log(\text{hardness})) + 1.227]}$

Notes: *1) hardness measured as mg/L as $CaCO_3$; 2) the short-term hardness equation is applicable from 28 to 796 mg $CaCO_3$ /L and should not be applied outside of this range; 3) the long-term hardness equation is applicable from 10 to 699 mg $CaCO_3$ /L and should not be applied outside of this range.*

Guidelines for the protection of fresh water aquatic life at various hardness values

Water hardness (mg/L as $CaCO_3$)	Short-term exposure (mg Cl^-/L)	Long-term exposure (mg Cl^-/L)
Lower limit*	***403***	***50***
Soft (50)	***478***	***108***
Moderately hard (150)	***660***	***181***
Hard (300)	***810***	***251***
Upper limit (796 and 699)**	***1079***	***375***

Notes: *1) the short-term hardness equation is applicable from 28 to 796 mg $CaCO_3$ /L and should not be applied outside of this range; 2) the long-term hardness equation is applicable from 10 to 699 mg $CaCO_3$ /L and should not be applied outside of this range.*
** 403 mg Cl-/L is the lower limit short-term benchmark value that applies to waters less than 28 mg CaCO3/ L. 50 mg Cl-/L is the lower limit long-term WQG value that applies to waters less than 10 mg CaCO3/ L.*
*** 1079 mg Cl-/L is the upper limit short-term benchmark value that applies to waters greater than 796 mg CaCO3/ L. 375 mg Cl-/L is the upper limit long-term WQG value that applies to waters greater than 699 mg CaCO3/ L.*

Base on the findings from this assessment it was determined that future research should focus on chronic endpoints for those species most sensitive to chloride, which are predominantly made up of unionid mussels, fingernail calms, algae, and daphnids. These taxa were found to occur beneath the 20th percentile on the long-term SSD. In addition, future research should focus on determining chronic hardness-toxicity relationships which are not well established for many of the most sensitive organisms. Toxicity testing on specific organisms or taxonomic groups where hardness-toxicity relationships are unclear, would also be highly beneficial in improving the accuracy of future fresh water aquatic guidelines for chloride. Table 8.1 lists the recommended species and their priority level, along with a rational for inclusion, desirable exposure durations, and lifestages.

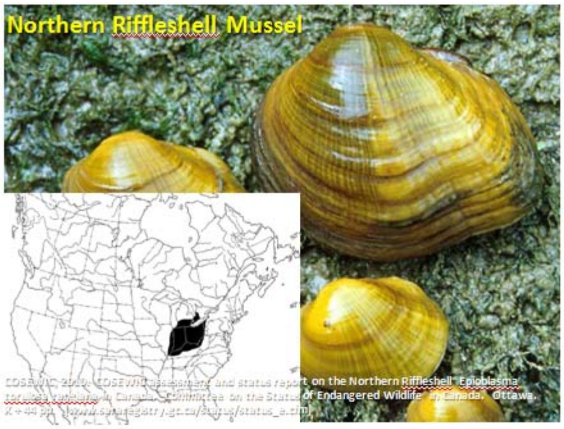
1 INTRODUCTION AND SCOPE

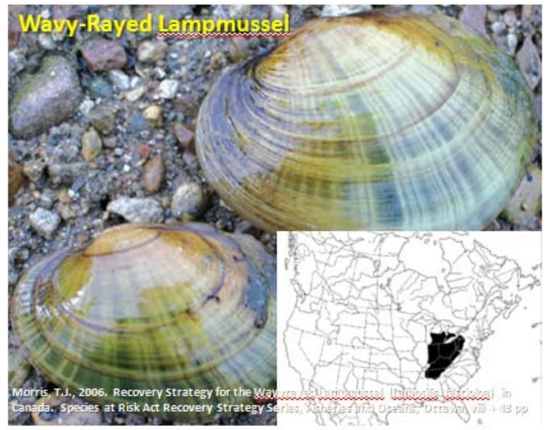
Chloride is a major ion in aquatic ecosystems, entering solution through the weathering of soils and parent geological material containing salts of sodium (NaCl), potassium (KCl), calcium ($CaCl_2$), and magnesium ($MgCl_2$). Although chloride is among the most commonly occurring ions in the natural environment (others including: sulphate, sodium, potassium, calcium, magnesium, and bicarbonate), the ion is a potentially harmful contaminant at elevated concentrations. Managing the impacts of anthropogenic chloride can be particularly challenging due to the anion's high solubility and resulting mobility. Additionally, the chloride ion does not readily react or interact with other compounds, hence it does not biodegrade, readily precipitate, volatilize, bioaccumulate, or absorb onto mineral surfaces. As a result, concentrations remain high in surface water and sediment pore water, and low in sediment (Evan and Frick 2001, Mayer *et al.* 1999).

The primary source of anthropogenic chloride is the application of road salt for winter motorist safety. Annual application of road salt in Canada is estimated at approximately 5 million tonnes / year (EC and HC 2001), primarily in the form of NaCl (97%), followed by $CaCl_2$ (2.9%), and less commonly in the form of $MgCl_2$ and KCl (0.1%) (Environment Canada 2004). The loading of chloride on aquatic ecosystems in proximity to urban areas has emerged as a major issue resulting in the Priority Substances List Assessment Report for Road Salts (Environment Canada 2004) identifying chloride salts as toxic under subsections 64 (a) and (b) of the *Canadian Environmental Protection Act, 1999*. Produced water, brought out of formation during oil extraction is another source of anthropogenic chloride which is of relevance in oil producing provinces. Other sources of anthropogenic chloride include municipal waste water production, irrigation drainage, and other industrial effluents.

The Canadian Council of Ministers of the Environment (CCME) has recently revised the Water Quality Guideline for chloride (from 230 to 120 mg/L for chronic exposure), applicable to freshwater aquatic life receptors where COSEWIC (Committee on the Status of Endangered Wildlife in Canada) Endangered or Special Concern Pelecypoda (clams, mussels) are not present (CCME 2011). Toxicity testing data demonstrate that the glochidia lifestage of Pelecypoda can be especially sensitive to the adverse effects associated with chloride exposure. Based on the Protection Clause (CCME 2007), for aquatic systems where the COSEWIC species at risk, *Lampsilis fasciola* and *Epioblasma torulosa rangiana* are present, a guideline ranging from 24 to 42 mg Cl^- /L is applicable.

The Pelecypoda species *Lampsilis fasciola* (COSEWIC special concern) and *Epioblasma torulosa rangiana* (COSEWIC endangered) were the most sensitive species in the long-term dataset and had a relatively strong influence on the chloride Species Sensitivity Distribution (SSD) and resulting chloride water quality guideline. The two species are shown below (Epioblasma torulosa rangiana or Northern Riffleshell, and Lampsilis fasciola or Wavy-rayed lampmussel). These species are encountered within a localized area of Canada, essentially within the Ontario Great Lakes region (shown in the map inserts below, black marked area of Canada and the United States).





COSEWIC Endangered: Northern Riffleshell (Epioblasma torulosa rangiana).
COSEWIC Special Concern: Wavy-rayed Lampmussel (Lampsilis fascioloa).

While the COSEWIC Endangered Northern Riffleshell is not located in Alberta or any species within the same genera (Clifford 2012, Clarke 1973), the Lampsilis genera, of the Family Unionidae is encountered in Alberta. A number of other Pelecypoda species are present in Alberta, three of which have been tested for chloride sensitivity and are included in the present database: *Lampsilis silquoidea*, *Musculium secures*, and *Sphaerium simile* (Appendix A)

CCME (2011) indicated there was a need for the generation of more toxicity data incorporating aspects of hardness. Given that Canadian surface water bodies can have relatively harder water, key components of this report are:

1. To assess available information and relationships between hardness and chloride toxicity towards various toxicologically sensitive species; and,
2. Where necessary to recommend additional toxicity testing studies that may be required to develop a suitably defensible database for a hardness modified chloride aquatic life guideline.

Furthermore, additional work examining the relative toxicity of different chloride salts (*e.g.*, KCl, $MgCl_2$, $CaCl_2$) may be warranted. Although the relative toxicity of major chloride salts has been established for particular species, (Mount *et al.* 1997, Waller *et al.* 1996, Khangarot 1991, Patrick *et al.* 1968, Jones *et al.*1940, 1941), a broader understanding of these relationships over a wider range of taxa is warranted. This addition research task was included herein and builds on previous efforts to establish the relative toxicities of major chloride salts (Evans and Frick 2001, Bright and Addison 2002).

2 METHODOLOGY

The methods utilized in this assessment can generally be described in four phases. For the first phase, an extensive literature search was utilized to establish a toxicological database for chloride. Secondly, this data was critically examined to insure that various minimum data quality requirements were met as outlined by the CCME (2007). Thirdly, acceptable data was categorised by acute or chronic endpoints, and a collation process was utilized to establish acute and chronic SSDs for the derivation of a short-term benchmark concentration and a long-term Water Quality Guideline (WQG) respectively. Sufficient data was available to construct acute SSDs for the other major chloride salts (KCl, $CaCl_2$, and $MgCl_2$), thus at this point a comparative analysis of the relative toxicity of various chloride-cation pairings was conducted. Finally, the unadjusted SSDs were adjusted to a standard water hardness value of 50 mg $CaCO_3$ /L. This was accomplished by analysing hardness-toxicity relationships among a range of different organisms which provided a short- and long-term pooled slope allowing for SSDs data points to be adjusted based on hardness. Equations were then established to calculate the hardness dependent short-term benchmark and the long-term WQG. The following four sections outline this process in greater detail.

2.1 TOXICOLOGICAL DATABASE COMPILATION

In order to derive short-term benchmarks and long term guidelines for chloride, a toxicological database was compiled using data from the scientific literature which included peer-reviewed articles, government guideline deviation documents, and technical reports. Citation searches were conducted using existing reviews, the ECOTOX database (US EPA 2014a), and the peer reviewed citation index Web of Science (Thomson Reuters), spanning a publication date range of 1916 to 2014.

Toxicity data collected and cited by CCME as part of their guideline derivation process (CCME 2011) were obtained and critically reviewed, as many of these studies are fundamental to the derivation of chloride aquatic life guidelines. Additional studies identified in the literature searches, regardless of publication date, were critically reviewed to determine if they should be incorporated in the guideline derivation process.

The aquatic toxicological database is comprised of literature examining the toxicity of the common chloride salts NaCl, KCl, $CaCl_2$, and $MgCl_2$. Studies examining non-chloride salts, whole effluents, and multiple salt toxicity were preliminarily included and reviewed, although the data were excluded from analysis herein, in terms of guideline derivation, in alignment with typical protocols for developing aquatic life guidelines and since the inclusion of mixture and whole effluent data can introduce additional complexities. The data were however considered in discussions of relative toxicity between different chloride salts

In addition to effect concentrations, toxicity endpoints, and exposure durations; other experimental variables recorded included: information on other ions in solution (water hardness, major ion concentrations) organism data (taxonomic, life history, geographic distribution), and environmental conditions (temperature, pH, dissolved oxygen, and light exposure). A table representing the entire database is presented in Appendix A.

In total, 190 studies were included in the database, representing 1405 data entries. Sixty nine percent of these data represented sodium chloride, while 20%, 8%, and 3% represented KCl, $CaCl_2$, and $MgCl_2$, respectively. The data included in this review represents an expanded data set from that used in the CCME (2011) guideline derivation, with 69 additional citations equivalent to 363 additional data points. In addition to the single salt data, 30 publications dealing with multiple salt solutions, whole effluents, and non-chloride salts were compiled and reviewed though they are not summarized here as it was outside the scope of the report.

2.2 SHORT-TERM AND LONG-TERM DATA QUALITY REQUIREMENTS

The guideline derivation conducted herein parallels that conducted by CCME (2011) and uses a statistical (SSD) approach to determine short-term benchmarks and long-term guidelines. The protocols and minimum data requirements for this type of derivation are outlined in CCME (2007). Short-term benchmarks are intended to estimate severe effects and to protect most aquatic organisms from lethality during intermittent events (*e.g.* spill events), whereas long-term WQGs are meant to protect all forms of aquatic life from any adverse effects over indefinite exposure periods (CCME 2007, CCME 2011). To determine short and long-term SSDs, data classified as primary or secondary was used (CME 2007), while unacceptable data was excluded. Data was deemed to be unacceptable for various reasons, most commonly when experimental controls were not reported, environmental conditions were inappropriate (*e.g.* test temperatures too high), the ionic composition of the test medium was unknown, or the species tested was not appropriate for Canadian waters (*e.g.* tropical species). A number of data points from particular studies were found to be unacceptable and are indicated in Appendix A. Studies which were evaluated and found to be entirely unacceptable for the analysis conducted here are referenced at the end of Appendix A.

Separate short and long-term SSD data requirements were followed as outlined by the CCME (2007, 2011), which are shown in Table 2.1 and specify the requirement for data from multiple taxa. For the short-term SSD derivation, severe effects data (LC_{50} or equivalent (*e.g.* EC_{50} for immobility in small invertebrates)) over short-term durations may be utilized with additional requirements depending on the taxa examined. For long-term guideline derivation, low and no effect data is preferred in the following order: EC/ICx representing a no-effect threshold > EC/IC_{10} > $EC/IC_{11\text{-}25}$ > MATC > NOEC > LOEC > $EC/IC_{26\text{-}49}$ > nonlethal EC/IC_{50}. Applicable endpoints must be reported across long-term durations with additional requirements depending on the taxa examined (Table 2.1).

Table 2.1. Minimum short and long-term SSD requirements

Taxa	Studies (#)	Species (#)	Endpoints	Required exposure durations (days)
		SHORT-TERM		
Fishes	3	3 (1 salmonid & 1 non-salmonid)	Severe effects (EC/LC_{50})	≤ 4
Invertebrates	3	3 (1 planktonic)	Severe effects (EC/LC_{50})	≤ 4
Amphibians	None	None	Severe effects (EC/LC_{50})	≤ 4
Algae	None	None	Severe effects (EC/LC_{50})	≤ 1 (≤ 2 if data deficient)
Plants	None	None	Severe effects (EC/LC_{50})	Case-by-case
		LONG-TERM		
Fishes	3	3 (1 salmonid & 1 non-salmonid)	Low & no effect *	≥ 21 (larva: ≥ 7)
Invertebrates	3	3 (1 planktonic)	Low & no effect *	≥ 7 (short-lived: ≥ 4) (lethal: ≥21)
Amphibians	None	None	Low & no effect *	≥ 21 (larva: ≥ 7)
**** Algae**	1 (or plant)	1 (or plant)	Low & no effect *	≥ 1
**** Plants**	1 (or algae)	1 (or algae)	Low & no effect *	Case-by-case

Notes: *1) Requirements are derived from CCME 2007; 2) Toxicity data for amphibians is highly desirable but not required; 3) For semi-aquatic organisms, data must represent fully aquatic life stages.*
**** Low & no effect data:*** *preferred endpoints: EC/ICx representing a no-effect threshold > EC/IC10 > EC/IC11-25 > MATC > NOEC > LOEC > EC/IC26-49 > nonlethal* EC/IC_{50}.
***** Algae/Plants:*** *If a toxicity study indicates that a plant or algal species is among the most sensitive species in the data set, then this substance is considered to be phyto-toxic and three studies on nontarget freshwater plant or algal species are required.*

2.3 SPECIES SENSITIVITY DISTRIBUTION (SSD) – GUIDELINE DERIVATION

Two chloride toxicity SSDs were calculated (one short-term and one long-term) using data meeting the requirements outlined in Section 2.2. Because of data limitations, only chloride toxicity data from NaCl endpoints could be used in chronic SSD derivation. The long-term dataset for the other three chloride salts (KCl, $CaCl_2$, and $MgCl_2$) did not meet the minimum requirements for Type A (SSD) guideline derivation (CCME 2007). As a result, the complete guideline derivation presented here is based on NaCl data only. Similar data limitations also occurred in the CCME 2011 derivation where

KCl and $MgCl_2$ toxicity was completely excluded from the analysis. Their rational for this approach was based on evidence from a number of studies (Section 3.1) showing that the toxicity of these chloride salts stems from the cation rather than chloride (CCME 2011), thus establishing a Canadian WQG for chloride did not necessitate analysis of KCl and $MgCl_2$. For $CaCl_2$, the CCME (2011) cited evidence that NaCl and $CaCl_2$ possess similar toxicities (Section 3.1), which was used to provide the rational for combining the datasets for these two salts. The methods utilized here however are unique. Rather than combining the two datasets, an analysis of protectiveness was conducted to determine whether the guideline derived for NaCl was protective to all $CaCl_2$ toxicity endpoints available in the database (Section 7).

The methods utilized to establish both short- and long-term SSDs involved plotting the chloride effect concentrations for each species by the proportion of total species affected. A cumulative distribution function was then fitted to the data where the water quality guideline was defined as the chloride concentration corresponding to the 5th percentile of the total species affected. It was necessary to derive SSDs unadjusted for water hardness as additional endpoints were available in the current dataset compared to the CCME derivation conducted in 2011. As a result of this expanded dataset, it was expected that both the short- and long-term guidelines would differ from CCME (2011).

Only one endpoint per species may be plotted on an SSD, therefore multiple results for the same species were accounted for by following the methods outlined by the CCME (2011, 2007). The geometric mean of the effect concentrations was taken when multiple entries existed for the same species, endpoint, exposure duration, and lifestage. Data points were deemed inappropriate for averaging if any of these variables differed, or if environmental conditions across the relevant data points were not comparable. In addition, to reduce bias towards any one study, the arithmetic mean was used to represent multiple comparable endpoints for the same species reported from a single study. This methodology parallels the CCME approach (CCME 2011).

In some cases, there was more than one effect concentration available for a given species, but the endpoint, exposure duration, and/or lifestage differed (geometric mean could not be taken). In these cases the most sensitive data point was taken, or professional judgment was utilized to select a representative species effect concentration.

Following this selection procedure, each data point was ranked according to sensitivity and its position on the SSD determined using the Hazen plotting position represented by the following equation (Aldenberg *et al.* 2002, Newman *et al.* 2002):

$$HPP = (i - 0.5) / N \qquad \text{(Eq. 2.1)}$$

where:

HPP = Hazen plotting position
i = the species rank (ascending toxicity values)
N = the total number of species included in the SSD

The plotting position for a given species was interpreted as the proportion of species affected and these positional rankings, along with the corresponding effect concentrations were used to create the SSD.

Using Benchmark Dose Software (BMDS) version 2.40 (US EPA 2013a), six cumulative distribution functions (Gama, Logistic, Log-Logistic, Probit, Log-Probit, and Weibull) were fit to the Hazen plotted data. The most appropriate function was selected based on goodness-of-fit, model feasibility, and minimizing the scaled residuals. Using the selected model, a 5% Benchmark Dose (BMD), the concentration of chloride at which 5% of species are predicted to be affected, was determined for both the shot-term and long-term datasets along with a benchmark dose lower confidence limit (BMDL). This approach differed from the CCME (2011) methods in that, there, a customized Microsoft Excel-based software package, SSD Master Version 2.0 (Rodney *et al*., 2008) was used. With this approach five models were tested (Normal, Logistic, Gompertz, Weibull, and Fisher-Tippett).

Despite the limitations to the chronic datasets of KCl, $CaCl_2$, and $MgCl_2$. Sufficient acute data was available for KCl and $CaCl_2$ to calculate short-term SSDs. The data for $MgCl_2$ did not meet the short-term requirments outlined in Table 2.1 but despite this an SSD was constructed for comparative purposes. The same methods of SSD derivation outlined above were utilized. Results from this analysis allowed for a comparison of the relative toxicity of chloride under various cation pairings along with a comparison to relationships established in the literature (Section 3.1).

2.4 HARDNESS ADJUSTMENT – GUIDELINE DERIVATION

It is well known that water hardness ameliorates the toxicity of metals to aquatic organisms. The competing effect of hardness ions (Ca^{2+} and Mg^{2+}) towards metal ions for binding sites on cell membranes is a key component of the well-established Biotic Ligand Model used to predict metal toxicity (Paquin *et al.* 2002). Although the mechanism is less clear, growing evidence has demonstrated an inverse relationship between major ion toxicity and water hardness (Section 5.1). Various regulating bodies have begun to incorporate hardness amelioration into fresh water guidelines. The US EPA, the Great Lakes Environmental Centre (GLEC), and the Illinois Natural History Survey (INHS) in collaboration with the state of Iowa have developed an algorithm for the adjustment of a chloride water quality guidelines based on hardness (IDNR 2009a, 2009b). Within Canada, British Columbia has recently established a hardness adjusted guideline for the sulphate ion which draws (among other sources) from research conducted by Elphick et al. (2011a) and the Pacific Environmental Science Centre (PESC) (BCME 2013). Finally, site specific guidelines for chloride in Canada such as the EKATI and Snap Lake diamond mines (Northwest Territories) now incorporate a hardness ameliorating factor (Rescan 2007).

To explore potential relationships between chloride toxicity (short and long-term) and water hardness, data was compiled for species in which effect concentrations were available across a wide range of water hardness values. Two separate analyses were conducted using the short-term and long-term data sets. Log-log relationships were used to characterize hardness-toxicity relationships following the methods outlined by Stephan *et al.* (1985). An approach utilized by both the CCME and the U.S. Environmental Protection Agency (US EPA) in their respective hardness adjusted guidelines for cadmium (CCME 2014, US EPA 2001). This is also the preferred method to derive hardness adjusted guidelines for chloride (CCME 2011). In order for species data to be included, effect concentrations are required over a range of hardness such that the highest hardness is at least three times the lowest, and the highest is also at least 100 mg/L higher than the lowest (CCME 2014, US EPA 2001, Stephan *et al.* 1985). Data points that met these criteria across comparable endpoints, exposure durations, and life stages, were plotted together with Log(hardness) and Log(chloride effect concentration) as the independent (x-axis) and responding (y-axis) variables respectively. Slopes for the hardness-toxicity relationship were calculated for each species using linear regression.

To determine if a hardness-toxicity relationship could be compared across all species within the two data sets, an F-test was used to evaluate the null hypothesis that individual species slopes were not significantly similar to one another. Following the rejection of this null hypothesis, an analysis of covariance (ANCOVA) was conducted using the statistical software JMP 11 (SAS Institute *Inc.*) to calculate a pooled slope as per previously established methods (CCME 2014, US EPA 2001). The pooled slope is derived from a least squares regression analysis using a pooled data set, where every variable is adjusted relative to its mean. The slope of this regression line represents the best estimate of the toxicity-hardness relationship across all species included in the analysis (US EPA 2001). Following these statistical procedures, two pooled slopes were obtained based on the analysis of short- and long-term data.

The pooled slope values relating chloride toxicity to water hardness were used to adjust all toxicity data points used in the short-term and long-term SSDs to a standardized hardness of 50 mg/L. This relatively low hardness concentration was chosen as a starting point from which subsequent guidelines in harder water could be calculated. The equations to derive the short- and long-term hardness adjusted effect concentrations were as follows:

$$\mathbf{ST}\boldsymbol{EC}_{\boldsymbol{x}\mathbf{(50\ mg\ CaCO3\ /L)}} = \mathbf{10}^{\{\ (\ [\ \log(50)\ -\ \log(\text{hardness})\ ]\ \times\ \text{STPS})\ +\ \log(\text{STECx})\ \}} \qquad \textbf{\textit{(Eq. 2.2)}}$$

$$\mathbf{LT}\boldsymbol{EC}_{\boldsymbol{x}\mathbf{(50\ mg\ CaCO3\ /L)}} = \mathbf{10}^{\{\ (\ [\ \log(50)\ -\ \log(\text{hardness})\ ]\ \times\ \text{LTPS})\ +\ \log(\text{LTECx})\ \}} \qquad \textbf{\textit{(Eq. 2.3)}}$$

where:

ST$EC_{x(50\ mg\ CaCO3\ /L)}$:	a given short-term effect concentration normalized to 50 mg $CaCO_3$/L
ST*ECx:*	*a* given short-term effect concentration
STPS:	the pooled slope derived from short-term data
LT$EC_{x(50\ mg\ CaCO3\ /L)}$:	a given long-term effect concentration normalized to 50 mg $CaCO_3$/L
LT*ECx:*	a given short-term effect concentration
LTPS:	the pooled slope derived from long-term data

* hardness (permanent) measured as $CaCO_3$ equivalents

Two separate short- and long-term hardness adjusted SSDs were created for the 50 mg/L hardness adjusted data and the 5th percentile short-term benchmark and long-term guideline were calculated as outlined in Section 2.3. Using the 5th percentile chloride concentration (representing the y co-ordinate on the fitted line), a hardness of 50 mg/L (representing the x co-ordinate on the fitted line), and the pooled slope value (m), the equation could be solved for the y-intercept. In other words, if the equation log(y) = m·log(x) + b is rearranged to solve for b (i.e., the y-intercept), the following result is obtained: *b = log([Chloride $^{5th\%}$]) – ((pooled slope) x log(hardness))*. Therefore, the resulting equation to derive the short-term benchmark and long-term WQG concentrations were:

$$\textit{Short-term Benchmark} = 10^{\{ STPS\ (\log[hardness]) - STb \}} \qquad \textit{(Eq.2.4)}$$

$$\text{Long-term WQG} = 10^{\{ LTPS\ (\log[hardness]) - LTb \}} \qquad \textit{(Eq.2.5)}$$

where:

STPS = the pooled slope derived from short-term data
STb = the y-intercept derived from short-term data
LTPS = the pooled slope derived from long-term data
LTb = the y-intercept derived from short-term data
* hardness (permanent) measured as $CaCO_3$ equivalents

To assess the protectiveness of the short- and long-term hardness dependant guidelines, all acceptable chloride effect concentrations (log transformed and uncorrected for hardness) were plotted against log hardness. This analysis was conducted separately for the short- and long-term datasets where the respective benchmark and WQG were plotted as a straight lines. Any values occurring below these guidelines were examined in detail. This analysis was an important component of the overall guideline derivation as it incorporated $CaCl_2$ data which could not be assessed by Type A (SSD) methods. Including the $CaCl_2$ data here provided a measure of the effectiveness of the guidelines towards calcium chloride toxicity. The assessment of protectiveness is presented in Section 7 and is based on methods utilized by the CCME (2014).

3 ACUTE TOXICOLOGICAL GUIDELINE EXCLUDING HARDNESS

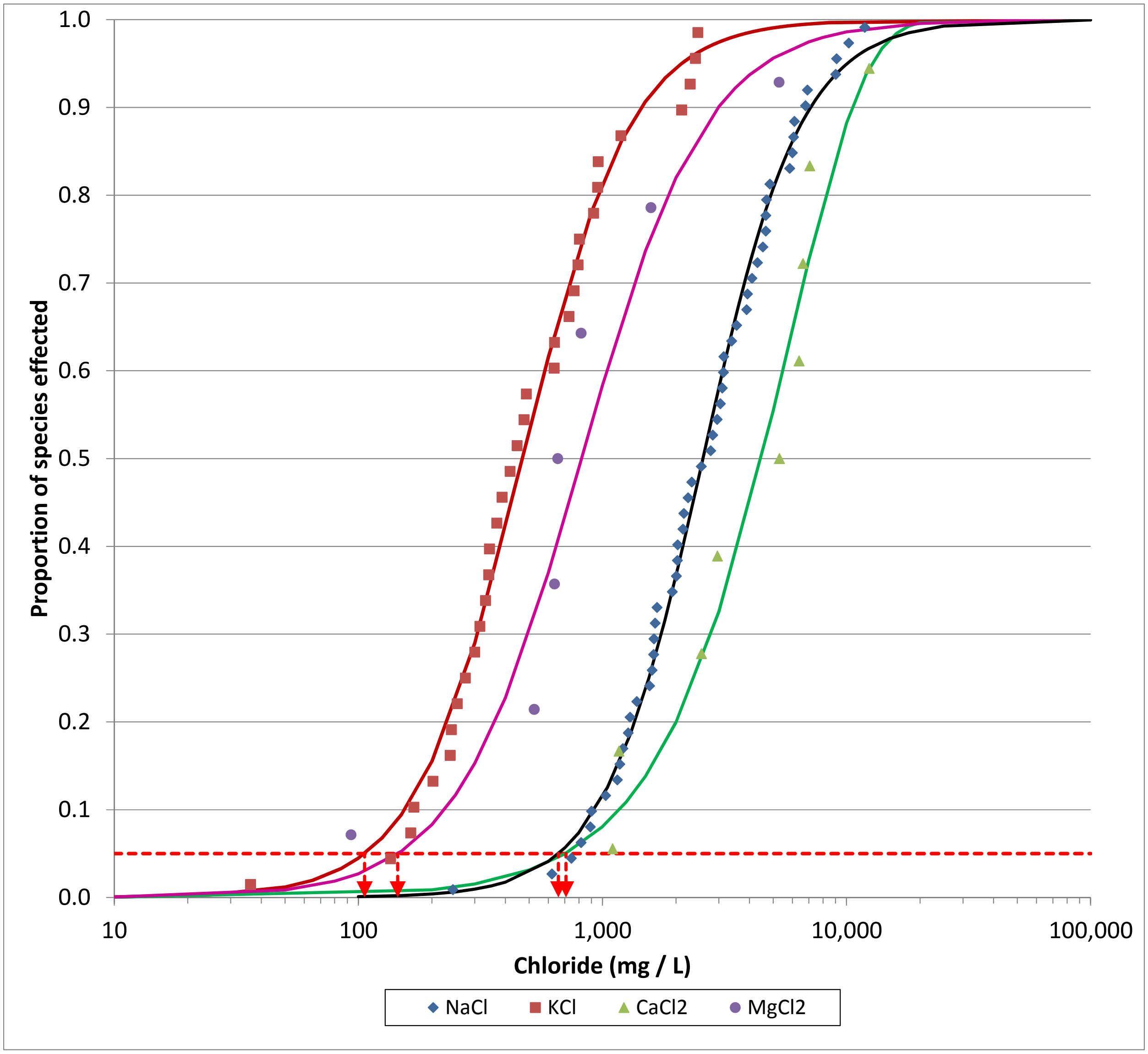
The following section outlines the results from toxicological analysis of the acute dataset. To determine the relative toxicity of the four major chloride salts (NaCl, KCl, $CaCl_2$, and $MgCl_2$), cumulative distribution functions are established and corresponding BMDs calculated. Determining the relative toxicity of these salts formed an important part of the analysis and allowed for comparison with published work on major ion toxicity. Subsequently, the derivation of the short-term benchmark concentration for chloride is outlined using data for NaCl toxicity. A detailed summary of the data utilized in the short-term SSD is provided along with an overview of the derived guideline.

3.1 COMPARATIVE TOXICITY OF CHLORIDE SALTS (NaCl, KCl, $CaCl_2$, $MgCl_2$)

In the natural environment, chloride occurs primarily as salts of sodium, potassium, calcium, and magnesium. Depending on the cation pairing, the toxicity of chloride to aquatic organisms has been shown to vary, which suggests that toxicity may be more related to the cation paired with chloride. To compare relative toxicity, SSDs for each salt were estimated based on the short-term severe effect (EC/LC_{50}) data collected in the database. Chloride effect concentrations are plotted against their corresponding Hazen plotting position in Figure 3.1. Associated 5 % BMDs and BMDLs were calculated and are presented in Table 3.1. All of the data points utilized to construct the short-term SSDs for potassium, calcium, and magnesium chloride are presented in Appendix C. The data points making up the sodium chloride SSD are examined in detail in Section 3.2.

The data for $MgCl_2$ did not meet the CCME (2007) minimum protocols for short-term SSD derivation. More specifically, it did not meet the requirement for three different fish species (at least one of which is a salmonid and one of which is a non-salmonid). Only a single LC_{50} for *Pimephales promelas* of 1,579 mg Cl^- /L was available (Mount *et al.* 1997). Although not meeting the SSD guideline derivation requirements, it was assumed that the $MgCl_2$ data was sufficient for comparative purposes.

Figure 3.1. Comparative toxicity of the common salts of chloride – severe endpoints (EC/LC_{50})



***Notes**: 1) estimated SSDs using cumulative distribution models (blue: NaCl, red: KCl, green: $CaCl_2$, purple: $MgCl_2$); 2) $MgCl_2$ does not meet the minimum data requirements for short-term SSD derivation as outlined in CCME (2007).*

Table 3.1. Derived BMDs for various chloride salts – severe endpoints (EC/LC_{50})

Salt	BMD (mg Cl-/L)[A]	BMDL (mgCl- /L)[B]	Best-fit model
KCl	**106**	102	Log-Logistic
***$MgCl_2$**	**145**	128	Log-Logistic
NaCl	**659**	645	Log-Logistic
$CaCl_2$	**703**	591	Weibull

[A] ***BMD:*** *Benchmark Dose, the salt concentration corresponding to the 5th percentile of species effected*
[B] ***BMDL:*** *Benchmark Does Lower confidence limit*
***** *$MgCl_2$ did not meet the minimum data requirements for short-term SSD derivation as outlined in CCME (2007).*

Figure 3.1 demonstrates the important effect corresponding cations have on chloride toxicity. Based on the overall SSDs, KCl was the most toxic salt and approximately an order of magnitude more toxic compared to NaCl. Although the regressions for $CaCl_2$ and especially $MgCl_2$ suffer from data limitations, it appears that the relative toxicity of the salt pairings is:

KCl > $MgCl_2$ > NaCl ≈> $CaCl_2$

Published research supports this ordering of relative chloride salt toxicity. For the diatom *Nitzschia linearisl,* 120 h EC_{50} (50% reduction in cell number) values varied from 637, 1474, and 2000 mg Cl^-/L, in solutions containing KCl, NaCl, and $CaCl_2$ respectively. Waller *et al.* (1996) demonstrated an 80% mortality in yellow perch exposed to 2,500 mg KCl/L (1,189 mg Cl^- /L) over a 24 h timeframe, whereas 10,000 mg/L of $CaCl_2$ (6,389 mg Cl^- /L) and NaCl (6,066 mg Cl^- /L) was required to cause 83% and 0% mortality respectively. Working with the flatworm *Polycelis nigra*, Jones (1940, 1941) found decreasing toxicity in 48 h exposures to KCl (317 mg Cl^- /L), $MgCl_2$ (2,830 mg Cl^- /L), $CaCl_2$ (4,600 mg Cl^- /L), and NaCl (6,736 mg Cl^- /L) respectively. Khangarot and Ray (1989) investigated the toxicity of NaCl, $CaCl_2$, and KCl to the water flea *D. magna* and found respective 48 h LC_{50} values of 621 mg Cl^- /L, 686 mg Cl^- /L, and 135 mg Cl^- /L, suggesting again the relatively toxicity of K > Na > Ca chloride salts. Since 2001, two reviews have also identified cation relationship in chloride toxicity. Work stemming from the Canadian Environmental Protection Act 1999 (Evans and Frick 2001) and a report to the British Columbia Ministry of Water, Land and Air Protection (Bright and Addison 2002) found that cation pairings effected toxicity as follows: KCl > $MgCl_2$ > $CaCl_2$ > NaCl. These results suggest a slightly different ordering in which $CaCl_2$ is more toxic than NaCl; however the most toxic salts were still found to be KCl followed by $MgCl_2$. The body of evidence presented above, along with the results generated herein, are generally consistent with an extensive evaluation of major ion toxicity conducted by Mount *et al.* (1997).

In the CCME (2011) guideline derivation for chloride, the work of Mount *et al.* (1997) was cited as the primary justification for grouping the toxicity data of NaCl with $CaCl_2$. It was reasoned that the toxicity of these two salts was due to the chloride ion, thus this data could be grouped and analysed together. However, $CaCl_2$ formed a relatively minor component of the CCME dataset with only three values utilized in short-term benchmark derivation (Baudouin and Scoppa (1974) 48h LC_{50} for *Daphnia hyaline*, *Eudiaptomus padanus padanus*, and *Cyclops abyssorum prealpinus*) and no values used in long-term WQG derivation. With this in mind, and the fact that calcium is associated with potential ameliorating effects on acute toxicity via water hardness and cell membrane permeability (Eddy 1975, Pic and Maetz 1981, Potts and Fleming 1970, Penttinen *et al.* 1998, Robertson 1941), it was analysed separately from the sodium chloride data. To ensure that the derived sodium chloride based guideline provides adequate protection for calcium chloride toxicity, an analysis of protectiveness (Section 7) is applied to all toxicity endpoints available in the database for NaCl and $CaCl_2$.

3.2 SHORT-TERM SPECIES SENSITIVITY DISTRIBUTION (SSD)

In total, 502 acute data points were obtained for sodium chloride across a wide range of aquatic organisms. Of these, a number were deemed unacceptable for guideline derivation or did not meet the requirements of short-term data (CCME 2007). From the remaining data points, a number were omitted or averaged so that only one data point represented a single species. In cases where multiple comparable endpoints (*i.e.* same species, duration, lifestage *etc.*) were available for a single species, arithmetic (intra-study comparisons) and geometric means (inter-study comparisons) were taken (see Section 2 for full discussion). The short-term SSD comprised 56 species , with values ranging from a 24 h EC_{50} (glochidia survival) of 244 mg Cl^-/L for the freshwater mussel *Epioblasma torulosa rangiana* to a 96 h LC_{50} of 11,881 mg Cl^- /L for the American eel (*Anguilla rostrata*) Table 3.2.

Table 3.2. EC/LC_{50} (severe endpoints) used in the derivation of the short-term SSD

Species	Common name	Endpoint	Endpoint criteria	EC/LC_{50} (mg Cl^- /L)	HPP [A]	Citation
Epioblasma torulosa rangiana	Freshwater mussel	24h EC_{50}	Glochidia survival	244	0.0089	Gillis (2011)
Daphnia magna	Water flea	48h EC_{50}	No heart beat	621	0.0268	Khangarot & Ray(1989)
Lampsilis fasciola	Freshwater mussel	24h LC_{50}	Glochidia survival	746	0.0446	***MEAN VALUE**
Lampsilis cardium	Freshwater mussel	24h EC_{50}	Glochidia survival	817	0.0625	Gillis (2011)
Lampsilis siliquoidea	Fatmucket Clam	24h EC_{50}	Glochidia survival	892	0.0804	***MEAN VALUE**
Sphaerium simile	Fingernail clam	96h LC_{50}	Mussel mortality	902	0.0982	***MEAN VALUE**
Villosa iris	Freshwater mussel	24h EC_{50}	Glochidia survival	1,031	0.1161	Pandolfo_etal (2012)
Ceriodaphnia dubia	Water Flea	48h LC_{50}	No visible movement and no response to prodding	1,151	0.1339	***MEAN VALUE**
Ambystoma maculatum	Spotted salamander	96h LC_{50}	Mortality	1,178	0.1518	Collins & Russell (2009)
Daphnia ambigua	Water flea	48h LC_{50}	Imobilization	1,213	0.1696	Harmon_etal (2003)
Elliptio lanceolata	Yellow lance	96h LC_{50}	Glochidia survival	1,274	0.1875	Wang & Ingersoll (2010)
Brachionus patulus	Rotifer	24h LC_{50}	Mortality	1,298	0.2054	Peredo-Alvarez_etal (2003)
Hyalella azteca	Amphipod	96h LC_{50}	Survival	1,382	0.2232	Elphick_etal (2011a)
Hydra attenuata	Hydra	96h LC_{50}	Mortality	1,559	0.2411	Santos_etal(2007)
Daphnia pulex	Water flea	48h LC_{50}	Mortaility	1,597	0.2589	***MEAN VALUE**
Elliptio complanata	Freshwater mussel	24h EC_{50}	Glochidia survival	1,620	0.2768	Bringolf_etal (2007)
Epioblasma brevidens	Freshwater mussel	24h LC_{50}	Glochidia survival	1,626	0.2946	Valenti_etal (2007)
Epioblasma capsaeformis	Freshwater mussel	24h LC_{50}	Glochidia survival	1,644	0.3125	Valenti_etal (2007)
Villosa constricta	Freshwater mussel	24h EC_{50}	Glochidia survival	1,674	0.3304	Bringolf_etal (2007)

Table 3.2. EC/LC50 (severe endpoints) used in the derivation of the short-term SSD

Species	Common name	Endpoint	Endpoint criteria	EC/LC50 (mg Cl^- /L)	HPP [A]	Citation
Musculium transversum	Fingernail clam	96h LC_{50}	Mussel mortality	1,930	0.3482	Soucek_etal (2011)
Villosa delumbis	Freshwater mussel	24h EC_{50}	Glochidia survival	2,008	0.3661	Bringolf_etal (2007)
Brachionus calyciflorus	Rotifer	24h LC_{50}	Survival	2,027	0.3839	***MEAN VALUE**
Tricorythus sp.	Mayfly	96h LC_{50}	Mortality	2,033	0.4018	Goetsch & Palmer (1997)
Pycnopsyche sp.	Caddisfly	96h LC_{50}	Mortality	2,139	0.4196	Blasius & Merritt (2002)
Hyla chrysoscelis	Cope's gray tree frog	96h LC_{50}	Mortality	2,156	0.4375	***MEAN VALUE**
Isonychia bicolor	Mayfly	96h LC_{50}	Mortality	2,245	0.4554	Echols_etal (2009)
Pseuacris triseriata	Chorus frog	96h LC_{50}	Mortality	2,320	0.4732	Garibay&Hall(2007)
Physa gyrina	Freshwater snail	96h LC_{50}	Mortality	2,540	0.4911	Birge_etal(1985)
Rana clamitans	Green Frog	96h LC_{50}	Mortality	2,776	0.5089	***MEAN VALUE**
Pseudacris crucifer	Spring Peeper	96h LC_{50}	Mortality	2,830	0.5268	Collins&Russell(2009)
Lirceus fontinalis	Isopod	96h LC_{50}	Mortality	2,950	0.5446	Birge_etal(1985)
Gyraulus parvus	Freshwater snail	96h LC_{50}	Mortality	3,043	0.5625	***MEAN VALUE**
Lithobates sylvaticus	Wood frog	96h LC_{50}	Mortality	3,096	0.5804	***MEAN VALUE**
Baetis tricaudatus	Mayfly	48h EC_{50}	Immobilization	3,130	0.5982	***MEAN VALUE**
Rana temporaria	Common frog	96h $LC_{47.6}$	Mortality	3,140	0.6161	Viertel(1999)
Lithobates pipiens	Northern Leopard frog	96h LC_{50}	Mortality	**3,385	0.6339	Doe(2010)
Chironomus xanthus	Midge	96h LC_{50}	Mortality	3,543	0.6518	Santos_etal(2007
Lemna minor	Common duckweed	48h EC_{50}	Mortality	3,900	0.6696	Simmons(2012)
Anaxyrus americanus	American toad	96h LC_{50}	Mortality	3,926	0.6875	Collins&Russell(2009)
Lumbriculus variegatus	California blackworm	96h LC_{50}	Survival	4,094	0.7054	***MEAN VALUE**
Nephelopsis obscura	Leech	96h LC_{50}	Mortality	4,310	0.7232	Environ (2009)
Pimephales promelas	Fathead minnow	96h LC_{50}	No visible movement and no reponse to prodding	4,543	0.7411	***MEAN VALUE**
Gammarus pseudolimnaeus	Amphipod	96h LC_{50}	Mortality	4,671	0.7589	Blasius&Merritt(2002)
Hexagenia spp.	Mayfly	48h LC_{50}	Mortality	4,671	0.7768	Wang&Ingersoll(2010)
Chironomus dilutus / tentans	Midge	96h LC_{50}	Mortality	4,697	0.7946	***MEAN VALUE**
Chironomus attenatus	Midge	48h LC_{50}	Mortality	4,850	0.8125	Thornton&Sauer(1972)
Rana catesbeiana	Bullfrog	96h LC_{50}	Mortality	5,846	0.8304	Environ (2009)
Lepidostoma sp.	Caddisfly	96h LC_{50}	Mortality	6,000	0.8482	Williams_etal(1999)
Cyprinella leedsi	Bannerfin Shiner	96h LC_{50}	Mortality	6,070	0.8661	Environ (2009)
Tubifex tubifex	Tubificid worm	96h LC_{50}	Survival	6,119	0.8839	***MEAN VALUE**

Table 3.2. EC/LC$_{50}$ (severe endpoints) used in the derivation of the short-term SSD

Species	Common name	Endpoint	Endpoint criteria	EC/LC$_{50}$ (mg Cl$^-$/L)	HPP [A]	Citation
Lepomis macrochirus	Common bluegill	96h LC$_{50}$	Immobilization	6,790	0.9196	***MEAN VALUE**
Chironomus riparius	Midge	48h LC$_{50}$	Mortality	6,912	0.9018	Wang&Ingersoll(2010)
Oncorhynchus mykiss	Rainbow trout	96h LC$_{50}$	Survival	9,033	0.9375	***MEAN VALUE**
Gambusia affinis	Mosquito fish	96h LC$_{50}$	Mortality	9,099	0.9554	Al-Daham&Bhatti(1977)
Gasterosteus aculeatus	Three-spined stickleback	96h LC$_{50}$	Mortality	10,200	0.9732	Garibay&Hall(2007)
Anguilla rostrata	American eel	96h LC$_{50}$	Mortality	11,881	0.9911	***MEAN VALUE**

[A] ***HPP:*** *Hazen plotting position. See Section 2 for description*
**** MEAN VALUE:*** *data shown is the mean value taken from multiple studies. Intra-study comparisons use arithmetic value, inter-study comparisons use geometric mean. Raw values used presented in Table 3.2.*
***This value was reported as 3,385 mg Cl⁻/L in SSD derivation but as 3,397 mg Cl⁻/L in Appendix I (CCME 2011). Since the orignianl publication was not avalible, the smaller more conservative value is used here.*

Table 3.3. Raw data used in the derivation of short-term SSD averaged data points

Species	Common name	Endpoint	Effect conc. (mg Cl$^-$/L)	Arithmetic mean[A]	Geometric mean	Citation
Lampsilis fasciola	Freshwater mussel	24 h EC$_{50}$	1,868		**746**	Valenti_etal(2007)
Lampsilis fasciola	Freshwater mussel	24 h EC$_{50}$	1,116			Bringolf_etal(2007)
Lampsilis fasciola	Freshwater mussel	24 h EC$_{50}$	113	199		Gillis(2011)
Lampsilis fasciola	Freshwater mussel	24 h EC$_{50}$	285			Gillis(2011)
Lampsilis siliquoidea	Fatmucket Clam	24 h EC$_{50}$	1,213	1426	**892**	Cope_etal(2008)
Lampsilis siliquoidea	Fatmucket Clam	24 h EC$_{50}$	1,638			Cope_etal(2008)
Lampsilis siliquoidea	Fatmucket Clam	24 h EC$_{50}$	334			Bringolf_etal(2007)
Lampsilis siliquoidea	Fatmucket Clam	24 h EC$_{50}$	1,430	1491		Gillis(2011)
Lampsilis siliquoidea	Fatmucket Clam	24 h EC$_{50}$	763			Gillis(2011)
Lampsilis siliquoidea	Fatmucket Clam	24 h EC$_{50}$	1,430			Gillis(2011)
Lampsilis siliquoidea	Fatmucket Clam	24 h EC$_{50}$	1,962			Gillis(2011)
Lampsilis	Fatmucket Clam	24 h	1,870			Gillis(2011)

Table 3.3. Raw data used in the derivation of short-term SSD averaged data points

Species	Common name	Endpoint	Effect conc. (mg Cl^-/L)	Arithmetic mean[A]	Geometric mean	Citation
siliquoidea		EC_{50}				
Sphaerium simile	Fingernail clam	96 h LC_{50}	740		**902**	Soucek_etal(2011)
Sphaerium simile	Fingernail clam	96 h LC_{50}	1,100			Soucek_etal(2011)
Ceriodaphnia dubia	Water Flea	48 h LC_{50}	1,189		**1151**	Mount_etal(1997)
Ceriodaphnia dubia	Water Flea	48 h LC_{50}	1,068			Elphick_etal (2011a)
Ceriodaphnia dubia	Water Flea	48 h LC_{50}	507	477		Hoke_etal(1992)
Ceriodaphnia dubia	Water Flea	48 h LC_{50}	447			Hoke_etal(1992)
Ceriodaphnia dubia	Water Flea	48 h LC_{50}	977	1385		Soucek_etal(2011)
Ceriodaphnia dubia	Water Flea	48 h LC_{50}	861			Soucek_etal(2011)
Ceriodaphnia dubia	Water Flea	48 h LC_{50}	1,249			Soucek_etal(2011)
Ceriodaphnia dubia	Water Flea	48 h LC_{50}	1,402			Soucek_etal(2011)
Ceriodaphnia dubia	Water Flea	48 h LC_{50}	1,589			Soucek_etal(2011)
Ceriodaphnia dubia	Water Flea	48 h LC_{50}	1,779			Soucek_etal(2011)
Ceriodaphnia dubia	Water Flea	48 h LC_{50}	1,836			Soucek_etal(2011)
Ceriodaphnia dubia	Water Flea	48 h LC_{50}	1,600	1648		Tietge_etal(1997)
Ceriodaphnia dubia	Water Flea	48 h LC_{50}	1,696			Tietge_etal(1997)
Ceriodaphnia dubia	Water Flea	48 h LC_{50}	1,395			Cowgill&Milazzo(1990)
Ceriodaphnia dubia	Water Flea	48 h LC_{50}	1,347			Echols_etal(2012)
Ceriodaphnia dubia	Water Flea	48 h LC_{50}	964			Harmon_etal(2003)
Ceriodaphnia dubia	Water Flea	48 h LC_{50}	1,413			Valenti_etal(2007)
Daphnia pulex	Water flea	48 h LC_{50}	2,042		**1597**	Gardner&Royer(2010)
Daphnia pulex	Water flea	48 h LC_{50}	1,504	1241		Robison(2011)
Daphnia pulex	Water flea	48 h LC_{50}	1,134			Robison(2011)
Daphnia pulex	Water flea	48 h LC_{50}	1,341			Robison(2011)
Daphnia pulex	Water flea	48 h LC_{50}	1,068			Robison(2011)
Daphnia pulex	Water flea	48 h LC_{50}	1,159			Robison(2011)
Daphnia pulex	Water flea	48 h LC_{50}	1,159	1745		Palmer_etal (2004)
Daphnia pulex	Water flea	48 h LC_{50}	1,775			Palmer_etal (2004)
Daphnia pulex	Water flea	48 h LC_{50}	1,805			Palmer_etal (2004)
Daphnia pulex	Water flea	48 h LC_{50}	2,242			Palmer_etal (2004)
Daphnia pulex	Water flea	48 h LC_{50}	*1,470			Birge_etal(1985)
Brachionus calyciflorus	Rotifer	24 h LC_{50}	1,645		**2027**	Elphick_etal (2011a)
Brachionus calyciflorus	Rotifer	24 h LC_{50}	2,223			Calleja_etal(1994)
Brachionus calyciflorus	Rotifer	24 h LC_{50}	2,277			Peredo-Alvarez_etal(2003)
Hyla chrysoscelis	Cope's gray tree	96 h LC_{50}	1,855		**2156**	Brown_etal(2012)

Table 3.3. Raw data used in the derivation of short-term SSD averaged data points

Species	Common name	Endpoint	Effect conc. (mg Cl^-/L)	Arithmetic mean[A]	Geometric mean	Citation
	frog					
Hyla chrysoscelis	Cope's gray tree frog	96 h LC_{50}	1,869			Brown_etal(2012)
Hyla chrysoscelis	Cope's gray tree frog	96 h LC_{50}	2,174			Brown_etal(2012)
Hyla chrysoscelis	Cope's gray tree frog	96 h LC_{50}	2,167			Brown_etal(2012)
Hyla chrysoscelis	Cope's gray tree frog	96 h LC_{50}	1,871			Brown_etal(2012)
Hyla chrysoscelis	Cope's gray tree frog	96 h LC_{50}	2,316			Brown_etal(2012)
Hyla chrysoscelis	Cope's gray tree frog	96 h LC_{50}	3,055			Brown_etal(2012)
Rana clamitans	Green Frog	96 h LC_{50}	2,479		**2776**	Brown_etal(2012)
Rana clamitans	Green Frog	96 h LC_{50}	3,109			Collins&Russell(2009)
Gyraulus parvus	Freshwater snail	96 h LC_{50}	3,078		**3043**	Soucek_etal(2011)
Gyraulus parvus	Freshwater snail	96 h LC_{50}	3,009			Soucek_etal(2011)
Lithobates sylvaticus	Wood frog	96 h LC_{50}	3,099		**3096**	Sanzo&Hecnar(2006)
Lithobates sylvaticus	Wood frog	96 h LC_{50}	3,755			Jackman(2010)
Lithobates sylvaticus	Wood frog	96 h LC_{50}	4,586			Harless_etal(2011)
Lithobates sylvaticus	Wood frog	96 h LC_{50}	1,721			Collins&Russell(2009)
Baetis tricaudatus	Mayfly	48 h EC_{50}	2,875		**3130**	Lowell_etal(1995)
Baetis tricaudatus	Mayfly	48 h EC_{50}	3,233			Lowell_etal(1995)
Baetis tricaudatus	Mayfly	48 h EC_{50}	3,300			Lowell_etal(1995)
Lumbriculus variegatus	California blackworm	96 h LC_{50}	3,100		**4094**	Elphick_etal (2011a)
Lumbriculus variegatus	California blackworm	96 h LC_{50}	5,408			Environ (2009)
Pimephales promelas	Fathead minnow	96 h LC_{50}	3,876		**4543**	Mount_etal(1997)
Pimephales promelas	Fathead minnow	96 h LC_{50}	4,079			Elphick_etal (2011a)
Pimephales promelas	Fathead minnow	96 h LC_{50}	3,740	4014		Tietge_etal(1997)
Pimephales promelas	Fathead minnow	96 h LC_{50}	4,288			Tietge_etal(1997)
Pimephales promelas	Fathead minnow	96 h LC_{50}	4,640			Adelman_etal(1976)
Pimephales promelas	Fathead minnow	96 h LC_{50}	6,570			Birge_etal(1985)
Chironomus dilutus	Midge	96 h LC_{50}	5,867		**4697**	Elphick_etal (2011a)
Chironomus dilutus	Midge	96 h LC_{50}	3,761			Wang&Ingersoll(2010)
Tubifex tubifex	Tubificid worm	96 h LC_{50}	5,648		**6119**	Elphick_etal (2011a)

Table 3.3. Raw data used in the derivation of short-term SSD averaged data points

Species	Common name	Endpoint	Effect conc. (mg Cl^-/L)	Arithmetic mean[A]	Geometric mean	Citation
Tubifex tubifex	Tubificid worm	96 h LC_{50}	4,278	5143		Soucek_etal(2011)
Tubifex tubifex	Tubificid worm	96 h LC_{50}	6,008			Soucek_etal(2011)
Tubifex tubifex	Tubificid worm	96 h LC_{50}	7,886			Wang&Ingersoll(2010)
Lepomis macrochirus	Common bluegill	96 h LC_{50}	7,853		**6790**	Trama_(1954)
Lepomis macrochirus	Common bluegill	96 h LC_{50}	*5,870			Birge_etal(1985)
Oncorhynchus mykiss	Rainbow trout	96 h LC_{50}	6,030		**9033**	Elphick_etal (2011a)
Oncorhynchus mykiss	Rainbow trout	96 h LC_{50}	9,886			Dow_etal(2010)
Oncorhynchus mykiss	Rainbow trout	96 h LC_{50}	12,363			Vosyliene_etal(2006)
Anguilla rostrata	American eel	96 h LC_{50}	10,847		**11881**	Hinton&Eversole(1978)
Anguilla rostrata	American eel	96 h LC_{50}	13,012			Hinton&Eversole(1979)

[A] ***Arithmetic mean:*** *the geometric mean was preferentially taken. Arithmetic means were used to collate multiple data points for a single study when multiple studies were available for a species. This ensured that no one study had a greater influence on the derived SSD value.*
** These values were incorrectly entered as 892 and 3,543mg Cl^-/L in CCME (2011). The correct values of 1,470 and 5,870 mg Cl^-/L for D. pulex and L. macrochirus are utilized here.*

3.2.1 Evaluation of Short-Term Toxicological Data

This section summarizes important data points for four taxonomic groups (fish, invertebrates, amphibians, and plants/algae), and outlines situations where professional judgment was required to arrive at the SSD effect concentration presented in Table 3.2. Note that this section deals only with data used to establish the short-term SSD.

Short-term toxicity to fish

Acute chloride toxicity data was collected for 35 species of fish and is presented in Appendix A. Of these species, 96 h LC_{50} values from seven species were included in the short-term SSD. The other fish species were excluded from the analysis as the data did not meet requirments (*e.g.* less than four day exposure durations, species native to tropical climates, inappropriate endpoints such as LC_0 / LC_{100}). Fish were found to be quite tolerant of high chloride exposures over acute timeframes. The most sensitive species was found to be the Fathead minnow (*P. promelas*), with a 96h LC_{50} of 3,386 mg Cl^- /L (Mount *et al.* 1997) and a collated SSD value of 4,543 mg Cl^- /L (Table 3.3). The greatest tolerance to chloride was found for the catadromous American eel (*A. rostrata*), with a 96 h LC_{50} of 13,012 mg Cl^- /L. This species was the most tolerant organism across the entire short-term SSD. The species included in the short-term SSD match those used by the CCME (2011), however values for half of these (*P. promelas*, *L. macrochirus*, and *O. mykiss*) differ since additional data was utilized during data collation. It is also important to note that an LC_{50} for *L. macrochirus* was entred incorrectly in the CCME (2011) guideline derivation. There they used a value of 3,543 mg Cl^- /L, however after careful consideration and examination of the original paper (Birge *et al.* 1985), the value of of 5,840 mg Cl^- /L was utilized. This value is also in better agreement with the other LC_{50} value avalible for *L. macrochirus* of 7,853 mg Cl^- /L (Trama 1954).

Short-term toxicity to amphibians

Ten species of amphibians were included in the short-term SSD. As with the fish, all endpoints were 96 h LC_{50} values. Acute chloride toxicity data from six species was excluded from the analysis due to various reasons such as exposure durations being more than four days, issues with experimental conditions, or where species were native to tropical regions. Similar to the CCME (2011) findings, the most sensitive species was the spotted salamander (*A. maculatum*) with a 96 h LC_{50} of 1,178 mg Cl^- /L and the most tolerant species was the bullfrog (*R. catesbeiana*) with a 96 h LC_{50} of 5,846 mg Cl^- /L (Environ 2009). The current amphibian dataset is similar to that used in the CCME (2011), however, a 2,156 mg Cl^- /L 96 h LC_{50} for the Cope's grey tree frog (*H. chrysoscelis*) is included from a 2012 study conducted by Brown *et al.* Additionally, LC_{50} values for the chorus (*P. triseriata*), wood (*L. sylvatica*), and green (*R. clamitans*) frogs differ from those derived by the CCME (2011) due to the availability of additional information and subsequent averaging.

Short-term toxicity to invertebrates

For the short-term SSD, invertebrates were represented by 38 data points which included 3 additions to the 35 used in the CCME (2011) dataset. The additions included the hydra *H. attenuate* with a 96 h LC_{50} of 1,559 mg Cl^- /L (Santos *et al.* 2007), a mayfly (*Tricorythus* sp.) with a 96 h LC_{50} of 2,033 mg Cl^- /L, and a midge (*C. xanthus*) with a 96 h LC_{50} of 3,543 mg Cl^- /L. In general, invertebrates are more sensitive to acute chloride exposures when compared to vertebrates and make up all of the values below the 15th percentile. These sensitive data points remain similar to those used in the CCME (2011) guideline derivation with the exception of *L. siliquoidea*, *V. iris*, and *C. dubia* where values have been updated with either more recent data (*V. iris:* Pandolfo *et al.* 2012; *C. dubia*: Echols *et al.* 2012) or with data from studies not included in the CCME (2011) derivation (*C. dubia*: Tietge *et al.* 1997, Harmon *et al.* 2003; *L. siliquoidea*: Cope *et al.* 2008).
Bivalves made up a large proportion of the most sensitive species (6 of the 8 most sensitive species) and included the COSEWIC designated special concern wavy-rayed lampmussel (*L. fasicola*) with a 24 h LC_{50} (glochidia) of 746 mg Cl^- /L (Valenti *et al.* 2007, Bringolf *et al.* 2007, Gillis 2011) and the endangered rainbow mussel (*V. iris*) with a 24 h LC_{50} (glochidia) of 1,031 mg Cl^- /L (Pandolfo *et al.* 2012). This new data from Pandolfo *et al.* (2012) resulted in a lower 24h EC_{50} (glochidia survival) compared to the CCME (2011) derivation. The northern riffleshell mussel (*E. torulosa rangiana*) with a 24 h LC_{50} (glochidia) of 244 mg Cl^- /L was the most sensitive species in the SSD. This species has been designated as endangered by COSEWIC. Further information on freshwater bivalve chloride sensitivities, glochidia lifestage endpoints, and life history characteristics, can be found in CCME (2011).

The four water flea species *D. magna*, *C. dubia*, *D. ambigua*, and *D. pulex* had effect concentrations at or below the 20th percentile of the SSD with 48 h LC_{50} values of 621, 1,151, 1,213, and 1,597 mg Cl^- /L respectively. Along with the bivalves, water fleas were some of the most sensitive species to short-term chloride exposures. *D. magna* was the second most sensitive species to acute chloride exposures. Derived values for *C. dubia* were higher than estimates in the CCME derivation, due to the addition of data from Harmon *et al.* (2003), Tietge *et al.* (1997), and Echols *et al.* (2012). The derived 48 h LC_{50} for *D. pulex* is also higher than estimates in CCME (2011). This is due to the addition of data from Gardner and Royer (2010) and Robison (2011). In addition, an LC_{50} for *D. pulex* was entered incorrectly in the CCME (2011) guideline derivation where a value of 892 mg Cl^- /L was used. After careful consideration and examination of the original paper (Birge *et al.* 1985), the value of 1,470 mg Cl^- /L was utilized as opposed to the value used by CCME. This value is also in better agreement with the other LC_{50} values avalible for *D. pulex* (Table 3.3).

3.2.2 Derivation of the Short-Term SSD and Benchmark Concentration

The SSD chloride concentrations (Table 3.2) were plotted against their corresponding Hazen plotting position values. Cumulative distribution functions were then fitted to the data using the maximum likelihood approach of the BMDS software (US EPA 2013a). The best model was selected based on goodness -of-fit and model feasibility. Using BMDS software, the Log-Logistic Function provided the best overall fit for the data with the lowest AIC (Akaike's Information Criteria) value. This model also minimized the scaled residuals of each of the data points. The equation of the model was:

$$y = 1 / [1 + e^{-(b + m * \ln(x))}] \qquad \textbf{(Eq. 3.1)}$$

Where y is the proportion of species affected and x is the chloride concentration. The slope of the line (m) and the y-intercept (b) were calculated to be **2.16** and **-16.97** respectively. The Log-Probit model also provided a good fit to the data.

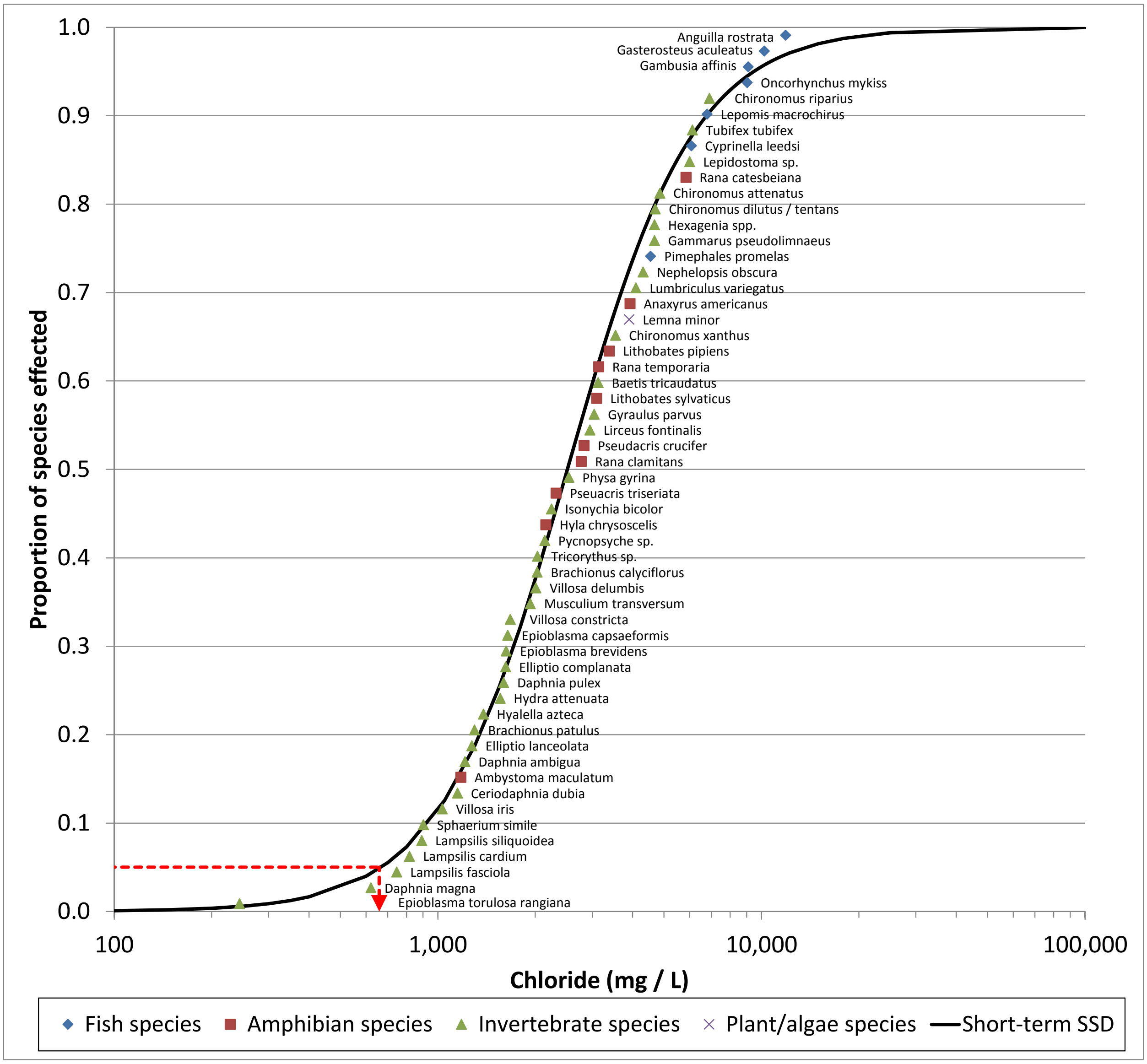
Figure 3.2 shows the short-term SSD made up of the EC/LC_{50} (severe endpoints) chloride concentrations and the corresponding Hazen plotting position values (Table 3.2), along with the fitted Log-Logistic function (Eq. 3.1). The short-term benchmark concentration, defined as the 5th percentile along the derived Log-Logistic function was **659** mg/L chloride with a lower-bound confidence limit of **645** mg/L chloride.

Compared to CCME (2011) the short-term benchmark concentration derived here is approximately 20 mg/L higher (CCME value = 640 mg/L). This difference likely stems from changes to species data points occurring low on the SSD. For instance, because of greater data availability, values for *L. siliquoidea, C. dubia*, and *D. pulex* were updated from 709 to 892 mg Cl^- /L; 1080 to 1151 mg Cl^- /L, and 1248 to 1597 mg Cl^- /L respectively.

Since the methods used in the current SSD derivation differed from those used by the CCME (2011), the CCME dataset was analysed using the BMDS software for comparative purposes. Using the

Log-Logistic function on the CCME dataset, the derived short-term guideline was 604 mg Cl^- /L, 36 mg Cl^- /L lower than the value derived by the CCME (2011).

Figure 3.2. Short-term SSD – severe endpoints (EC/LC$_{50}$) for NaCl



Notes:*1) SSD derived by plotting severe (EC/LC$_{50}$) chloride effect concentrations for 56 species of aquatic organism against their corresponding Hazen plotting position (HPP); 2) Red hashed line indicates the 5th percentile (y-axis) and the resulting short-term benchmark concentration (x-axis) of 659 mg Cl^-/L.*

4 CHRONIC TOXICOLOGICAL EFFECTS EXCLUDING HARDNESS

The following section outlines the results from toxicological analysis of the chronic dataset. The derivation of the long-term WQG for chloride is outlined using data for NaCl toxicity. A detailed summary of the data utilized in the long-term SSD is provided along with an overview of the derived guideline.

4.1 LONG-TERM SPECIES SENSITIVITY DISTRIBUTION (SSD)

In total, 455 chronic data points were obtained for sodium chloride across a wide range of aquatic organisms. and 38 of these were deemed unacceptable for guideline derivation based on number of reasons (see Section 2 for further discussion). Of the remaining 417, 332 could be classified as long-term data based on the requirements of the CCME (2007). From these 332 data points, 300 were omitted or collated so that only one data point represented a single species. In cases where multiple comparable endpoints (*i.e.* same species, duration, lifestage *etc.*) were available for a single species, arithmetic (intra-study comparisons) and geometric means (inter-study comparisons) were taken (see Section 2 for full discussion). Thirty two (32) species made up the long-term SSD, with values ranging from a 24 h EC_{10} (glochidia survival) of 24 mg Cl^- /L for the freshwater mussel *Lampsilis fasciola* to a 144 h LOEC of 7820 mg Cl^- /L for a Cyanobacteria (*Synechocystis sp.*) Table 4.1.

Table 4.1. No and low effect concentrations used in the derivation of the long-term SSD

Species	Common name	Endpoint	Endpoint criteria	No/low effect concentration (mg Cl^- /L)	HPP [A]	Citation
Lampsilis fasciola	Freshwater mussel	24 h EC_{10}	Glochidia survival	24	0.0156	Bringolf_etal (2007)
Epioblasma torulosa rangiana	Freshwater mussel	24 h EC_{10}	Glochidia survival	42	0.0469	Gillis (2011)
Musculium securis	Fingernail clam	1440-1920 h LOEC	Reproduction (reduced natality - mean # newborns/ # parents)	121	0.0781	Mackie (1978)
Daphnia ambigua	Water flea	240 h EC_{10}	Mortality & Reproduction	259	0.1094	Harmon_etal (2003)
Pseudokirchneriella subcapitata	Green algae	96 h EC_{10}	Population Florescence	326	0.1406	***MEAN VALUE**
Ceriodaphnia dubia	Water flea	168 h IC_{25}	Reproduction	337	0.1719	***MEAN VALUE**
Daphnia pulex	Water flea	504 h IC10	Reproduction	368	0.2031	Birge_etal (1985)
Elliptio complanata	Freshwater mussel	24 h EC_{10}	Glochidia survival	406	0.2344	Bringolf_etal (2007)
Daphnia magna	Water flea	504 h IC_{25}	Reproduction	421	0.2656	Elphick_etal (2011a)
Hyalella azteca	Amphipod	672 h EC_{25}	Growth, dry weight/ amphipod	421	0.2656	Bartlett_etal (2012)
Lemna minor	Common duckweed	168 h MATC	Growth (# live thalli)	496	0.3281	Simmons (2012)
Tubifex tubifex	Tubificid worm	672 h IC10	Reproduction	519	0.3594	Elphick_etal (2011a)

Table 4.1. No and low effect concentrations used in the derivation of the long-term SSD

Species	Common name	Endpoint	Endpoint criteria	No/low effect concentration (mg Cl^-/L)	HPP [A]	Citation
Pimephales promelas	Fathead minnow	816 h LC_{10}	Mortality	591	0.3906	***MEAN VALUE**
Salmo trutta	Brown trout	192 h NOEC	Mortality	607	0.4219	Camargo & Tarazona (1991)
Villosa delumbis	Freshwater mussel	24 h EC_{10}	Glochidia survival	716	0.4531	Bringolf_etal (2007)
Villosa constricta	Freshwater mussel	24 h EC_{10}	Glochidia survival	789	0.4844	Bringolf_etal (2007)
Lumbriculus variegatus	California blackworm	672 h IC_{25}	Biomass	825	0.5156	Elphick_etal (2011a)
Oncorhynchus mykiss	Rainbow trout	168 h EC_{25}	Embryo viability	989	0.5469	Beak International Inc.
Brachionus patulus	Rotifer	336 h NOEC	Population increase	1,213	0.5781	Peredo-Alvarez_etal (2003)
Brachionus calyciflorus	Rotifer	48 h IC10	Reproduction	1,241	0.6094	Elphick_etal (2011a)
Xenopus laevis	African clawed frog	168 h LC_{10}	Mortality	1,307	0.6406	Beak International Inc.
Lampsilis siliquoidea	Freshwater mussel	96 h EC_{10}	Mussel mortality	1,474	0.6719	Bringolf_etal (2007)
Salvinia natans	Floating fern	336 h LOEC	Growth	1,773	0.7031	Jampeetong & Brix (2009)
Physa	Snail	1440 h NOEC	Mortality	2,000	0.7344	Williams_etal (1999)
Gammarus pseudolimnaeus	Amphipod	1440 h NOEC	Mortality	2,000	0.7344	Williams_etal (1999)
Stenonema modestum	Mayfly	336 h MATC	Development	2,047	0.7969	Diamond_etal (1992)
Chironomus dilutus	Midge	480 h IC10	Biomass	2,316	0.8281	Elphick_etal (2011a)
Lithobates pipiens	Northern Leopard frog	2592 h MATC	Mortality	3,431	0.8594	Doe (2010)
Chlorella minutissimo	Green algae	672 h MATC	Growth	6,066	0.8906	Kessler (1974)
Chlorella zofingiensis	Green algae	672 h MATC	Growth	6,066	0.8906	Kessler (1974)
Chlorella emersonii	Green alga	192-336 h MATC	Growth	6,824	0.9531	Setter_etal (1982)
Synechocystis sp.	Cyanobacteria	144 h LOEC	Physiological (polysaccharide production)	7,820	0.9844	Ozturk & Aslim (2010)

[A] ***HPP:*** *Hazen plotting position. See Section 2 for description*
*** *MEAN VALUE***: *data shown is the mean value taken from multiple studies. Intra-study comparisons use arithmetic value, inter-study comparisons use geometric mean. Raw values used presented in Table 4.2.*

Table 4.2. Raw data used in the derivation of long-term SSD averaged data points

Species	Common name	Endpoint	Effect conc. (mg Cl^-/L)	Arithmetic mean*	Geometric mean	Citation
Ceriodaphnia dubia	Water flea	168 h IC_{25}	454	362	**337**	Elphick_etal (2011a)
Ceriodaphnia dubia	Water flea	168 h IC_{25}	117			Elphick_etal (2011a)
Ceriodaphnia dubia	Water flea	168 h IC_{25}	264			Elphick_etal (2011a)
Ceriodaphnia dubia	Water flea	168 h IC_{25}	146			Elphick_etal (2011a)
Ceriodaphnia dubia	Water flea	168 h IC_{25}	454			Elphick_etal (2011a)
Ceriodaphnia dubia	Water flea	168 h IC_{25}	580			Elphick_etal (2011a)
Ceriodaphnia dubia	Water flea	168 h IC_{25}	521			Elphick_etal (2011a)
Ceriodaphnia dubia	Water flea	144-168 h IC_{25}	147	314		Lasier&Harden(2010)
Ceriodaphnia dubia	Water flea	144-168 h IC_{25}	456			Lasier&Harden(2010)
Ceriodaphnia dubia	Water flea	144-168 h IC_{25}	340			Lasier&Harden(2010)
Pimephales promelas	Fathead minnow	816 h LC_{10}	585		**591**	Rescan (2007)
Pimephales promelas	Fathead minnow	792 h LC_{10}	598			Birge_etal(1985)
Pseudokirchneriella subcapitata	Green algae	96 h EC_{10}	96		**326**	Simmons (2012)
Pseudokirchneriella subcapitata	Green algae	96 h EC_{10}	1270	1113		Geis & Hemming (2014)
Pseudokirchneriella subcapitata	Green algae	96 h EC_{10}	955			Geis & Hemming (2014)

**** Arithmetic mean:** the geometric mean was preferentially taken. Arithmetic means were used to collate multiple data points for a single study when multiple studies were available for a species. This ensured that no one study had a greater influence on the derived SSD value.*

4.1.1 Evaluation of Long-Term Toxicological Data

The remainder of this section summarizes important data points for four taxonomic groups (fish, invertebrates, amphibians, and plants/algae) and outlines situations where professional judgment was required to arrive at the SSD effect concentration presented in Table 4.1. Note that this section deals only with data used to establish the long-term SSD.

Long-term toxicity to fish

Although chronic endpoints are available for eight species of fish in the database, only data from three met the long-term SSD requirements: rainbow trout (*O. mykiss*), fathead minnow (*P. promelas*), and brown trout (*S. trutta*). For rainbow trout, a 168 h EC_{25} value (989 mg Cl^-/L) for embryo viability (Beak 1999) was selected since it was the most sensitive of the preferred (*sensu* CCME 2007) endpoints available. Two (792-816 h) LC_{10} values for embryo survival, 585 mg Cl^-/L (Rescan 2007) and 598 mg Cl^- /L (Birge *et al.* 2007)), were utilized to represent *P. promelas*. These values were used since they represent some of the most sensitive preferable endpoints (MATC, NOEC, and LOEC endpoints are less preferable). In their Canadian WQG derivation, the CCME (2011) took the same approach, using the same LC_{10} mortality endpoint from Birge *et al.* (2007) for *P. promelas* (Rescan (2007) data was not used in the CCME 2011 derivation). In the case of brown trout, a 192 h NOEC concentration of 607 mg Cl^- /L was utilized for fish at the fingerling lifestage. Although NOEC values are generally less preferred and requirements (CCME 2007) are for exposure durations of ≥ 504 h for juvenile fish, the CCME (2011) task group chose to include this data point in their long-term SSD. We also include it in the current long-term SSD dataset.

Long-term toxicity to amphibians

Unlike the acute SSD, amphibians were found to be less sensitive to chronic chloride exposures when compared to fish. Data for two amphibian species are included in the long-term SSD dataset: a 168 h LC_{10} of 1,307 mg Cl^- /L for the African clawed frog (*X. Laevis*) from Beak (1999) and a 108 d MATC (mortality) for the northern leopard frog (*L. pipiens*) from Doe (2010). The African clawed frog is an invasive species in Canada and generally would not be included in SSD derivation. In the 2011 CCME chloride WQG derivation, no explanation is given for its inclusion other than the fact that the Beak (1999) data provides the lowest effect concentration for the amphibian dataset. Additionally, for both of these species, severe (mortality) endpoints are utilized. This is not elaborated on in CCME (2011), however the concentrations represent the most sensitive endpoints for these species (except where no effect was observed (NOEC)). Values used in the current long-term SSD are the same as those used in CCME (2011).

Long-term toxicity to invertebrates

In general, invertebrates were found to be the most sensitive organisms to chronic chloride exposure with the lower 30% of the long-term SSD predominantly made up of bivalve and water flea taxa. Across the SSD, bivalves are represented by six species of freshwater mussels and clams. The sensitivity of these animals to chloride makes them especially important, particularly the glochidia lifestage, a larval form which occupies the two most sensitive positions on the long-term SSD. The 24 h EC_{10} values for the COSEWIC special concern wavy rayed lampmussel (*L. fasciola*) and the COSEWIC endangered northern riffleshell mussel (*E. torulosa reangiana*) were 24 (Bringolf *et al.* 2007) and 42 mg Cl^- /L (Gillis 2010) respectively. All of the bivalve data in the current long-term dataset is the same as those values used by the CCME (2011). As is outlined in CCME (2011), an exception is made for glochidia chronic endpoints. Because of the short duration of the lifestage, 24 h EC_{10} values (glochidia survival) are considered long-term endpoints.

Water fleas represent another sensitive species of invertebrate. All of the water flea long-term endpoints fell in close proximity to one another on the SSD, with 168-504 h EC/IC10-25s ranging from 259 (*D. ambigua;* Harmon *et al.* 2003) to 337 mg Cl^- /L (*C. dubia*; collated value) and 368 (*D. pulex*; Birge *et al.* 1985) to 421 mg Cl^- /L (Elphick *et al.* 2011a). These values all correspond to values utilized in the CCME (2011) long-term SSD derivation with the exception of *C. dubia*. Whereas only the 7 d IC_{25} (reproduction) endpoint of 454 mg Cl^- /L (Elphick *et al.* 2011a) was used in the CCME SSD derivation, the current dataset draws from the IC_{25} (reproduction) endpoints of both Elphick *et al.* (2011a) and Lasier and Harden (2010).

The 336 h NOEC of 1,213 mg Cl^- /L for the rotifer *Brachionus patulus* (Peredo-Alvarez *et al.* 2003) represents an addition to the CCME dataset. The most tolerant invertebrate species was found to be the midge, *C. tentans,* with a 20 d growth IC10 of 2,316 mg Cl^- /L (Elphick *et al.* 2011a).

Long-term toxicity to plants / algae

Two aquatic macrophytes and five algae species were included in the long-term SSD. The endpoint included for the macrophyte *L. minor* (duckweed) was a 168 h EC_{10} (growth) of 496 mg Cl^- /L (Simmons 2012), while a 336 h LOEC (growth) of 1773 mg Cl^- /L represented the floating fern *Salvinia. Natans* (Jampeetong and Brix 2009).

Vascular plants are used less frequently than algae in toxicity assays for sodium chloride and other chloride salts. Where macrophytes (vascular plants) are used, duckweed (*Lemna spp*.) is the most commonly selected test organism due to their small size, ease of culture and rapid reproduction. It has been suggested that the limited use of macrophytes in toxicological research may be attributed to high levels of variability between replicates (Lewis 1995). Such variability can be seen in the present database where chronic 168 h EC_{50} (growth) values varied between 1,525 to 4,167 mg Cl^- /L (Buckley *et al.* 1996, Keppeler 2009, Simmons 2012; Appendix A). Furthermore, the 168h EC_{10} (growth - # live thalli) currently used in the long-term SSD (496 mg Cl^- /L; Simmons 2012) is less than half that of the 168 h MATC value used in the CCME derivation (1,171 mg Cl^- /L; Taraldsen and Norberg-King 1990). Evidence of chloride (NaCl) sensitivity has been found in field studies using semi-aquatic bryophytes. Wilcox (1984) and Wilcox and Andrus (1987) found that growth was retarded in two *Sphagum* species (*S. fimbriatum* and *S. recurvum*) exposed to chloride concentrations between 300 and 1500 mg/L. However, for *L. minor*, the low endpoint obtained by Simmons (2012) is anomalous compared to previous laboratory work on this species and positions *L. minor* with well-established sensitive species on the long-term SSD (i.e. at similar sensitivity to daphnids).

Algae are the major primary producers in the aquatic food chain; therefore setting a chloride WQG that is protective of these species is imperative for overall ecosystem protection. Despite this, algae have not been traditionally used in standard bioassays and are relatively poorly represented in the ecotoxicology literature for chloride. Evidence suggests that some species of algae may be particularly sensitive to various toxicants. A review using the Toxic Substances Control Act database found that algae were more sensitive than invertebrates and fish in 50% of reports while being less sensitive in 30% (Lewis 1995). In the CCME 2011 guideline derivation for chloride, all of the algae species included in the long-term SSD fell at the upper end of the distribution. These species (*Chlorella minutissimo*, *C. zofingiensis, and C. emersonii*) are also included in the present SSD, however, it is important to note that these are all green algae of the same genus. Further investigations found one species in particular, *Pseudokirchneriella subcapitata*, which was particularly sensitive to chloride. In the database three studies (Geis *et al.* 2000, Santos *et al.* 2007, and Simmons 2012) and one unpublished toxicity assessment (Geis and Hemming 2014) examine the toxicity of chloride towards this species. 72-96 h IC_{50} values (cell density) for this species range between 528 mg Cl^- /L (Santos *et al.* 2007) and 780 mg Cl^- /L (Simmons 2007), whereas 96 h EC_{50} values (population estimate based on fluorescence) vary more widely from 1,820 mg Cl^- /L (Geis *et al.* 2000) to 674 mg Cl^- /L (Simmons 2012). The long-term value included in the present SSD was derived by averaging 96 h EC_{10} (fluorescence) results from Geis and Hemming (1270 and 955 mg Cl^- /L) with the lower effect concentration found by Simmons (96 mg Cl^- /L). Note that the dilution water used by Simmons contained 15 mg $CaCO_3$/L, a hardness comparable to Very Soft Reconstituted Water (US EPA classification). This low hardness concentration could be a factor contributing to results which are approximately 10-fold lower than comparable toxicity assays performed by Geis and Hemming where dilution waters of 85 and 170 mg $CaCO_3$ were used.

4.1.2 Derivation of the Long-Term SSD and Water Quality Guideline

The SSD chloride concentrations (Table 4.1) were plotted against their corresponding Hazen plotting position values. Cumulative distribution functions were then fitted to the data using the maximum likelihood approach of the BMDS software (US EPA 2013a). The best model was selected based on goodness -of-fit and model feasibility. Using BMDS software, the Log-Logistic Function provided the best overall fit for the data with the lowest AIC (Akaike's Information Criteria) value. This model also minimized the scaled residuals of each of the data points. The equation of the model was:

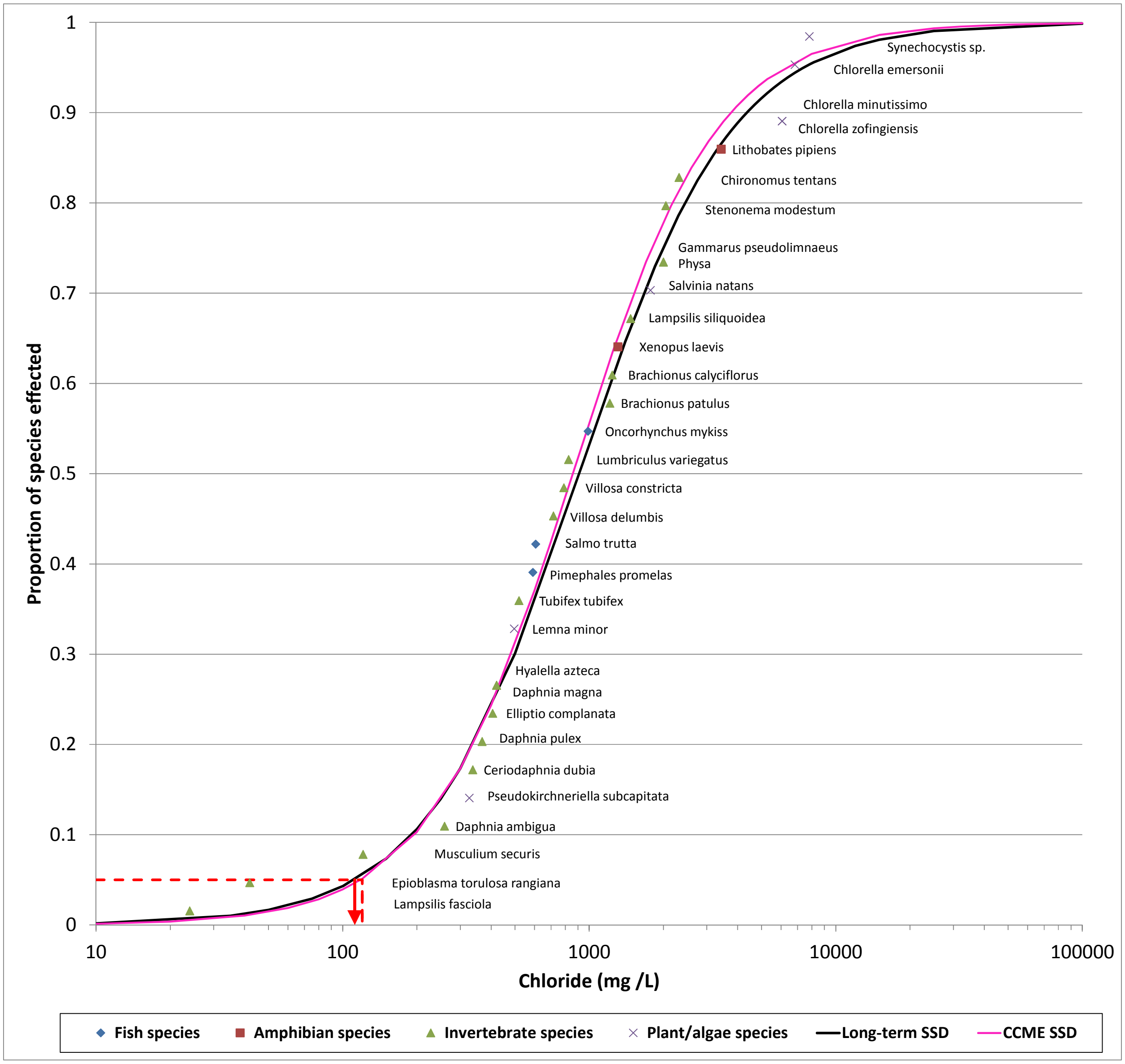
$$y = 1 / [1 + e^{-(b + m * \ln(x))}] \qquad \textbf{(Eq. 3.1)}$$

Where y is the proportion of species affected and x is the chloride concentration. The slope of the line (m) and the y-intercept (b) were calculated to be **1.40** and **-9.57** respectively.

Figure 4.1 shows the long-term SSD made up of the no and low effect endpoints and corresponding Hazen plotting position values (Table 4.1), along with the fitted Log-Logistic function (Eq. 3.1). The long-term WQG, defined as the 5th percentile along the derived Log-Logistic function was **112** mg/L chloride with a lower-bound confidence limit of **106** mg/L chloride.

Compared to CCME (2011) the long-term WQG concentration derived here is 8 mg/L lower (CCME value = 120 mg/L). The more sensitive guideline is the result of new additions to the SSD dataset, along with revised effect concentrations for a number of species located relatively low on the curve. Three data points in particular are important, and likely the primary factors driving the greater sensitivity of the current model. One is a 96h EC_{10} endpoint of 96 mg Cl^- /L for the green algae *P. subcapitata* (Simmons 2012). This data point represents a departure from the CCME derivation where algae species were only represented high on the curve with effect concentrations > 6000 mg Cl^- /L (See Section 4.1.1 for further discussion). Two data points representing *C. dubia* and *L. minor* have also been reduced from 454 to 337 mg Cl^- /L and 1171 to 496 mg Cl^- /L respectively with the addition of data from Elphick *et al.* (2011a), Lasier and Harden (2010), and Simmons (2012).

Figure 4.1. Long-term SSD – no and low effect endpoint toxicity data for NaCl



***Notes**:1) SSD derived by plotting no and low effect concentrations (preferred endpoints: EC/ICx representing a no-effect threshold > EC/IC10 > EC/IC11-25 > MATC > NOEC > LOEC > EC/IC26-49 > nonlethal EC/IC$_{50}$) for 32 species of aquatic organism against their corresponding Hazen plotting position (HPP); 2) Red hashed line indicates the 5th percentile (y-axis) and the resulting long-tern water quality guidelines derived by the CCME (x-axis at hashed line) and derived in this report (x-axis at red arrow) of 112 mg Cl^{-}/L.*

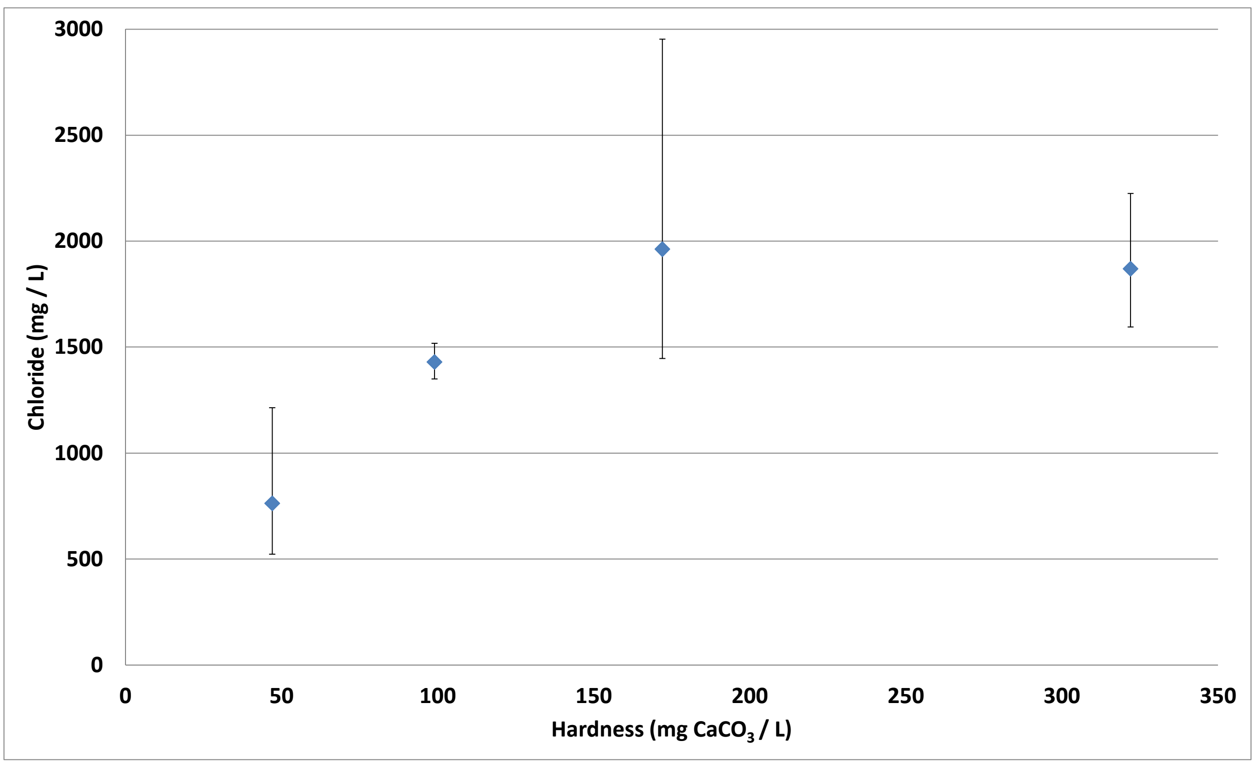
5 HARDNESS MODIFICATION

As has been conducted by regulatory agencies for various substances of concern, including chloride, the influence of hardness on toxicity was evaluated for deriving a hardness-based WQG for aquatic life. The background to this concept, including theoretical considerations, is provided in Section 5.1. Sections 5.2 and 5.3 examine hardness relationships based on short-term and long-term toxicity datasets, respectively. Information in these sections are used to derive short-term and long-term pooled hardness-toxicity slopes, along with the associated hardness adjusted SSDs (adjusted to 50 mg $CaCO_3$ / L hardness), which can be used to express short-term benchmarks and long-term WQGs based on water hardness.

5.1 BACKGROUND AND THEORETICAL CONSIDERATIONS

Hardness has been identified as an important variable regarding the toxicity of chloride salts towards aquatic organisms. For example, Gillis (2011) studied the effects of water hardness on *Lampsilis siliquoidea* (Fatmucket mussel), a species found in Alberta. The 24 hour EC_{50} values in soft (47 mg/L $CaCO_3$), moderately hard (99 mg/L $CaCO_3$), hard (172 mg/L $CaCO_3$), and very hard (322 mg/L $CaCO_3$) reconstituted water were 763, 1,430, 1,962, and 1,870 mg/L chloride. Within this range of hardness, an approximate 2-fold decrease in toxicity was observed with harder water (Figure 5.1).

Figure 5.1. Hardness-toxicity relationship established for *Lampsilis siliquoidea* (Gillis 2011)



Note: *data is for 24 h EC50 (glochidia survival) values including 95% confidence intervals*.

The CCME (2011) summarized studies where hardness reduced chloride toxicity (by up to 5-fold) for a variety of aquatic species including the Wavy-rayed lampmussel, water flea, fingernail clam, tubificid worm, snail, and isopod. And Gillis (2011) determined hardness reduced the toxicity of chloride the towards Fatmucket mussel. Similar relationships have been observed for metals such as zinc and cadmium, although the mechanism may be distinct for chloride salts (CCME 2011). The issue is complicated for chloride salts by the presence of toxicity studies showing results as equivocal or where the amelioration of chloride toxicity due to increasing water hardness was relatively minor (or negligible), for species such as water flea, fingernail clam, fathead minnow, snail, and damselfly. However, in some studies where minimal hardness effects were observed, the hardness levels were relatively low (*e.g.*, < 100 mg/L $CaCO_3$), suggesting the studies were of insufficient power to discern a hardness related effect.

The CCME (2011) stated "Jurisdictions will have the option of adjusting for site-specific hardness conditions, if they so choose, with the development of site-specific water quality guidelines (or objectives)". Furthermore, the CCME (2011) stated "CCME will re-visit the chloride guidelines when sufficient studies are available". Studies in this regard refers to chronic toxicity endpoints and a hardness relationship that meets required parameters defined in guidance from the US Environmental Protection Agency (US EPA 2001), "...such as the highest hardness is at least 3 times the lowest and the highest hardness is at least 100 mg/L higher than the lowest)".

In contrast to major ions, it is well known that water hardness ameliorates the toxicity of metals to aquatic organisms. For metals, the mitigating mechanism involves competition for binding sites on the surface of cell membranes (Paquin *et al.* 2002). The US EPA (2014b) National Recommended Water Quality Criteria outlines seven metals in which guidelines are derived based on hardness. Table 5.1 outlines the functions utilized the derived chronic guidelines for cadmium, chromium III, copper, lead, nickel, and zinc.

Table 5.1. Summary of chronic hardness dependent WQGs for metals

Metal	m_C	b_C	Freshwater conversion factor (FC)	Chronic guidelines (µg /L) at hardness of 100 mg / L
Cadmium	0.741	-4.719	1.101672 - [(ln hardness)(0.041838)]	0.25
Chromium III	0.819	0.685	0.86	74
Copper	0.855	-1.702	0.96	*BLM calc
Lead	1.273	-4.705	1.46203 - [(ln hardness)(0.145712)]	2.5
Nickel	0.846	0.058	0.997	52
Zinc	0.847	0.884	0.986	118
**Silver	m_A = 1.72	b_A = -6.59	Acute = 0.85	Acute = 3.2

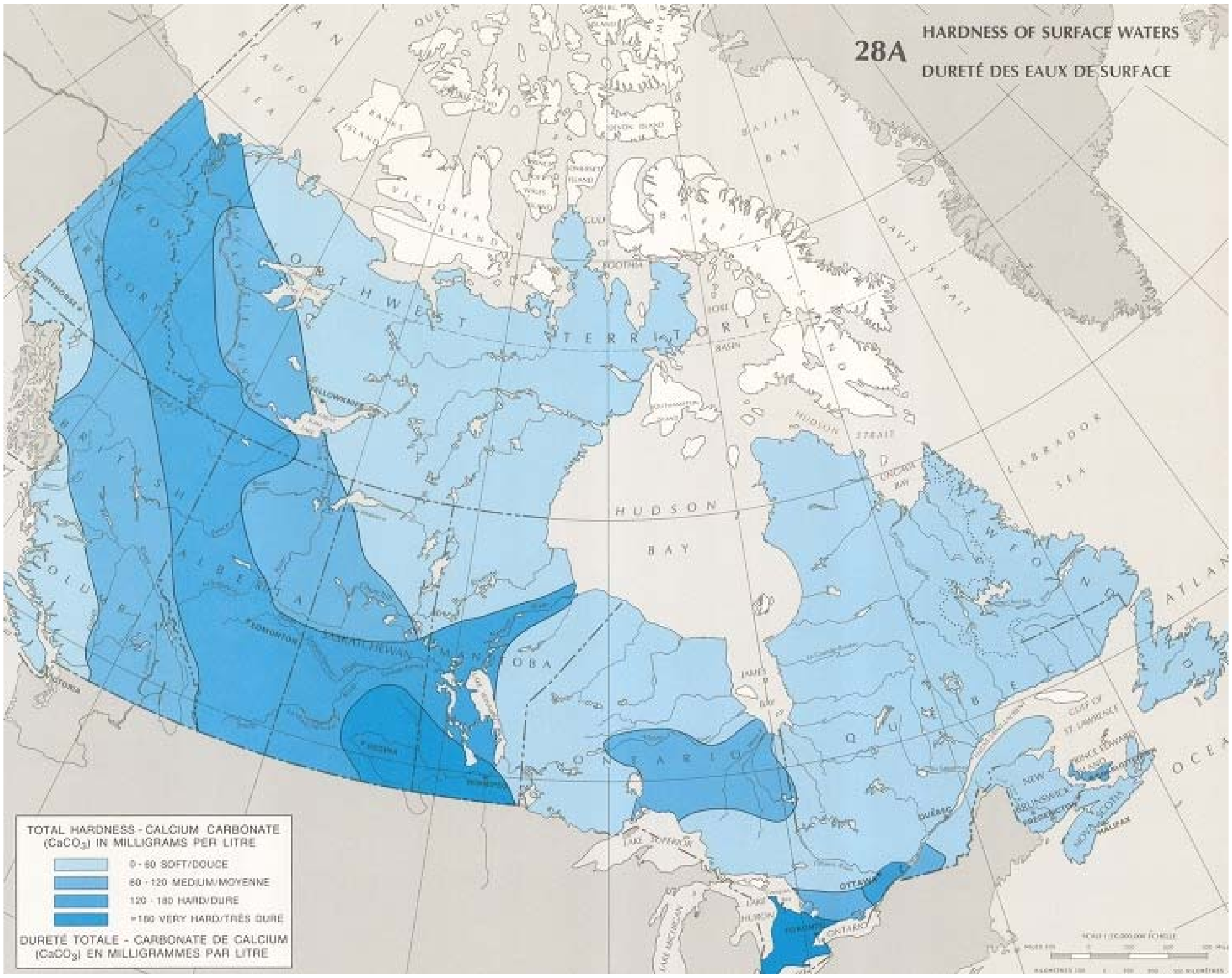
**** BLM calc:*** *guidelines for copper are calculated using the Biotic Ligand Model.*
***** Silver:*** *guidelines provided are acute, chronic hardness-adjusted guidelines have not been developed.*

Precedent has been set by regulatory agencies for developing major ion water quality guidelines that incorporate aspects of hardness. The US EPA, the Great Lakes Environmental Centre (GLEC), and the Illinois Natural History Survey (INHS) in collaboration with the state of Iowa have developed an algorithm for the adjustment of a chloride water quality guidelines based on hardness (IDNR 2009a). The Iowa acute and chronic chloride guidelines are derived using the equations 287.8 * $(\text{hardness})^{0.205797}(\text{sulphate})^{-0.07452}$; and 177.87 * $(\text{hardness})^{0.205797}(\text{sulphate})^{-0.07452}$ respectively (IDNR 2009b). Within Canada, British Columbia has recently established a hardness adjusted guideline for the sulphate ion which draws (among other sources) from research conducted by Elphick *et al.* (2011a) and the Pacific Environmental Science Centre (PESC). The approved 30-day average water quality guidelines are 128, 218, 309, and 429 mg SO4 /L for very soft (0-30 mg/L), soft / moderately soft (31-75 mg/L), moderately soft / hard (76-180 mg/L), and very hard (181-250 mg/L) water respectively (BCME 2013).

Water hardness has also been identified as an ameliorating factor in aquatic guideline policy. In their National Water Quality Management Strategy, the Australian and New Zealand governments recognise that greater water “softness” in freshwater ecosystems dominated by chloride and sodium can cause a greater risk to biota, from classes of contaminants for which water hardness and acid buffering capacity may ameliorate toxicity (ANZECC, 2000). Finally, the effects of hardness amelioration have been incorporated into site specific guidelines for chloride in Canada. For the EKATI diamond mine (Northwest Territories), a site specific guideline (calculated as: 124 * ln (hardness) -128) was established for waters with hardness varying from 10 to 160 mg/L (Rescan 2007). A similar site-specific hardness dependent guideline has been recommended for the Snap Lake diamond mine (Northwest Territories) (De Beers 2013). Given the growing application of hardness derived guidelines to major ion toxicity, an important component of any future research on the toxicity of chloride towards sensitive aquatic organisms is to incorporate varying hardness levels and produce information that can be used to improve the accuracy of guidelines developed on a provincial or national scale.

Variability in water hardness across Canada is graphically presented below (Figure 5.2), and it should be noted that hardness definitions differ. In Alberta, the following scheme has been used for water hardness (Alberta Government 2011): 1) Soft (0 to 50 mg/L); 2) Moderately Soft (50 to 100 mg/L); 3) Moderately Hard (100 to 200 mg/L); 4) Hard (200 to 400 mg/L); 5) Very Hard (400 to 600 mg/L); and, 6) Extremely Hard (> 600 mg/L).

Figure 5.2. Regional water hardness trends in Canada



Note: *Total hardness of surface waters indicated as calcium carbonate ($CaCO_3$) in mg/L. Figure from NRCAN 1978*

Water hardness is defined as the sum of all polyvalent cations in solution. In natural aquatic systems, calcium ($Ca2^+$) and magnesium ($Mg2^+$) occur at much higher concentrations compared to other polyvalent cations, hence, water hardness can be measured based on these divalent cations alone. Hardness is generally expressed as total hardness in $CaCO_3$ equivalents using the following formula (Hiscock 2005):

Total hardness (mg CaCO3 /L) = 2.5[Ca2+] + 4.1[Mg2+] ***(Eq. 5.1)***

Numerous studies have shown an inverse relationship between water hardness and chloride toxicity. Naumann (1934), working with Daphnia magna, found that deleterious concentrations of $CaCl_2$ and KCl could be ameliorated with increased hardness. Garrey (1916) reported a reduction in chloride (NaCl, KCl, $MgCl_2$) toxicity towards minnows (Notropis sp.) using increasing concentrations of $CaCl_2$. More recently, Grizzle and Mauldin (1995) reported a 13-fold reduction in chloride (NaCl) toxicity towards juvenile striped bass (Morone saxatilis) by increasing calcium concentrations from 3 to 100 mg/L. Examining various reconstituted water formulations and NaCl toxicity, Lasier *et al.* (2006) demonstrated reduced Ceriodaphnia dubia reproductive success in lower hardness concentrations. At a chloride concentration of 565 mg/L, reproduction was reduced by 21 % after water hardness was lowered from 100 to 45 mg CaCO3 /L.

The exact mechanism by which calcium and magnesium ameliorate chloride toxicity may be variable between species, and different in the magnitude and direction of effect. Several researchers have shown that calcium is more important than magnesium in ameliorating toxicity (Leblanc and Surpenant 1984, Jackson *et al.* 2000, Welsh *et al.* 2000). One plausible rationale is that calcium reduces membrane permeability, thereby protecting organisms from the toxic effects of other ions. Evidence for this has been found in fish (Eddy 1975, Pic and Maetz 1981, Potts and Fleming 1970, Penttinen *et al.* 1998) and invertebrates (Robertson 1941). Ca^{2+} and Mg^{2+} are also known to reduce the toxicity of metals by competing for binding sites on the surface of cell membranes (*e.g.* Paquin *et al.* 2002). The same mechanism has been suggested to be responsible for the hardness amelioration of NaCl (and $NaSO_4$) toxicity (Soucek *et al.* 2011, Davies and Hall 2007, Elphick *et al.* 2011a).

In their 1997 work, Mount *et al.* tested the toxicity of 12 salts (NaCl, Na_2SO_4, $NaHCO_3$, KCl, K_2SO_4, $KHCO_3$, $CaCl_2$, $CaSO_4$, $MgCl_2$, $MgSO_4$, $CaCO_3$, and $MgCO_3$) on two daphnids and one fish species (*C. dubia*, *D. magna*, and *P. promelas*). Based on their results, they suggest that the amelioration of chloride (NaCl, KCl, $CaCl_2$, and $MgCl_2$) and other major ion toxicity may have been caused by a multi-ion effect rather than the result of hardness itself (*i.e.* specifically Ca^{2+} and Mg^{2+} ions). As an example, the C. dubia 48 h LC50 values for NaCl and $CaCl_2$ of 1187 and 1172 mg Cl^- / L are highlighted. Here it is reasoned that if hardness was an important ameliorating factor, the exposure with $CaCl_2$ would be expected to produce a higher LC50 compared to NaCl, results which were not found. However, this assertion does not account for the extreme hardness concentration in the $CaCl_2$ assay (1702 mg CaCO3 /L) which, viewed another way could be considered a toxic level of water hardness. Indeed, ameliorating effects of hardness are not expected to follow linear relationships. As the Gillis (2011) data suggests (Figure 5.1) there is an upper bound at which increasing water hardness no longer provides an ameliorating benefit to a given organism. Figure 5.1 demonstrates that for *L.* siliquoidea, the upper threshold likely occurs at a hardness concentration between 172 and 322 mg $CaCO_3$ / L. The CCME (2011) cites the Mount *et al.* (1997) study as potential evidence that amelioration of chloride toxicity may be based on a multi-ion effect rather than a hardness effect. However, the methods utilized in this study are not designed to evaluate the influence of water hardness on chloride toxicity and hardness ranges are extreme between treatment groups (moderately hard reconstituted water is the only test medium utilized). Thus, no conclusions about the ameliorating effect of water hardness on chloride toxicity should be drawn from this paper.

What the Mount *et al.* (1997) results do demonstrate is that major ion toxicity is ameliorated by a multiple ion effect in complex solutions containing multiple salts. Within the literature, this study is unique. Very few studies have examined the toxicity of complex solutions at an individual ion basis. Such research requires a sophisticated experimental design with a large number of ion combinations (in the case of Mount *et al.* 1997, over 2,900 ion solutions), thus data outlining the ameliorating effect that multiple ions have on one another is limited. Regardless, the majority of the studies examined in the current literature review demonstrate a reduction in chloride toxicity in solutions of higher hardness. In consideration of the Mount *et al.* (1997) results, Elphick *et al.* (2011a) suggests that in addition to its observed ameliorating influence, water hardness may represent a proxy for higher overall ionic strength or more balanced ionic ratios for major ions.

5.1.1 Effects of Water Hardness on the Toxicity of Chloride to Aquatic Life

Table 5.2 summarizes literature that has directly tested the toxicity modifying effects of water hardness on chloride salt toxicity over acute and chronic timeframes. In these studies, 11 species of aquatic organisms were exposed to sodium or potassium chloride salts in 20 individual investigations of hardness-toxicity relationships. Of these 20 investigations 4 found no relationship between water hardness and the toxicity of chloride to the species tested. The studies outlined in Table 5.2 are examined in more detail in the following discussion.

Table 5.2. Studies investigating the effect of water hardness on chloride toxicity to aquatic life

Species	End-point	Exp. time (h)	ST/LT (SSD)[A]	Data Quality[B]	Effect Concentration (mg Cl^- /L)	Hardness (mg $CaCO_3$ /L)	Toxicity - hardness relationship Δ Cl^- : Δ Hardness (logarithmic slope)	Lifestage	Citation
Sodium chloride (NaCl)									
ACTINOPTERYGII									
Pimephales promelas (Fathead minnow)	LC_{50}	96	Acute	?	2,457 (ave: 2,790, 2,123)	44.0	**Not comparable due to lifestage differences**	524 h old	* US EPA (1991)
Pimephales promelas (Fathead minnow)	LC_{50}	96	Acute	?	2,244	300.0		<24 h old	* US EPA (1991)
Pimephales promelas (Fathead minnow)	LC_{50}	96	ST	?	4,167	84.8	**-0.5 (-0.01) No apparent effect of hardness on toxicity**	N/S	WISLOH (2007)
Pimephales promelas (Fathead minnow)	LC_{50}	96	ST	?	4,127	169.5		N/S	WISLOH (2007)
BRANCHIOPODA									
Ceriodaphnia dubia (Water flea)	LC_{50}	48	Acute	?	1,436 (ave: 1,638, 1,274, 1,395)	44.0	**1.0 (0.09)**	<24 h old	US EPA (1991)
Ceriodaphnia dubia (Water flea)	LC_{50}	48	Acute	?	1,698	300.0		<24h old	US EPA (1991)
Ceriodaphnia dubia (Water flea)	IC_{25} (repro)	168	LT	P/S	117	10	**3.0 (0.54)**	<24 h old	Elphick_etal (2011a)
Ceriodaphnia dubia (Water flea)	IC_{25} (repro)	168	LT	P/S	264	20		<24 h old	Elphick_etal (2011a)
Ceriodaphnia dubia (Water flea)	IC_{25} (repro)	168	LT	P/S	146	40		<24 h old	Elphick_etal (2011a)
Ceriodaphnia dubia (Water flea)	IC_{25} (repro)	168	LT	P/S	454	80		<24 h old	Elphick_etal (2011a)
Ceriodaphnia dubia (Water flea)	IC_{25} (repro)	168	LT	P/S	580	160		<24 h old	Elphick_etal (2011a)
Ceriodaphnia dubia (Water flea)	IC_{25} (repro)	168	LT	P/S	521	320	**No effect of hardness from 160 to 320 mg $CaCO_3$ /L**	<24 h old	Elphick_etal (2011a)
Ceriodaphnia dubia (Water flea)	IC_{50} (repro)	168	LT	P/S	161	10	**4.6 (0.62)**	<24 h old	Elphick_etal (2011a)
Ceriodaphnia dubia (Water flea)	IC_{50} (repro)	168	LT	P/S	301	20		<24 h old	Elphick_etal (2011a)
Ceriodaphnia dubia (Water flea)	IC_{50} (repro)	168	LT	P/S	481	40		<24 h old	Elphick_etal (2011a)
Ceriodaphnia dubia	IC_{50}	168	LT	P/S	697	80		<24 h old	Elphick_etal (2011a)

Table 5.2. Studies investigating the effect of water hardness on chloride toxicity to aquatic life

Species	End-point	Exp. time (h)	ST/LT (SSD)[A]	Data Quality[B]	Effect Concentration (mg Cl^- /L)	Hardness (mg $CaCO_3$ /L)	Toxicity - hardness relationship Δ Cl^- : Δ Hardness (logarithmic slope)	Lifestage	Citation
(Water flea)	(repro)								
Ceriodaphnia dubia (Water flea)	IC_{50} (repro)	168	LT	P/S	895	160		<24 h old	Elphick_etal (2011a)
Ceriodaphnia dubia (Water flea)	IC_{50} (repro)	168	LT	P/S	700	320	**No effect of hardness from 160 to 320 mg $CaCO_3$ /L**	<24 h old	Elphick_etal (2011a)
Ceriodaphnia dubia (Water flea)	LC_{50}	168	Chronic	P/S	132	10	**3.5 (0.67)**	<24 h old	Elphick_etal (2011a)
Ceriodaphnia dubia (Water flea)	LC_{50}	168	Chronic	P/S	316	20		<24 h old	Elphick_etal (2011a)
Ceriodaphnia dubia (Water flea)	LC_{50}	168	Chronic	P/S	540	40		<24 h old	Elphick_etal (2011a)
Ceriodaphnia dubia (Water flea)	LC_{50}	168	Chronic	P/S	1134	80		<24 h old	Elphick_etal (2011a)
Ceriodaphnia dubia (Water flea)	LC_{50}	168	Chronic	P/S	1240	160		<24 h old	Elphick_etal (2011a)
Ceriodaphnia dubia (Water flea)	LC_{50}	168	Chronic	P/S	1303	320		<24 h old	Elphick_etal (2011a)
Ceriodaphnia dubia (Water flea)	LC_{50}	48	ST	P/S	977	28	**1.2 (0.22)**	<24 h old	Soucek_etal(2011) [derived from: GLEC & INHS (2008)]
Ceriodaphnia dubia (Water flea)	LC_{50}	48	ST	P/S	861	47		<24 h old	Soucek_etal(2011) [derived from: GLEC & INHS (2008)]
Ceriodaphnia dubia (Water flea)	LC_{50}	48	ST	P/S	1,249	96		<24 h old	Soucek_etal(2011) [derived from: GLEC & INHS (2008)]
Ceriodaphnia dubia (Water flea)	LC_{50}	48	ST	P/S	1,402	187		<24 h old	Soucek_etal(2011) [derived from: GLEC & INHS (2008)]
Ceriodaphnia dubia (Water flea)	LC_{50}	48	ST	P/S	1,589	388		<24 h old	Soucek_etal(2011) [derived from: GLEC & INHS (2008)]
Ceriodaphnia dubia (Water flea)	LC_{50}	48	ST	P/S	1,779	565		<24 h old	Soucek_etal(2011) [derived from: GLEC & INHS (2008)]
Ceriodaphnia dubia (Water flea)	LC_{50}	48	ST	P/S	1,836	796		<24 h old	Soucek_etal(2011) [derived from: GLEC & INHS (2008)]
Ceriodaphnia dubia (Water flea)	IC_{25} (repro)	144-168	LT	P/S	147	40 (45 mg/L alkalinity)	**11.2 (0.74)**	<24 h old	Lasier&Harden(2010)
Ceriodaphnia dubia (Water flea)	IC_{25} (repro)	144-168	LT	P/S	340	40 (101 mg/L alkalinity)		<24 h old	Lasier&Harden(2010)
Ceriodaphnia dubia (Water flea)	IC_{25} (repro)	144-168	LT	P/S	456	85 (66 mg/L alkalinity)		<24 h old	Lasier&Harden(2010)
Ceriodaphnia dubia (Water flea)	IC_{50} (repro)	144-168	LT	P/S	342	40 (45 mg/L alkalinity)		<24 h old	Lasier&Harden(2010)
Ceriodaphnia dubia (Water flea)	IC_{50} (repro)	144-168	LT	P/S	563	40 (101 mg/L alkalinity)		<24 h old	Lasier&Harden(2010)

Table 5.2. Studies investigating the effect of water hardness on chloride toxicity to aquatic life

Species	End-point	Exp. time (h)	ST/LT (SSD)[A]	Data Quality[B]	Effect Concentration (mg Cl^- /L)	Hardness (mg $CaCO_3$ /L)	Toxicity - hardness relationship Δ Cl^- : Δ Hardness (logarithmic slope)	Lifestage	Citation
Ceriodaphnia dubia (Water flea)	IC_{50} (repro)	144-168	LT	P/S	653	85 (66 mg/L alkalinity)		<24 h old	Lasier&Harden(2010)
Ceriodaphnia dubia (Water flea)	EC_{22} (repro)	168	LT	P/S	342	45.0	**Reduced toxicity with Increasing hardness: 2.2-fold increase in hardness results in higher ECx values (chloride concentration constant)**	<24 h old	Lasier_etal(2006)
Ceriodaphnia dubia (Water flea)	EC_{35} (repro)	168	LT	P/S	342	46.0		<24 h old	Lasier_etal(2006)
Ceriodaphnia dubia (Water flea)	EC_{17} (repro)	168	LT	P/S	342	99.0		<24 h old	Lasier_etal(2006)
Ceriodaphnia dubia (Water flea)	EC_{13} (repro)	168	LT	P/S	342	100.0		<24 h old	Lasier_etal(2006)
Ceriodaphnia dubia (Water flea)	EC_{54} (repro)	168	LT	P/S	565	45.0	**Reduced toxicity with Increasing hardness: 2.2-fold increase in hardness results in higher ECx values (chloride concentration constant)**	<24 h old	Lasier_etal(2006)
Ceriodaphnia dubia (Water flea)	EC_{59} (repro)	168	LT	P/S	565	46.0		<24 h old	Lasier_etal(2006)
Ceriodaphnia dubia (Water flea)	EC_{44} (repro)	168	LT	P/S	565	99.0		<24 h old	Lasier_etal(2006)
Ceriodaphnia dubia (Water flea)	EC_{33} (repro)	168	LT	P/S	565	100.0		<24 h old	Lasier_etal(2006)
Ceriodaphnia dubia (Water flea)	LC_{50}	48	ST	?	1,677	84.8	**-2.1 (-0.16) No apparent effect of hardness on toxicity**	N/S	WISLOH (2007)
Ceriodaphnia dubia (Water flea)	LC_{50}	48	ST	?	1,499	169.5		N/S	WISLOH (2007)
							BIVALVIA		
Sphaerium simile (Fingernail clam)	LC_{50}	96	ST	P/S	740	51	**2.5 (0.30)**	Juvenile	Soucek_etal(2011) [derived from: GLEC & INHS (2008)]
Sphaerium simile (Fingernail clam)	LC_{50}	96	ST	P/S	1,100	192		Juvenile	Soucek_etal(2011) [derived from: GLEC & INHS (2008)]
Sphaerium tenue (Fingernail clam)	LC_{50}	96	ST	U (no mention of control survival)	698	20.0	**-0.4 (-0.03) No apparent effect of hardness on toxicity**	N/S	Wurtz&Bridges(1961)
Sphaerium tenue (Fingernail clam)	LC_{50}	96	ST	U (no mention of control survival)	667	100.0		N/S	Wurtz&Bridges(1961)
Lampsilis siliquoidea (Wavy-rayed lampmussel)	EC_{50}	24	ST	P/S	763	47.0	**4.0 (0.45)**	Glochidia	Gillis(2011)

Table 5.2. Studies investigating the effect of water hardness on chloride toxicity to aquatic life

Species	End-point	Exp. time (h)	ST/LT (SSD)[A]	Data Quality[B]	Effect Concentration (mg Cl^- /L)	Hardness (mg $CaCO_3$ /L)	Toxicity - hardness relationship Δ Cl^- : Δ Hardness (logarithmic slope)	Lifestage	Citation
Lampsilis siliquoidea (Wavy-rayed lampmussel)	EC_{50}	24	ST	P/S	1,430	84.6		Glochidia	Gillis(2011)
Lampsilis siliquoidea (Wavy-rayed lampmussel)	EC_{50}	24	ST	P/S	1,430	99.0		Glochidia	Gillis(2011)
Lampsilis siliquoidea (Wavy-rayed lampmussel)	EC_{50}	24	ST	P/S	1,962	172.0		Glochidia	Gillis(2011)
Lampsilis siliquoidea (Wavy-rayed lampmussel)	EC_{50}	24	ST	P/S	1,870	322.0		Glochidia	Gillis(2011)
GASTROPODA									
Gyraulus parvus (Planorbid snail)	LC_{50}	96	ST	P/S	3,078	56	**-0.4 (-0.01) No apparent effect of hardness on toxicity**	Adult	Soucek_etal(2011) [derived from: GLEC & INHS (2008)]
Gyraulus parvus (Planorbid snail)	LC_{50}	96	ST	P/S	3,009	212		Adult	Soucek_etal(2011) [derived from: GLEC & INHS (2008)]
Physa heterostropha (Snail)	LC_{50}	96	ST	U (no mention of control survival)	2,487	20.0	**11.8 (0.20)**	N/S	Wurtz&Bridges(1961)
Physa heterostropha (Snail)	LC_{50}	96	ST	U (no mention of control survival)	3,427 (ave: 3,034, 3,761)	100.0		N/S	Wurtz&Bridges(1961)
CLITELLATA									
Tubifex tubifex (Tubifex worm)	LC_{50}	96	ST	P/S	4,278	52	**11.7 (0.24)**	Adult	Soucek_etal(2011) [derived from: GLEC & INHS (2008)]
Tubifex tubifex (Tubifex worm)	LC_{50}	96	ST	P/S	6,008	197.1		Adult	Soucek_etal(2011) [derived from: GLEC & INHS (2008)]
INSECTA									
Argia sp. (Damselfly)	LC_{50}	96	ST	U (no mention of control survival)	1,3952	20.0	**7.6 (0.03)**	nymph	Wurtz&Bridges(1961)

Table 5.2. Studies investigating the effect of water hardness on chloride toxicity to aquatic life

Species	End-point	Exp. time (h)	ST/LT (SSD)[A]	Data Quality[B]	Effect Concentration (mg Cl^- /L)	Hardness (mg $CaCO_3$ /L)	Toxicity - hardness relationship Δ Cl^- : Δ Hardness (logarithmic slope)	Lifestage	Citation
Argia sp. (Damselfly)	LC_{50}	96	ST	U (no mention of control survival)	1,4558	100.0		nymph	Wurtz&Bridges(1961)
					MALACOSTRACA				
Asellus communis (Isopod)	LC_{50}	96	ST	U (no mention of control survival)	3,094	20.0	**23.9 (0.30)**	N/S	Wurtz&Bridges(1961)
Asellus communis (Isopod)	LC_{50}	96	ST	U (no mention of control survival)	5,004	100.0		N/S	Wurtz&Bridges(1961)
					Potassium chloride (KCl)				
					BIVALVIA				
Musculium transversum (Fingernail clam)	LC_{50}	96	ST	P/S	907	243	**19.2 (3.57)**	Juvenile	Anderson_etal (1978)
Musculium transversum (Fingernail clam)	LC_{50}	96	ST	P/S	2,267	314		Juvenile	Anderson_etal (1978)
					CHROMADOREA				
Caenorhabditis elegans (Nematode)	LC_{50}	48	ST	U (soil nematode)	18,613	76	**128.11 (0.54)**	72 h old	Cressman&Williams(1994)
Caenorhabditis elegans (Nematode)	LC_{50}	48	ST	U (soil nematode)	19,766	85		72 h old	Cressman&Williams(1994)

[A] ***ST/LT (SSD):*** *SSD experimental duration requirements for long-term data (CCME 2007). "ST" or "LT" indicates that the data point meets the requirements for short or long-term SSD data respectively. "Chronic" or "Acute" indicates that the data is classified as chronic or acute, but does not meet the specific requirements for SSD derivation.*

[B] ***Data quality:*** *"P/S" indicates that toxicity data is classified as primary or secondary and can be used in guideline derivation. "U" indicates data points that are deemed unacceptable for guideline derivation."?" Indicates that unclassified data (original document could not be obtained for review).*

* **US EPA (1991):** results for *P. promelas* were deemed incomparable for examining hardness-toxicity relationships because of differences in lifestages (<24 h versus 524 h old). Note however that these two endpoints are compared by the CCME (2011).

Soucek et al. 2011

This 2011 publication stems from work originally published by the Great Lakes Environmental Center and the Illinois Natural History Survey in association with the US EPA (GLEC and INHS, 2008). The project was initiated to guide the state of Iowa in the derivation of WQGs for chloride and sulfate. Tests were conducted on a water flea (*Ceriodaphnia dubia*), a fingernail clam (*Sphaerium simile*), a tubifex worm (*Tubifex tubifex*), and a planorbid snail (*Gyraulus parvus*). *C. dubia* was exposed to chloride in waters ranging in hardness from 28 to 796 mg $CaCO_3$/L, with a constant sulfate concentration of 65 mg/L. Mean 48 h LC_{50} values ranged from 977 to 1,836, demonstrating a strong correlation between increasing water hardness and reduced toxicity (power function R^2 = 0.85). The other 3 test organisms were exposed to two hardness concentrations, soft water (51-56 mg $CaCO_3$ /L) and hard water (192-210 mg $CaCO_3$ /L). The most sensitive species, *S. simile*, along with *T. tubifex*, both demonstrated increasing 96 h LC_{50} values with increasing hardness. An ameliorating effect of hardness was not observed for *G. parvus* (see Section 5.1.2 for further discussion).

Gillis 2011

Gillis (2011) examined chloride toxicity in relation to water hardness using the glochidia life-stage of the freshwater mussel *Lampsilis siliquoidea*. Using 24 h EC 50 values for glochidia survival (ability to close valve), a 6.8-fold increase in hardness (47-322 mg $CaCO_3$) corresponded to a 2.5-fold decrease in toxicity.

Elphick et al. 2011a

Chronic relationships have been established by Elphick *et al.* (2011a), Lasier and Harden (2010) and Lasier et al (2006). Elphick *et al.* used both reproductive (IC_{25} and IC_{50} values) and mortality (LC_{50}) endpoints to examine hardness effects over durations of168 hours for *C. dubia*. Increases in hardness from 10 to 160 mg $CaCO_3$/L demonstrated 5- and 5.6-fold increases in IC_{25} and IC_{50} values, although hardness concentrations of 320 mg $CaCO_3$ /L produced no ameliorating effect when compared to the 160 mg $CaCO_3$ /L treatment. Examining mortality endpoints, a 9.8-fold reduction in toxicity was observed after a 32-fold increase in hardness, representing a substantial ameliorating effect (logarithmic slope = 0.67).

Lasier and Harden 2010 and Lasier et al. 2006

Using a *C. dubia* three-brood reproduction test, Lasier and Harden (2010) found chloride to be significantly less toxic in moderate-hardness water when compared to low-hardness water (85 versus 40 mg $CaCO_3$ /L). Alkalinity was also found to have an ameliorating impact, as IC_{25} and IC_{50} values were increased in low-hardness moderate-alkalinity water compared to exposures in low-hardness low-alkalinity water. Of all the studies examined, the results of Lasier and Harden (2010) demonstrated the largest ameliorating effect of hardness on chloride toxicity (NaCl exposures) with a logarithmic slope of 0.74. These results corroborate earlier work by Lasier *et al.* (2006) where the same 168 h three-brood test was performed on *C. dubia*. Here, two solutions with static chloride concentrations of 342 and 565 mg/L were exposed to varying levels of water hardness. It was found that a 2.2-fold increase in hardness (45-100 mg $CaCO_3$ /L) resulted in reduced deleterious impacts on water flea reproduction.

Wurtz and Bridges 1961

In an earlier study by Wurtz and Bridges (1961) four species of aquatic organisms (fingernail clam: *Sphaerium tenue*, snail: *Physa heterostropha*, isopod: *Asellus communis*, Damselfly: *Argia* sp.) were tested for their tolerance to elevated chloride. 96 h TLm values (Median Tolerance Limit, equivalent to LC_{50}) were tested across two hardness levels (20 and 100 mg $CaCO_3$ /L). All species, with the exception of *S. tenue*, showed an inverse relationship between chloride toxicity and higher hardness concentrations. For *S. tenue*, 48 h LC_{50} values did increase in harder water (LC_{50} of 1183 and 940 for hard and soft waters respectively) but at 24, 72, and 96 h no ameliorating effects were demonstrated. One potential problem with this analysis was highlighted by the CCME (2011), where it was suggested that the lower range of hardness tested (20 to 100 mg $CaCO_3$ /L) may not be sufficient to elucidate any toxicity-hardness relationships. Testing the same genus of clam (*Sphaerium simile*), Soucek *et al.* (2011) showed that an ameliorating relationship could be established over a higher and broader hardness range (51-192 mg $CaCO_3$ /L). Because control group survival was not reported for any species throughout this article, the work did not meet the data requirements for SSD derivation (CCME, 2007).

US EPA 1991

Data from toxicological work conducted by the Environmental Research Laboratory in Duluth (ERL-Duluth) was presented in US EPA 1991 along with other sources as a summary of toxicological data for 4 aquatic organisms. For *C. dubia,* 48 h LC_{50} values showed an inverse relationship to water hardness, although this ameliorating effect was small (logarithmic slope = 0.09). The ERL-Duluth data also presented 96 h LC_{50} values for *Pimephales promelas* exposed to NaCl in both soft (44 mg $CaCO_3$ /L) and very hard (300 $CaCO_3$ /L) reconstituted waters, however, this data was deemed incomparable since the age of tested organisms varied across hardness treatments (524 h and < 24 h fish used in soft and hard water treatments respectively). Note that this differs from the CCME (2011) where these two values are compared and cited as evidence against an ameliorating effect of hardness on chloride toxicity. The US EPA 1991 data should be interpreted with caution as the original ERL-Duluth data is not provided. Other than life-stage and water hardness, no other environmental or toxicity modifying factors were specified, similarly, no control group survival is specified.

WISLOH 2007

Data presented by WISLOH 2007 (originally referenced in IDNR 2009b) suggests that hardness does not ameliorate chloride toxicity for the water flea *C. dubia* or the cyprinid fish *P. promelas*. Of the studies examining the influence of hardness on chloride toxicity, *C. dubia* represents the most studied species with four peer-reviewed articles examining mortality and reproductive endpoints. In all of these studies, ameliorating relationships were established, such that the data from WISLOH 2007 is anomalous. For *P. promelas*, no other studies are available to compare to the WISLOH results, although research has shown that hardness ameliorates the toxicity of sulfate, another major anion (Elphick *et al.* 2011b). The original WISLOH data was not obtained, thus it was not possible to confirm that minimum data requirements were met (CCME 2007). For the CCME 2011 chloride guideline derivation, the original data was similarly unavailable, thus it was rejected for WQG derivation. Despite this, this work is cited by the CCME as evidence showing no relationship between water hardness and chloride (NaCl) toxicity.

Anderson et al. 1978; Cressman and Williams 1994

Studies examining the toxicity of chloride in the form of potassium, calcium, or magnesium are less common in the literature and even fewer have examine hardness-toxicity relationships using these chloride salts. For potassium chloride, two studies have examined hardness relationships. Anderson *et al.* (1978) found that increasing hard water solutions from 243 to 314 mg $CaCO_3$ /L caused a significant reduction in toxicity for the fingernail clam *Musculium transversum* (96 h LC_{50} increase from 907 to 2267 mg Cl^- /L). Additionally, For the soil nematoad *Caenorhabditis elegans,* a small change in hardness (76 to 85 mg CaCO3 /L) was found to increase 48 h LC50 values by a factor of 1.1 (Cressman and Williams 1994).

5.1.2 Studies Showing no Ameliorating Effect of Hardness on Chloride Toxicity

After reviewing the literature, four instances were found in which no ameliorating effect of hardness was observed on chloride toxicity:

1) **Wurtz and Bridges 1961 – *Sphaerium tenue;***
2) **WISLOH 2007 – *Ceriodaphnia dubia;***
3) **WISLOH 2007 – *Pimephales promelas;***
4) **Soucek *et al.* 2011 – *Gyraulus parvus.***

Of these occurrences, three were presented in studies / data tables with data quality issues such that the results cannot be used in guideline derivation. In Wurtz and Bridges 1961, no control survival was reported and in WISLOH (2007) the original data was not available. In the case of the *C. dubia* results reported by WISLOH, four other peer-reviewed articles demonstrated ameliorating effects of hardness for this species, such that the data from WISLOH (2007) is considered herein as anomalous. Similarly, subsequent tests on the clam genus *Sphaerium* (Soucek *et al.* 2011) have demonstrated hardness amelioration where this was not observed previously by Wurtz and Bridges (1961). For the Wurtz and Bridges (1961) data, one possible explanation for the neutral response of *Sphaerium tenue* may be the limited hardness range tested. Toxicity treatments were conducted in soft (20 mg $CaCO_3$ /L) and hard (100 mg $CaCO_3$ /L) water and it is possible that this range was not sufficient to observe a response when accounting for potential variability in the assay.

In the case of fish, the un-verified WISLOH results for *P. promelas* directly tested the effect of hardness on chloride toxicity. Although these results did not support a hardness-toxicity relationship, the inverse relationship between hardness and major ion toxicity has been established for *P. promelas* using sulfate (Elphick *et al.* 2011b).

Although the amelioration of chloride toxicity to the planorbid snail *P. heterostropha* was demonstrated by Wurtz and Bridges (1961), such a relationship was not found by Soucek *et al.* (2011) using *G. parvus*. The authors suggest that the unique respiration mechanism of planorbid snails (they lack gills) may explain the unique results, however this seems unlikely in light of the results of Wurtz and Bridges (1961) who found an inverse hardness-toxicity relationship for *P. heterostropha* (also a planorbid snail). Soucek *et al.* (2011) tested *G. parvus* of variable ages (3-5 mm diameter). If snails across these different age ranges respond differently to elevated chloride salt concentrations than the results of this work may have been affected, however no indication of age related effects was identified in the study.

It is important to note that the hardness-toxicity assessment conducted on *G. parvus* in this study utilized different $Ca^{2+}:Mg^{2+}$ ratios between the soft and hard water treatments (56 and 212 mg $CaCO_3$ /L respectively). During the soft water treatment the ratio of calcium to magnesium ions was 4.44 (13.5 mg Ca^{2+}/L : 3.04 mg Mg^{2+}/L), whereas the hard water treatment had a ratio of 2.23 (49.9 mg Ca^{2+}/L : 22.4 mg Mg^{2+}/L). It is known that different species specific hardness-toxicity modifying effects occur under different ratios of calcium to magnesium (Welsh *et al.* 2000). As a result, the two-fold difference in calcium to magnesium ratios between the soft and hard water treatments for *G. parvus* may explain the lack of an ameliorating effect of water hardness. Furthermore, if calcium is assumed to be of greater importance in ameliorating toxicity, as has been suggested in the literature (Leblanc and Surpenant 1984, Jackson *et al.* 2000, Welsh *et al.* 2000), then the absents of an ameliorating effect may be the result of a lower calcium to magnesium ratio in the hard versus soft water treatments.

The literature demonstrates that the bulk of evidence supports the hypothesis that an ameliorating relationship exists between increasing water hardness and chloride toxicity, thus applying a measure of this relationship to derived chloride guidelines is appropriate. However, further research is needed using species where this relationship has not been extensively explored, where weak and inconsistent relationships exist in the literature, or where data quality issues introduce an unacceptable level of uncertainty. One example is aquatic snails such as *G. parvus* and *P. heterostropha* in which conflicting results exist in the literature (Wurtz and Bridges 1961 versus Soucek *et al.* 2011). Another priority is to establish hardness-toxicity relationships for those species making up the lower portions of the acute and chronic SSDs. This will insure that any future hardness adjusted guidelines are protective of the most sensitive aquatic organisms. Further research using fingernail clam species of the genus *Sphaerium* is especially important since they are known to be sensitive to chloride (Section 3) and have been examined in the literature (*Sphaerium simile* in Soucek *et al.* 2011; *Sphaerium tenue* in Wurtz and Bridges (1961)). Other Bivalves such as *Epioblasma torulosa rangiana* (glochidia lifestage), *Musculium secures*, *Villosa iris* (glochidia lifestage), *Elliptio complanata* (glochidia lifestage), and members of the genus *Lampsilis* (glochidia lifestage) occur low on the acute and chronic SSDs and have limited or no hardness-toxicity relationships established for them (Section 8), as such they should also be a focus of further research.

5.2 HARDNESS ADJUSTED SHORT-TERM SSD

The following section outlines the results from toxicological analysis of the short-term dataset and WQG, adjusted for water hardness. The short-term hardness-toxicity relationships developed herein were used to establish an overall best-fit hardness-toxicity slope, and subsequently to adjust the SSD to a minimum hardness of 50 mg CaCO3 / L as well as other hardness thresholds. From this information, a mathematical relationship was developed that can be used to derive an acute (short-term) hardness-adjusted WQG, reflective of variable hardness levels and corresponding influence on toxicity.

5.2.1 Short-Term Hardness Adjustment Equation

From the short-term data, nine species had comparable effect concentration across the minimum hardness range required: *O. mykiss* (rainbow trout), *P. promelas* (fathead minnow), *L. siliquoidea* (freshwater mussel), *S. simile* (fingernail clam) *C. dubia* (water flea), *D. magna* (water flea), *L. variegatus* (California blackworm) and *T. tubifex* (tubificid worm), and *G. parvus* (Pulmonate snail). Appendix B summarizes the hardness-toxicity dataset which was utilized to analyze the relationship between water hardness and short-term toxicity. Comparing log chloride versus log hardness, linear regression was used to calculate a best-fit hardness-toxicity slope for each of the eight species. Slopes ranged from -0.017 to 0.476, with R^2 values between 0.534 and 0.998 (Table 5.3; Figure 5.3).

Within the dataset, toxicity-hardness relationships were also found for six additional species which were deemed unacceptable for hardness-toxicity slope calculations (Table 5.3). In most cases these data points were not included because effect concentrations were not available over the required minimum range of water hardness (Stephan et al 1985). Despite being excluded, most of these species still demonstrated an ameliorating of hardness. Exceptions to this occurred for the fish species *L. macrochirus* and the midge *C. dilutes*. However, in these cases, it is important to note the small sample size (n = 2) which reduces the statistical power of the data. In each case, the data comes from completely separate experiments, unlike the data limited *S. simile* (both data points from Soucek *et al.* 2011) which was used in hardness-toxicity slope calculations.

For *L. macrochirus*, the 96h LC_{50} reported by Birge *et al.* (1985) (5,870 mg Cl^- /L; Hardness: 102 mg $CaCO_3$ /L) was from a flow-through experiment, whereas the value reported by Trama (1954) (7,853 mg Cl^- /L; Hardness: 44 mg $CaCO_3$ /L) was from a static toxicity assay. The test design used is known to adversely alter toxicity tesing results (CCME 2007), therefore the two studies may have limited comparability.

For *C. dilutes*, comparisons were made between the 96 h LC_{50} values reported by Elphick *et al.* (2011a) and Wang and Ingersoll (2010), however the original data from Wang and Ingersoll was not obtained. Is unclear if experimental conditions may be impacting the comparability of these results.

Over a hardness range of 83 to 170 mg $CaCO_3$ /L, the effect concentrations reported for the glochidia of the freshwater mussel *L. fasciola* (Valenti *et al.* 2007, Bringolf *et al.* 2007, Gillis 2011) varied widely

(113-1868 mg Cl^- /L), thus no relationship could be determined from the data (R^2: 0.003). This 16 fold difference in chloride sensitivity was not unlike variations observed by Gillis (2011) who found an eight fold difference in *L. siliquoidea* (glochidia) 24 h EC_{50} values for mussels collected at two separate locations (113 and 1,430 mg Cl^- /L). As such, determining relationships between hardness and chloride toxicity for mussel glochidia may be best achieved under a single toxicity assay sampling from a single population. An example of this is the *L. siliquoidea* (glochidia) data reported by Gillis (2011) which was used to derive the short-term hardness-toxicity slope (Table 5.3).

Data for the fresh water snail *G. parvus* (Soucek *et al.* 2011) was included in the hardness-toxicity slope calculation. Here the two 96 h LC_{50} values of 3,078 and 3,009 mg Cl^- /L were reported for hardness values of 56 and 212 mg $CaCO_3$ /L respectively, suggesting that no hardness-toxicity relationship exists for this species. However, these findings may be the product of a two-fold difference in Ca^{2+}:Mg^{2+} ratios between the soft and hard water treatments (Section 5.1.2). Other work by Wurtz and Bridges (1961) has shown a strong ameliorating effect of water hardness on the freshwater snail *P. heterostropha* (Table 5.2). The Wurtz and Bridges results could not be included in the hardness-toxicity slope calculation as control survival was not reported. Despite this, these contrasting results suggest that further research is required to elucidate the effect of hardness on chloride toxicity towards fresh water snails.

An F-test was conducted on the slopes of the nine species deemed acceptable for the hardness-toxicity pooled slope calculation. Results from this test showed that the regression slopes for the nine species were not significantly different from one another (p = 0.630), thus an analysis of covariance (ANCOVA) was run to calculate a pooled slope across the whole dataset. The log transformed chloride effect concentration served as the dependant variable, the nine species formed the grouping variable, and the logarithm of hardness was the independent variable. From the ANCOVA analysis, the pooled slope was calculated to be **0.294** with a corresponding R^2 value of 0.941. The calculated pooled slope of 0.294 was then used to normalize all of the toxicity values in the short-term SSD (Section 3) to a hardness of 50 mg/L, using equation 2.2.

$$\mathbf{STEC_{x(50\ mg\ CaCO_3\ /L)} = 10^{\{([\log(50) - \log(hardness)] \times STPS) + \log(STECx)\}}} \qquad \textit{(Eq. 2.2)}$$

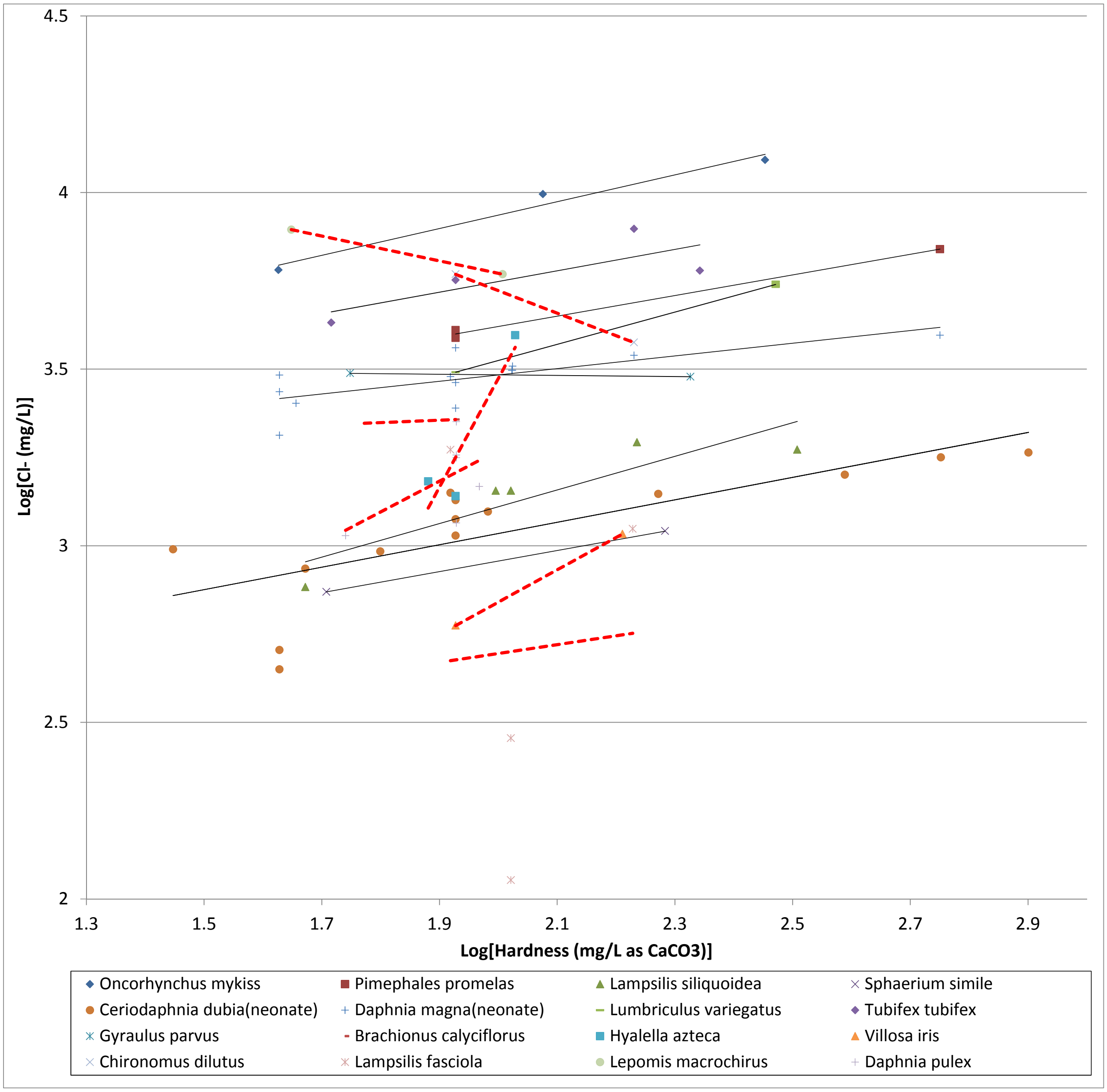
Where **STECx** $_{(50\ mgCaCO3\ /L)}$ is the short-term normalized effect concentration (in mg/L) and hardness was measured as $CaCO_3$ equivalents in mg/L.

Table 5.3. Short-term hardness-toxicity regression slopes

Species	n	Slope	R^2	df
Oncorhynchus mykiss **(LC_{50} - juveniles)**	3	0.380	0.973	1
Pimephales promelas **(LC_{50} - juveniles/larva)**	3	0.292	0.994	1
Lampsilis siliquoidea **(EC_{50} - glochidia)**	5	0.476	0.813	3
Sphaerium simile **(LC_{50} - juveniles)**	2	0.299	-	0
Ceriodaphnia dubia **(LC_{50} - adults)**	14	0.318	0.587	12
Daphnia magna **(LC_{50} - neonate)**	13	0.180	0.534	11
Lumbriculus variegatus **(LC_{50} - adults)**	5	0.456	0.998	3
Tubifex tubifex **(LC_{50} - adults)**	4	0.303	0.630	2
Gyraulus parvus	2	-0.017	-	0
POOLED SLOPE	**51**	**0.294**	**0.941**	**-**
**** Brachionus calyciflorus***	2	0.067	-	0
**** Hyalella azteca***	3	3.080	0.851	1
**** Villosa iris***	2	0.914	-	0
**** Chironomus dilutus***	2	-0.637	-	0
**** Lampsilis fasciola***	2	0.250	0.003	0
**** Lepomis macrochirus***	2	-0.352	-	0

** Data not used in hardness-toxicity slope calculation but plotted in Figure 5.3 for comparative purposes.*
Note: Summary of data points used here can be found in Appendix B

Figure 5.3. Short-term hardness-toxicity relationships



Note: *Red hashed regression lines represent data not meeting the hardness range or other requirements (Stephan et al. 1985). See Appendix B further details.*

5.2.2 Short-Term Hardness Adjusted SSD and Benchmark Concentration

In order to construct a hardness adjusted short-term SSD, 10 of the 56 species from the unadjusted curve (Table 3.1) were excluded, since no hardness data was available for these data points. The species excluded were: *A. maculatum* (spotted salamander), *H. attenuate* (hydra), *Pycnopsyche sp*. (caddisfly), *P. crucifer* (spring peeper) *L. sylvaticus* (wood frog), *A. americanus* (American toad), *G. pseudolimnaeus* (amphipod), *C. attenatus* (midge), *Lepidostoma sp*. (caddisfly), and *G. affins* (mosquito fish). With the exception of *A. maculatum* (HPP = 0.15), these excluded species were not found to be sensitive to chloride toxicity on the unadjusted SSD, therefore removing them was not expected to have a substantial impact on the SSD benchmark derivation.

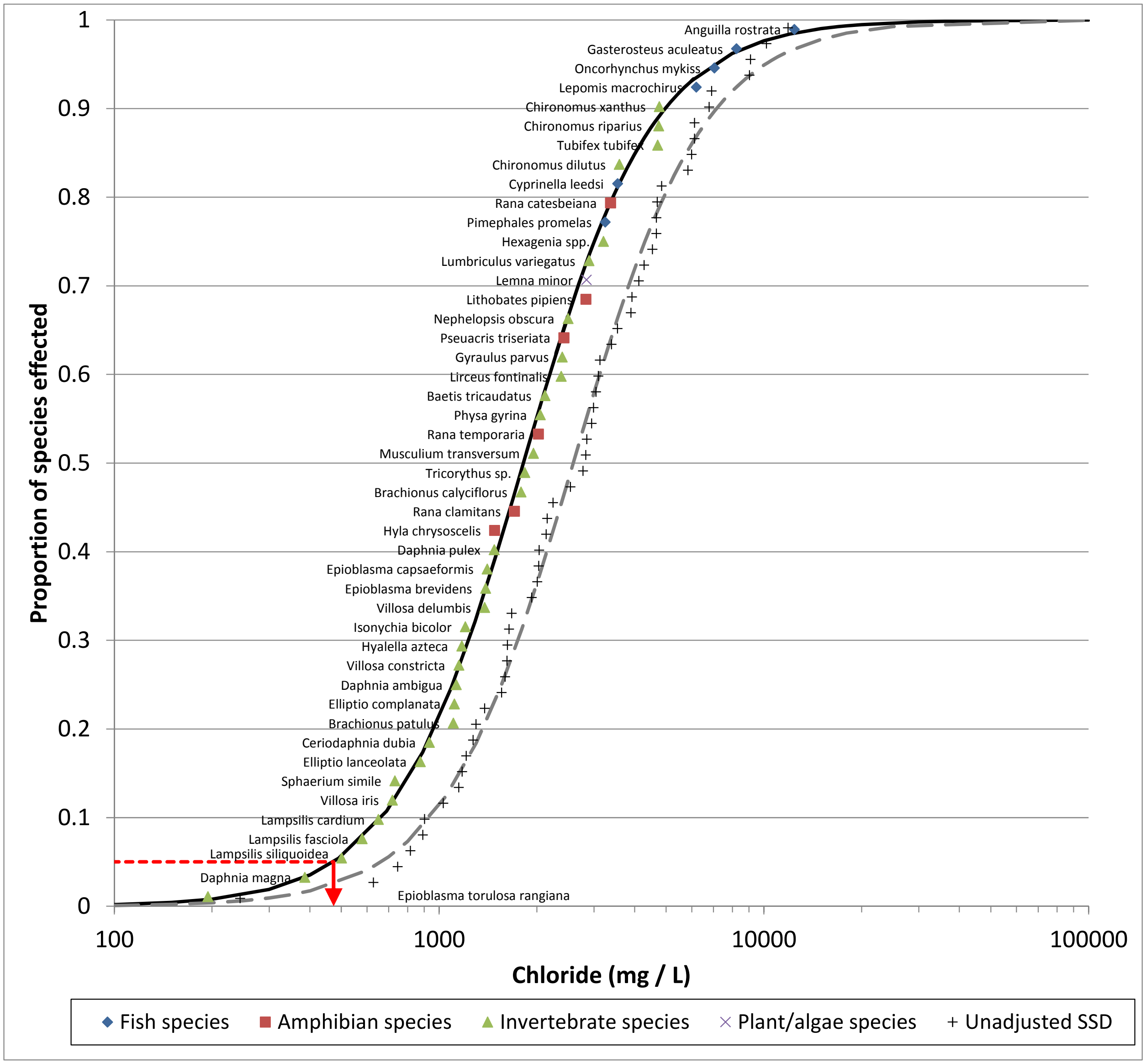
Using the remaining 46 species, a short-term SSD was constructed with effect concentrations normalized to a hardness of 50 mg $CaCO_3$ /L, using Eq. 2.2 and the pooled slope of 0.294 derived in the previous section. SSD derivation followed the same methods as described previously. The Log-Logistic Function provided the best overall fit for the data with the lowest AIC (Akaike's Information Criteria) value. This model also minimized the scaled residuals of each of the data points. The equation of the model was:

$$y = 1 / [1 + e^{-(b + m * \ln(x))}] \qquad \textit{(Eq. 3.1)}$$

Where y is the proportion of species affected and x is the chloride concentration. The slope of the line (m) and the y-intercept (b) were calculated to be 2.16 and -16.26 respectively. The Log-Probit model also provided a good fit to the data.

Figure 5.4 shows the short-term hardness adjusted SSD made up of EC/LC_{50} (severe endpoints) chloride concentrations normalized to 50 mg $CaCO_3$ /L with corresponding Hazen plotting position values. The short-term benchmark concentration, defined as the 5th percentile along the derived Log-Logistic function (Eq. 5.1) was **478** mg/L chloride with a lower-bound confidence limit of **463** mg/L chloride.

Figure 5.4. Hardness adjusted short-term SSD – severe endpoints (EC/LC50) for NaCl



***Notes**:1) SSD derived by plotting severe (EC/LC50) chloride effect concentrations adjusted to a hardness of 50 mg $CaCO_3$ /L for 46 species of aquatic organism against their corresponding Hazen plotting position (HPP); 2) Red hashed line indicates the 5th percentile (y-axis) and the resulting short-term benchmark concentration (x-axis) of 478 mg Cl^- /L; 3) Grey hashed curve and black crosses represent the unadjusted SSD (Figure 3.2).*

5.2.3 Hardness Equation for the Short-Term Benchmark

The short-term hardness equation, used for calculating benchmark concentrations across various hardness levels, is based on the US EPA procedure outlined in Stephan *et al.* (1985). The function was derived using the short-term hardness-toxicity slope of 0.294 calculated in Section 5.2.1 (m) and the derived short-term benchmark concentration of 478 mg Cl^- /L (y) at a hardness of 50 mg $CaCO_3$ /L (x) derived in Section 5.2.2. Since the slope (m), x, and y variables were known, the general equation log(y) = m*log(x)+b was rearranged to solve for b (y-intercept) which was found to be 2.179. Using this value the equation of the line to determine the long-term hardness dependant water quality guideline concentration can be expressed as:

$$\textit{Short-term benchmark} = 10^{[\,0.294\,(\log(\text{hardness})) + 2.179\,]} \qquad \textit{(Eq. 5.3)}$$

Where the benchmark concentration is measured in mg/L chloride and hardness is in $CaCO_3$ equivalents (mg/L).

Figure 5.5 provides a visual representation of the 50 mg $CaCO_3$ /L hardness adjusted SSD along with modeled curves at hardness concentration of 100, 200, 400, and 600 mg $CaCO_3$ /L. These values represent the upper bound thresholds for water classified as soft, moderately soft, moderately hard, hard, and very hard respectively (Alberta Government 2011). The short-term benchmark chloride concentrations corresponding to these hardness levels are indicated in Figure 5.5 and are summarized in the upper half of Table 5.4.

In order to compare the short-term dataset and the resulting benchmark WQGs to existing national WQGs, the CCME (2011) short-term SSD was adjusted for water hardness. The approach paralleled methods outlined previously, and involved transforming raw effect concentrations to a standard hardness of 50 mg $CaCO_3$ /L using Equation 2.2. The short-term pooled slope estimate (0.294) derived in Section 5.2.1 was utilized in data transformations. Nine data points from the original CCME (2011) dataset were not associated with water hardness concentrations and were therefore removed. The most sensitive of these data points was the Spotted Salamander (*A. maculatum*), which occurred below the 20^{th} percentile of the unadjusted SSD (CCME 2011). All other removed data points occurred above the 40^{th} percentile (*L. sylvaticus, P. crucifer, R. clamitans, G. pseudolimnaeus, C. attenatus, A. americanus, Lepidostoma sp., G. affinis*), and are expected to have a relatively minor influence on adjustments to the SSD. Three data points representing effect concentrations for $CaCl_2$ (all occurring above the 70^{th} percentile) were also removed (*D. hyaline, E. padanus padanus, C. abyssorum prealpinus*). A 50 mg $CaCO_3$ /L adjusted SSD was then modeled using the Log-Logistic function, where a short-term BMDL of 413 mg Cl^- /L was obtained. Using the modeled Log-Logistic function derived from the SSD analysis, a hardness equation was established to calculate short-term benchmark concentrations at various hardness levels, based on the underlying CCME (2011) dataset. These results, along with the equation used to derive them, are presented in the lower half of Table 5.4.

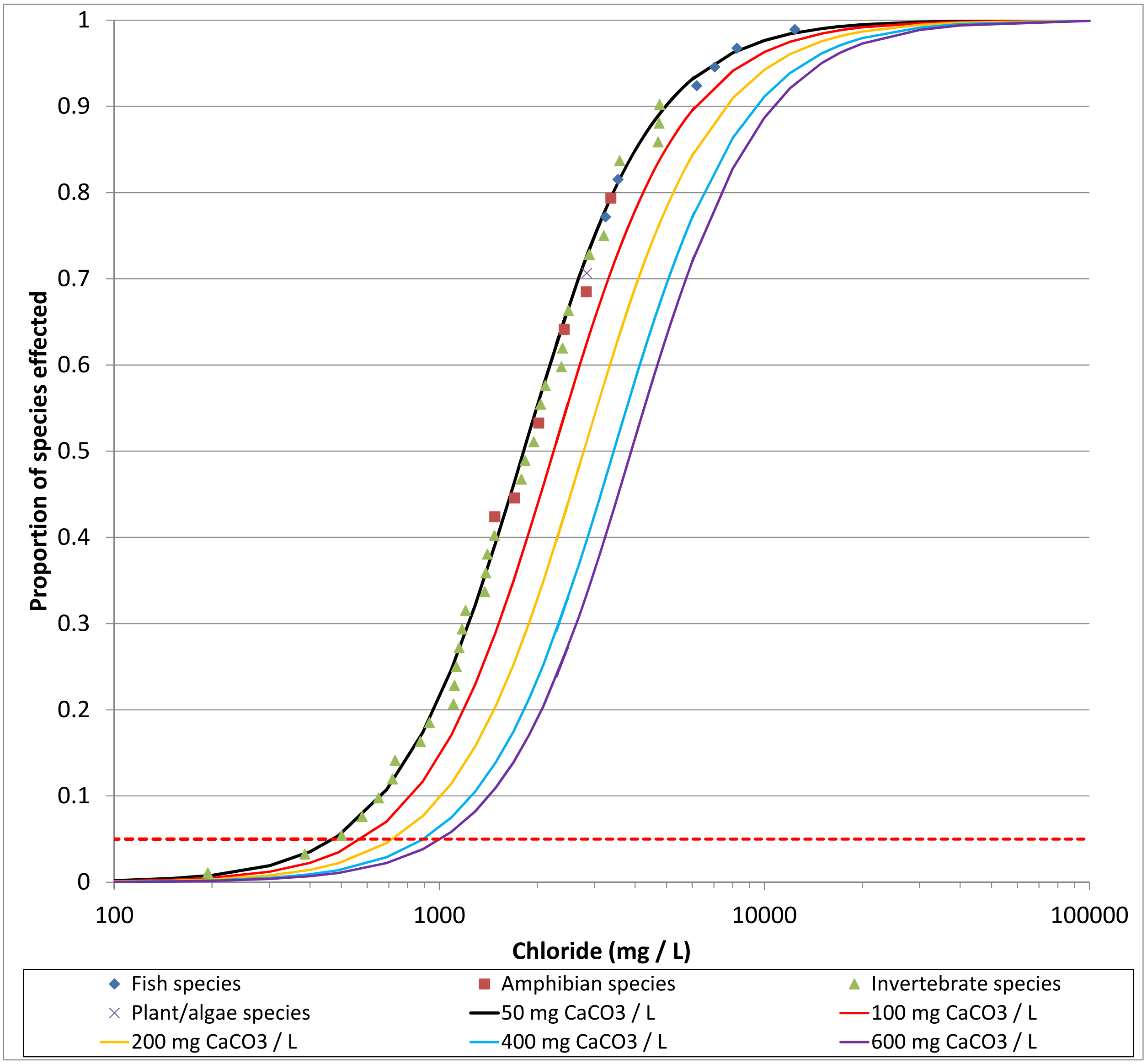
Table 5.4. Short-term hardness-adjusted benchmarks concentrations

Hardness (mg $CaCO_3$/L)	Short-term benchmark (mg Cl^-/L)
Current derivation	$(10^{[0.294 (\log(hardness)) + 2.179]})$
50 (soft)	**478**
100 (moderately soft)	**586**
200 (moderately hard)	**719**
400 (hard)	**881**
600 (very hard)	**993**
CCME 2011 data	$(10^{[0.294 (\log(hardness)) + 2.115]})$
50 (soft)	**413**
100 (moderately soft)	**506**
200 (moderately hard)	**621**
400 (hard)	**761**
600 (very hard)	**858**

Note: *The SSD utilized to derive CCME short-term hardness-adjusted guidelines was made up of n=39 data points, 12 less than the original 2011 dataset (n=51). This was the result of the loss of 9 data points where hardness data was not available and 3 data points which were based on $CaCl_2$ data in the original CCME dataset.*

From Table 5.4, it can be seen that the short-term guidelines are approximately 1.2 times higher than hardness adjusted guidelines using the original CCME (2011) dataset. This difference may be the result of changes to the modeled SSD curve caused by additions to the hardness adjusted dataset (current: n = 46; CCME: n=39), or effect concentrations which have been updated to higher values compared to the CCME (*e.g. L. siliquoidea*, *C. dubia*, and *D. pulex*; outlined in Section 3.2.2). In particular, data added since the CCME derivation has the potential to increase the calculated hardness-adjusted guidelines depending on the reported water hardness. For example, new 48h LC50s for *D. pulex* (Robinson 2011) corresponded to water hardness values of 38-66 mg $CaCO_3$/L, whereas hardness values associated with this species in the older CCME dataset ranged from 85-93 mg $CaCO_3$/L. When adjusted to a standard hardness of 50 mg $CaCO_3$/L, effect concentrations for the newer data would generally increase or remain unchanged, whereas CCME effect concentrations would be reduced. Data in the current dataset representing additions since the CCME (2011) derivation will affect the current short-term benchmark concentrations, depending on associated hardness concentrations.

Figure 5.5. Adjusted short-term SSD at various water hardness levels



***Notes**:1) Black curve represents the 50 mg $CaCO_3$ /L hardness adjusted SSD with associated species ; 2) Red hashed line indicates the 5th percentile (y-axis) and the resulting short-term benchmark concentrations (x-axis) of 478, 586, 719, 881, 993 mg Cl^- /L corresponding to hardness values of 50, 100, 200, 400, 600 mg $CaCO_3$ /L respectively.*

5.3 HARDNESS ADJUSTED CHRONIC SSD

The following section outlines the results from toxicological analysis of the long-term dataset and WQG, adjusted for water hardness. The long-term hardness-toxicity relationships developed herein were used to establish an overall best-fit hardness-toxicity slope, and subsequently to adjust the SSD to a minimum hardness of 50 mg CaCO3 / L as well as other hardness thresholds. From this information, a mathematical relationship was developed that can be used to derive a chronic (long-term) hardness-adjusted WQG reflective of variable hardness levels and corresponding influence on toxicity.

5.3.1 Long-Term Hardness Adjustment Equation

From the long-term data, four species had comparable effect concentration across the minimum hardness range required: *C. dubia* (water flea), *D. magna* (water flea), *L. minor* (duckweed), and P. subcapitata (green algae). Appendix B summarizes the hardness-toxicity dataset which was utilized to analyze the relationship between water hardness and long-term toxicity. Comparing log chloride versus log hardness, linear regression was used to calculate a best-fit hardness-toxicity slope for each of the three species. Slopes ranged from 0.332 to 0.583, with R^2 values between 0.488 and 0.958 (Table 5.5; Figure 5.6).

Two additional datasets were found to demonstrate hardness-toxicity relationships but were excluded from slope calculations. These included an additional IC_{50} (reproduction) dataset for *C. dubia* and a set of EC_{10-30} (glochidia survival) values for the freshwater mussel *L. fasciola*. The *C. dubia* IC_{50} values showed that hardness ameliorated chloride toxicity with a slope of 0.422 (R^2 = 0.578), however, the data was excluded since the IC_{25} values (reproduction) already available for this species were the more preferred endpoint. For *L. fasciola,* results from Bringolf *et al.* (2007) and Gillis (2011) also suggested an ameliorating effect of hardness on chloride toxicity but the data was excluded since it was not reported over a sufficiently wide hardness range.

An F-test was conducted on the four species represented by datasets deemed acceptable for hardness-toxicity slope calculations. Results from this test showed that the regression slopes for the eight species were not significantly different from one another (p = 0.905), thus an analysis of covariance (ANCOVA) was run to calculate a pooled slope across the whole dataset. The log transformed chloride effect concentration served as the dependant variable, the three species formed the grouping variable, and the logarithm of hardness was the independent variable. From the ANCOVA analysis, the pooled slope was calculated to be **0.473** with a corresponding R^2 value of 0.939.

The calculated pooled slope of 0.473 was then used to normalize all of the toxicity values in the long-term SSD (Section 4) to a hardness of 50 mg/L using Equation 2.3.

$$\mathbf{LT}\boldsymbol{EC}_{x(50\ mg\ CaCO3/L)} = 10^{\{([\log(50) - \log(\text{hardness})] \times \text{LTPS}) + \log(\text{LTECx})\}} \qquad \textit{(Eq. 2.3)}$$

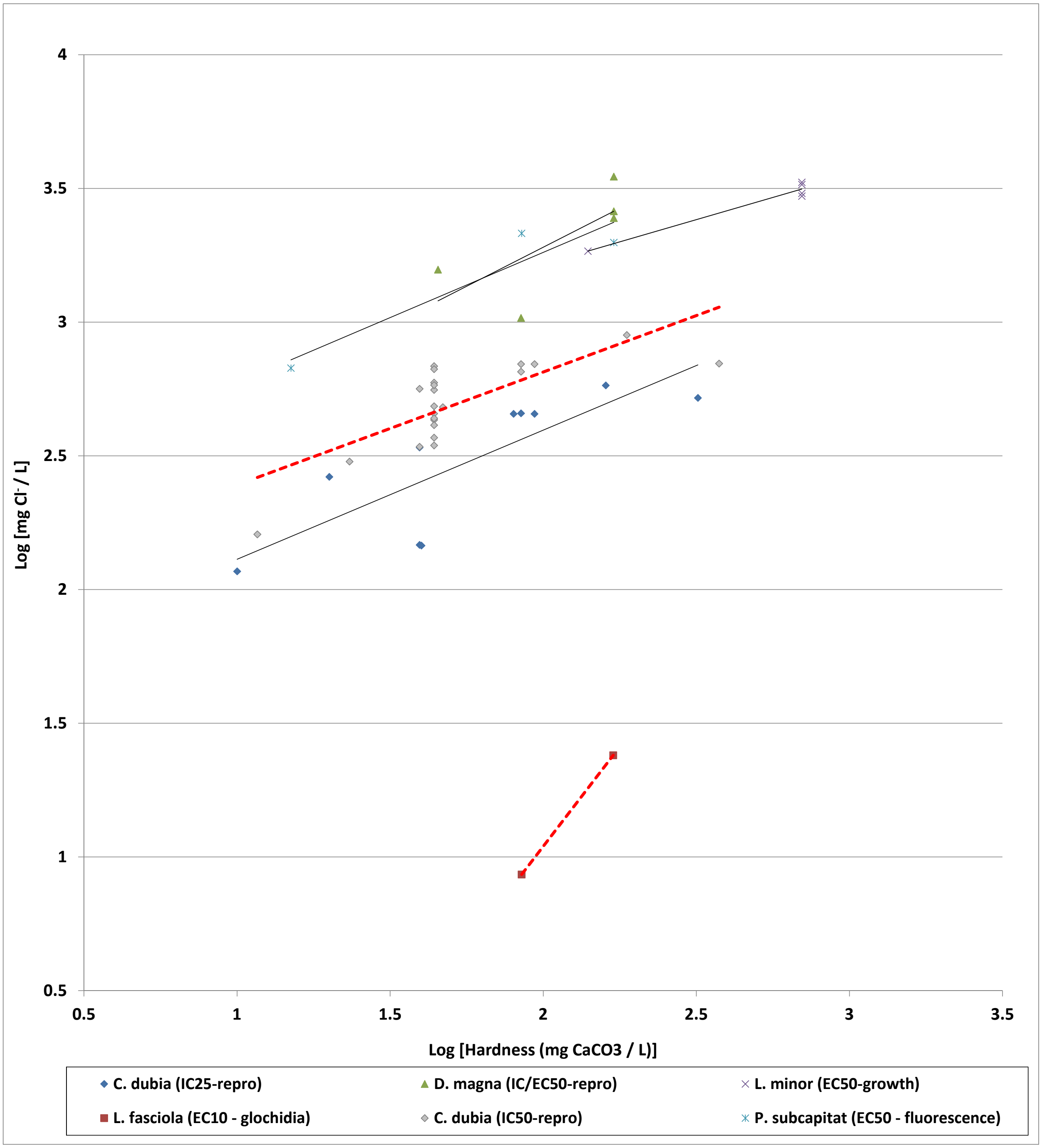
Where **LT*EC*$_{x(50\ mg\ CaCO3\ /L)}$** is the short-term normalized effect concentration measured in mg/L and hardness was measured as $CaCO_3$ equivalents in mg/L.

Table 5.5. Long-term hardness-toxicity regression slopes

Species	n	Slope	R^2	df
Ceriodaphnia dubia **(IC_{25} - repro)**	10	0.483	0.662	8
Daphnia magna **(IC/EC_{50} - repro)**	5	0.583	0.531	3
Lemna minor **(EC_{50} - growth)**	5	0.332	0.958	3
Pseudokirchneriella subcapitata ***(E_{50} –population florescence)***	3	0.488	0.888	1
POOLED SLOPE	**23**	**0.473**	**0.939**	**-**
****Ceriodaphnia dubia*** **(IC_{50} - repro)**	22	0.422	0.578	20
****Lampsilis fasciola*** **(EC_{10-30} glochidia survival)**	2	1.491	-	0

** Data not used in hardness-toxicity slope calculation but plotted in Figure 5.6 for comparative purposes.*
Note: Summary of data points used here can be found in Appendix B.

Figure 5.6. Long-term hardness-toxicity relationships



Note: *Red hashed regression lines represent data not meeting the hardness range or other requirements (Stephan et al. 1985). See Appendix B further details.*

5.3.2 Long-Term SSD and Water Quality Guideline

In order to construct a hardness adjusted long-term SSD, 7 of the 32 species from the unadjusted SSD curve (Table 4.1) were excluded since no hardness data was available for these data points. The species excluded were: *M. securis* (fingernail clam), *S. natans* (floating fern), a species of fresh water snail (*Physa sp.)*, *G. pseudolimnaeus* (Amphipod), and three species of algae (*C. zofingiensis*, *C. minutissimo*, and *Synechocystis sp.*). With the exception of *M. securis* (HPP = 0.11), these excluded species were not found to be sensitive to chloride toxicity on the unadjusted SSD, therefore removing them was not expected to have a substantial impact on the SSD benchmark derivation.

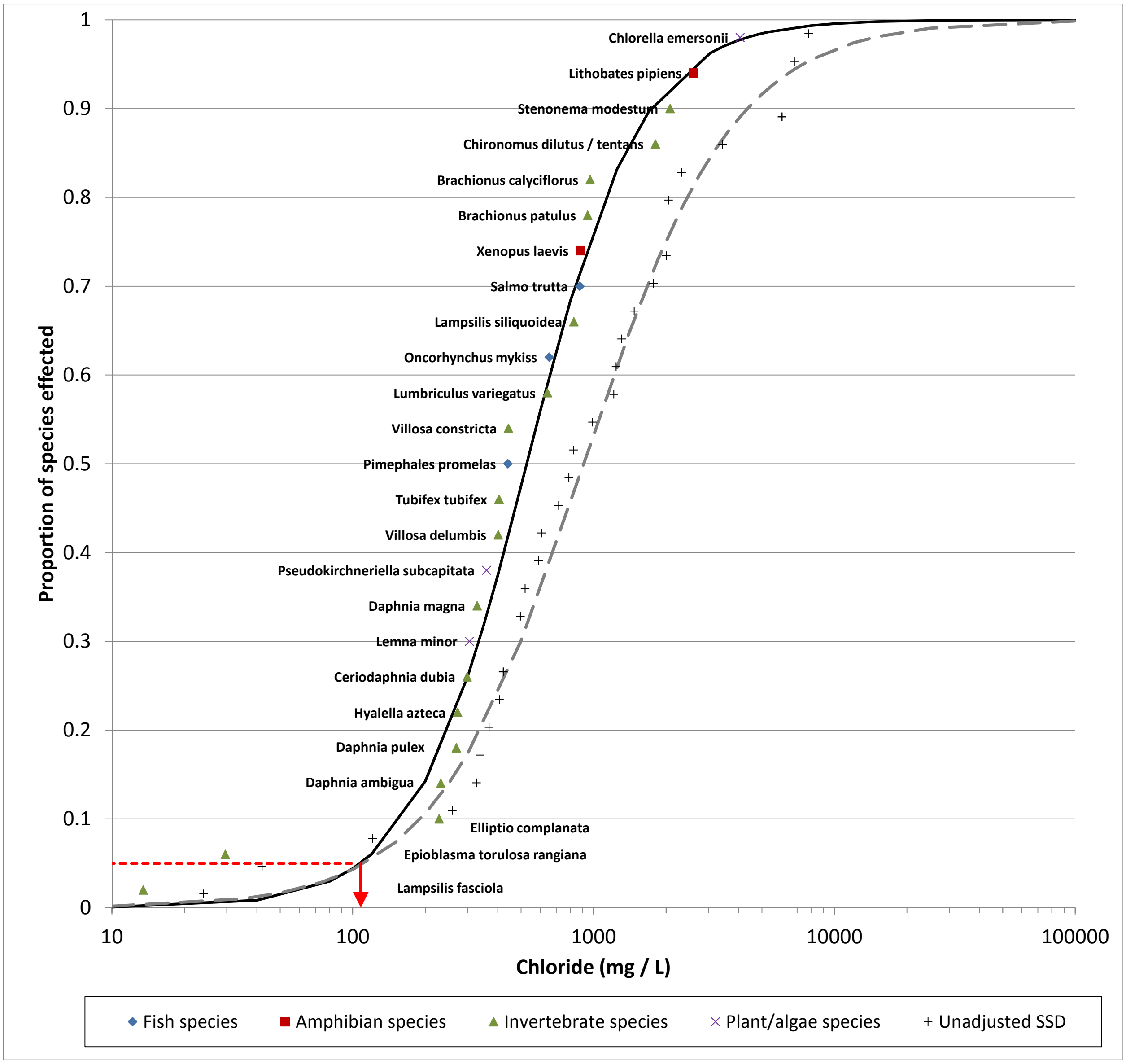
Using the remaining 25 species, a long-term SSD was constructed with effect concentrations normalized to a hardness of 50 mg $CaCO_3$ /L utilizing Eq. 2.3 and the pooled slope of 0.473 derived in the previous section. SSD derivation followed the same methods as described previously. The Log-Logistic Function provided the best overall fit for the data with the lowest AIC (Akaike's Information Criteria) value. This model also minimized the scaled residuals of each of the data points. The equation of the model was:

$$y = 1 / [\, 1 + e^{-(b + m * \ln(x))} \,] \qquad \textbf{(Eq. 3.1)}$$

Where y is the proportion of species affected and x is the chloride concentration. The slope of the line (m) and the y-intercept (b) were calculated to be 1.85 and -11.60 respectively.

Figure 5.7 shows the long-term hardness adjusted SSD made up of no and low effect concentrations normalized to 50 mg $CaCO_3$ /L with corresponding Hazen plotting position values. The short-term benchmark concentration, defined as the 5th percentile along the derived Log-Logistic function (Eq. 5.1) was **108** mg/L chloride with a lower-bound confidence limit of **103** mg/L chloride.

Figure 5.7. Hardness adjusted long-term SSD – no and low effect concentrations for NaCl



***Notes**:1) SSD derived by plotting no and low effect concentrations (preferred endpoints: EC/ICx representing a no-effect threshold > EC/IC10 > EC/IC11-25 > MATC > NOEC > LOEC > EC/IC26-49 > nonlethal EC/IC_{50}) adjusted to a hardness of 50 mg $CaCO_3$ /L for 32 species of aquatic organism against their corresponding Hazen plotting position (HPP); 2) Red hashed line indicates the 5th percentile (y-axis) and the resulting long-term WQG (x-axis) of 108 mg Cl^- /L; 3) Grey hashed curve and black crosses represent the unadjusted SSD (Figure 4.1).*

5.3.3 Hardness Equation for Calculating Long-Term Water Quality Guidelines

The long-term hardness equation, used for calculating water quality guidelines across various hardness levels is based on the US EPA procedure outlined in Stephan *et al.* (1985). The function was derived using the long-term hardness-toxicity slope of 0.473 calculated in Section 5.3.1 (m) and the derived long-term WQG of 108 mg Cl^- /L (y) at a hardness of 50 mg $CaCO_3$ /L (x) derived in Section 5.3.2. Since the slope (m), x, and y variables were known, the general equation log(y) = m*log(x)+b was rearranged to solve for b (y-intercept) which was found to be 1.227. Using this value the equation of the line to determine the long-term hardness dependant water quality guideline concentration can be expressed as:

$$\textit{Long-term WQG} = 10^{[\,0.473\,(\log(\text{hardness})) + 1.227]} \qquad \textit{(Eq. 5.2)}$$

Where the WQG concentration is measured in mg/L chloride and hardness is in $CaCO_3$ equivalents (mg/L).

Figure 5.8 provides a visual representation of the 50 mg $CaCO_3$ /L hardness adjusted SSD along with modeled curves at hardness concentration of 100, 200, 400, and 600 mg $CaCO_3$ /L. These values represent the upper bound thresholds for water classified as soft, moderately soft, moderately hard, hard, and very hard respectively (Alberta Government 2011). The long-term WQG chloride concentrations corresponding to these hardness levels are indicated in Figure 5.8 and are summarized in the upper half of Table 5.6

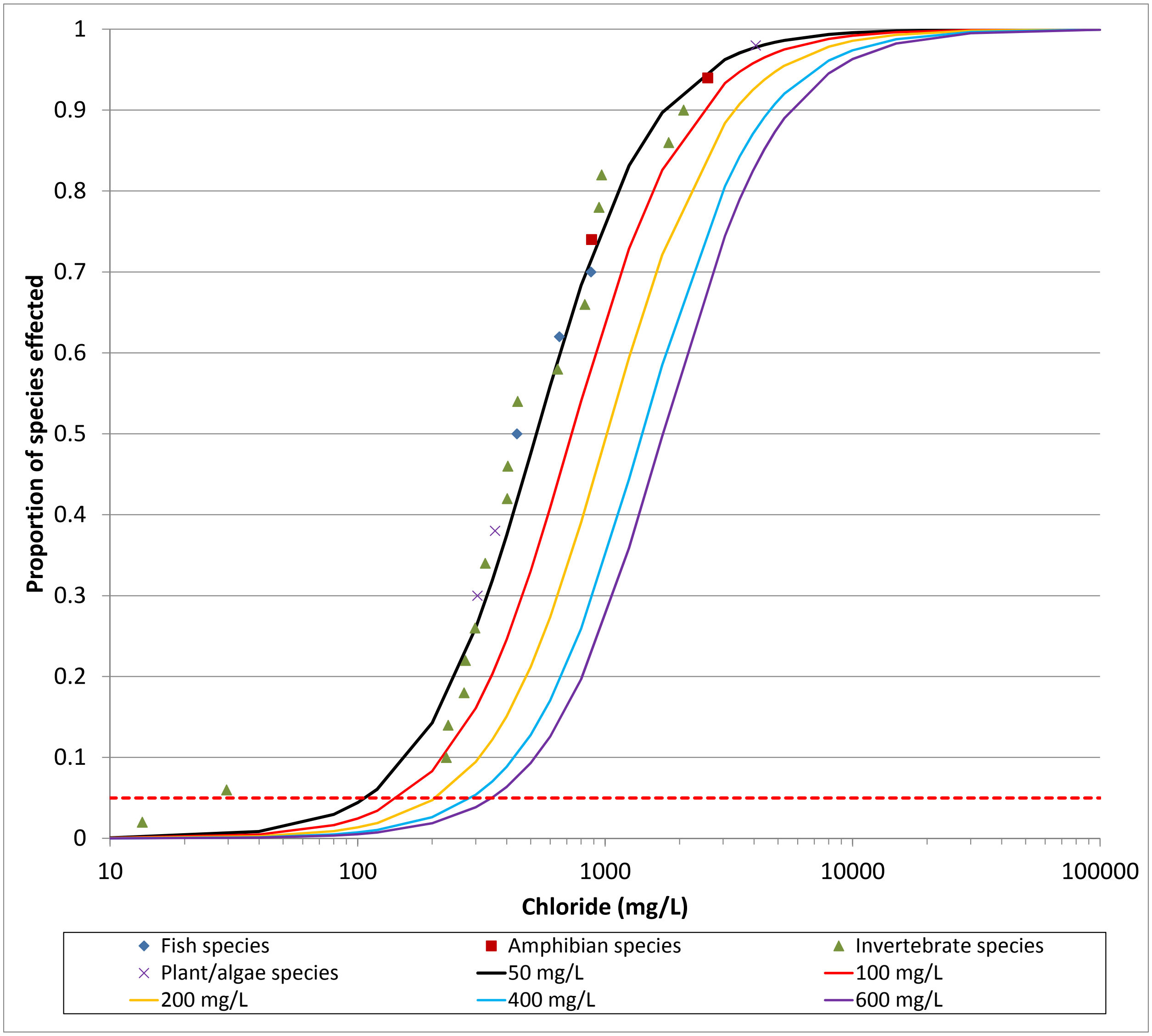
In order to compare the long-term dataset and the resulting WQGs to existing national guidelines, the CCME (2011) long-term SSD was adjusted for water hardness. The approach paralleled methods outlined previously and involved transforming raw effect concentrations to a standard hardness of 50 mg $CaCO_3$ /L using Equation 2.3. The long-term pooled slope estimate (0.473) derived in Section 5.3.1 was utilized in data transformations. Five data points from the original CCME (2011) dataset were not associated with water hardness concentrations and were therefore removed. The most sensitive of these data points was the Fingernail Clam *A. maculatum* that occurred below the 10^{th} percentile of the unadjusted SSD (CCME 2011). All other removed data points occurred above the 70^{th} percentile (*Physa sp., G. pseudolimnaeus, C. minutissimo, C. zofingiensis*), and is expected to have a minor influence on the results. A 50 mg $CaCO_3$ /L adjusted SSD was then modeled using the Log-Logistic function where a BMDL of 109 mg Cl^- /L was obtained. Using the modeled Log-Logistic function derived from the SSD analysis, a hardness equation was established to calculate long-term WQG concentrations at various hardness levels. These results, along with the equation used to derive them, are presented in the lower half of Table 5.5. From the results presented in Table 5.6 it can be seen that the long-term guidelines are approximately equivalent to the hardness adjusted guidelines using the original CCME (2011) dataset.

Table 5.6. Long-term hardness-adjusted WQG concentrations

Hardness ($mg\ CaCO_3/L$)	Long-term WQG ($mg\ Cl^-/L$)
Current derivation	***($10^{[0.473 (\log(hardness)) + 1.227]}$)***
50 (soft)	**108**
100 (moderately soft)	**149**
200 (moderately hard)	**207**
400 (hard)	**288**
600 (very hard)	**348**
CCME 2011 data	***($10^{[0.473 (\log(hardness)) + 1.234]}$)***
50 (soft)	**109**
100 (moderately soft)	**152**
200 (moderately hard)	**210**
400 (hard)	**292**
600 (very hard)	**354**

Note: *The SSD utilized to derive CCME long-term hardness-adjusted guidelines was made up of n=23 data points, 5 less than the original 2011 dataset (n=28). This was the result of the loss of 5 data points where hardness data was not available.*

Figure 5.8. Adjusted long-term SSD at various water hardness levels



***Notes**: 1) Black curve represents the 50 mg $CaCO_3$ /L hardness adjusted SSD with associated species; 2) Red hashed line indicates the 5th percentile (y-axis) and the resulting long-term WQG concentrations (x-axis) of 108, 149, 207, 288, 348 mg Cl^- /L corresponding to hardness values of 50, 100, 200, 400, 600 mg $CaCO_3$ /L respectively.*

6 ASSESSMENT OF GUIDELINE PROTECTIVENESS

To evaluate the protectiveness of the both the short- and long-term hardness adjusted guidelines, an assessment was conducted that involved plotting the applicable effect concentrations available in the database (short-term: severe effect LC/EC50s; long-term: no and low effect data) as a function of hardness. The associated short- and long-term guidelines were then plotted, and data points occurring below these respective guidelines examined in detail to assess protectiveness.

6.1 PROTECTIVENESS OF THE SHORT-TERM BENCHMARK

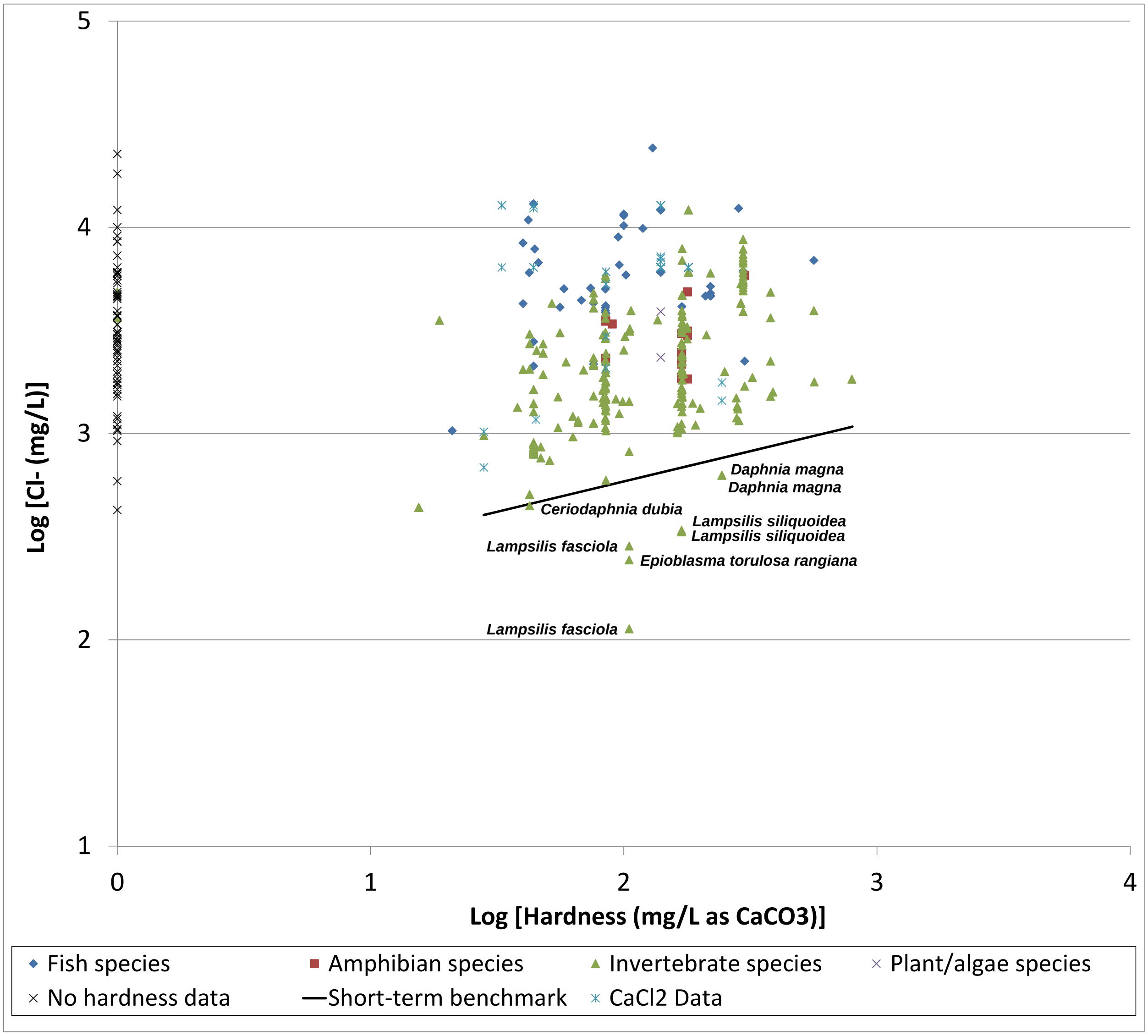
As outlined previously (Section 5.2.1), the hardness adjustment equation for the short-term benchmark was based on the hardness-toxicity relationship established for nine species. To test the protectiveness of this guideline, all acceptable chloride toxicity values (uncorrected for hardness) were plotted against their respective hardness values (Figure 6.1). These values were then compared to the derived short-term benchmark, which is represented by the straight line in the figure. All values falling below the derived guideline are examined in detail in order to determine if the derived guideline is adequately protective to all endpoints. The CCME used a similar approach in the derivation of guidelines for cadmium (CCME 2014).

Of the 361 data points plotted in Figure 6.1, eight fell under the short-term benchmark: one point for the water flea *C. dubia* (48 h LC_{50} from Hoke *et al.* 1992); two points for the water flea *D. magna* (24 and 48 h LC_{50} values from Khangarot and Ray 1989); one point for the fresh water mussel *E. torulosa rangiana* (24 h EC_{50} (glochidia survival) from Gillis 2011); two points for the fresh water mussel *L. fasciola* (both 24 h EC_{50} values (glochidia survival) from Gillis (2011); and, two points for *L. siliquoidea* (24 and 48 h EC_{50} values (glochidia survival). For four of the five species, there were a number of other data points above the short-term benchmark, specifically 28, 28, 4, and 8 for *C. dubia*, *D. magna*, *L. fasciola*, and *L. siliquoidea* respectively. Interestingly, all of the data points that fell below the short-term benchmark threshold in Figure 6.1 were incorporated into the short-term SSD (Tables 3.1 and 3.2). For *C. dubia*, *L. fasciola*, and *L. siliquoidea* the collated values used in the SSD (1151, 746, and 892 mg Cl^- /L respectively) all plot above short-term benchmark.

E. torulosa rangiana represents the second most sensitive effect concentration in the short-term data set (Figure 6.1), and the most sensitive species in the short-term SSD (Section 5.2.2). Only one short-term data point exists for this species (a 25 h EC_{50} (glochidia survival) of 244 mg Cl^- /L from Gillis 2011), thus no comparisons are possible across studies. Although *D. magna* is represented by 28 data points above the short-term benchmark (Figure 6.1) the species is the second most sensitive on the short-term SSD and with *E. torulosa rangiana*, represents the only other species occurring below the 5^{th} percentile benchmark value (478 mg Cl^- /L) on the 50 mg $CaCO_3$ /L hardness adjusted SSD. The same data points were also utilized in the 2011 CCME chloride guideline derivation. One point representing *C. dubia* occurs at the benchmark concentration threshold, however all other values for this species occur above the guideline. Although two values occur below the guideline for the mussel *L. fasciola*, the majority (5 out of 7) occur above. Similarly, for the mussel *L. siliquoidea*, the two values occurring below the guideline represent only 11% of the data for this species (the rest of the data points occur above the guideline), thus the guideline is protective for this species. Based on Figure 6.1, short-term exposures to levels of chloride exceeding the benchmark concentration may pose the greatest hazard to the glochidia lifestage of certain freshwater mussel species and to *Daphnia magna*. However, as the short-term benchmark concentration is intended for assessing the potential for severe effects following intermittent or short-lived chloride exposure, the protection clause does not apply (CCME 2007) and meeting the proposed long-term guideline will protect from severe effects.

Calcium chloride was also incorporated into Figure 6.1. No $CaCl_2$ data points were found to occur below the NaCl derived short-term benchmark. Thus, based on the current dataset, the short-term benchmark appears to be protective of aquatic organisms from exposures to $CaCl_2$. This is supported by comparative toxicity results (Section 3.1) in which sodium and calcium chloride salts were found to have similar toxicities with calcium being slightly less toxic. This guideline, however, will not be protective to chloride exposures from the other major chloride salts, KCl and $MgCl_2$, which are comparatively more toxic than NaCl towards aquatic organisms.

Figure 6.1. Protectiveness of the short-term benchmark



***Notes**: 1) All toxicity values, including those not utilized in the SSD derivation, are plotted; 2) Black line represents the short-term benchmark concentration over a continuous hardness range, constrained to the hardness range used in deriving the short-term hardness adjustment equation (Figure 5.3.). 3) Effect concentration with no reported hardness data are plotted for context (black x) and were set to an arbitrary hardness of 1 mg $CaCO_3$ /L.*

6.2 PROTECTIVENESS OF THE LONG-TERM WATER QUALITY GUIDELINE

The hardness adjustment applied to the long-term data was based on the slopes of the hardness-toxicity relationships of a limited number of species (n=4). The appropriateness of this guideline for extrapolation to other species, including sensitive species in the chronic SSD that drives the chronic water quality guideline, has limitations as these species may respond differently to hardness influence on chloride salt toxicity. In order to assess the protectiveness of the hardness-adjusted guideline to all aquatic organisms, acceptable toxicity values uncorrected for hardness were plotted against the hardness adjusted guideline (Figure 6.2). Some of the data points plotted on Figure 6.2 have been incorporated into the SSD (as well as CCME's 2011 SSD), but in other cases the data could not be included in the SSD for various reasons. Their assessment here provides a better understanding of the protectiveness of the derived guideline

Of the 404 acceptable long-term values plotted in Figure 6.2, four fell below the long-term WQG for chloride (Table 6.1). These include the 24h EC_{10} values of 24 mg Cl^- /L and 42 mg Cl^- /L for *Lampsilis fasciola* and *E. torulosa rangiana*, respectively. Both of these unionid mussels are considered species at risk by COSEWIC, *L. fasciola* is classified as special concern, while *E. torulosa rangiana* is classified as endangered. These results are similar to the CCME (2011) derivation where the same data points were the sole occurrences below the long-term SSD 5th percentile value of 120 mg Cl^- /L. As part of the CCME (2011) guideline derivation, the Protection Clause (CCME, 2007) was applied to ensure that these species at risk received adequate protection. The protection clause may be invoked for COSEWIC species at risk when no-effect or low-effect data points occur at levels lower than the proposed guideline. Based on the analysis conducted here, the same protection clause can be applied:

> "In areas where the COSEWIC special concern mussel (*L. fasciola*) or the COSEWIC endangered mussel (*E. torulosa rangiana*) are present, the protection clause can be implemented, resulting in a guideline value ranging from 24 to 42 mg Cl^- /L. In all other areas where non-endangered freshwater mussels are present, the long-term SSD 5th percentile value should be used as the guideline value"

Traditionally, toxicity assays using standard laboratory-cultured species have found daphnids to be the most sensitive species to chloride salt toxicity (IDNR 2009a, US EPA 1986). However, the results presented here suggest that freshwater mussels and clams may be more sensitive receptors to sodium chloride. The five closest points to the guideline regression are occupied by freshwater bivalves (labeled points in Figure 6.2), while the next most sensitive endpoints are 168 h IC_{25} and NOECs (reproduction) of 146-152 mg Cl^- /L for C. dubia at 39-47 mg $CaCO_3$ /L hardness (Elpick *et al.* 2011, Lasier and Harden 2010, Aragao and Pereira 2003).

All of the sensitive endpoints for freshwater mussels and clams are from studies examining the glochidia lifestage. Evidence suggests that free-living glochidia survive in the water column for durations varying between 24 h to 10 days (ASTM 2013, Cope *et al.* 2008). In the current dataset, glochidia endpoints at durations greater than 24h were available from four studies (Valenti *et al.* 2007, Pandlfo *et al.* 2012, Echols *et al.* 2012, Bringolf *et al.* 2007; Appendix A). Glochidia exposures at 48 h compared to 24 h generally showed either comparable effect concentrations or demonstrated slightly greater toxicity (*e.g.* Bringolf *et al.* (2007): *V.constricta* EC_{50} of 1674 (24h) and 1571 mg Cl^-/ L (48h); *E. complanata* EC_{50} of 1620 (24h) and 1353 mg Cl^-/ L (48h); and *L. fasciola* EC_{50} of 1116 (24h) and 1056 mg Cl^-/ L (48h)), with the exception of *V. delumbis* in which the 24 h exposure was more toxic (Bringolf *et al.* (2007): *V.delumbis* EC_{50} of 2008 (24h) and 2202 mg Cl^-/ L (48h)). However, as outlined by the CCME (2011), glochidia data was limited to 24 h exposures due to a lack of species-specific knowledge on glochidia lifespans. Recently, studies by Bringolf *et al.* (2013) have resulted in the recommendation of a maximum test duration of 24 hours for glochidia which has been adopted in the US EPA guideline for ammonia (US EPA 2013b). The current derivation utilizes glochidia data only at exposure durations of ≤ 24h.

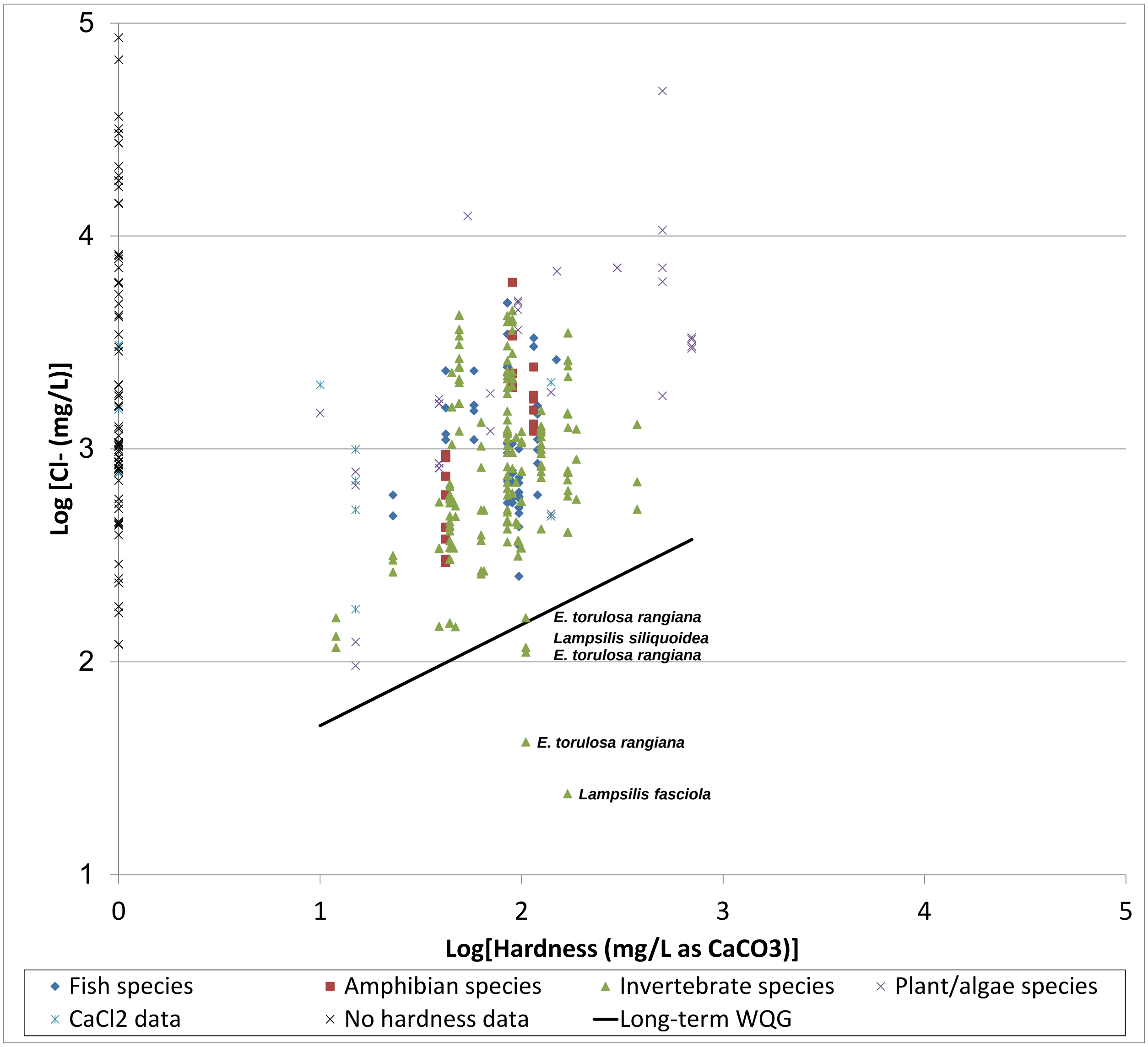
There was insufficient data (as outlined in Table 2.1) to conduct a Type A (SSD) analysis of the long-term $CaCl_2$ data set, and as a result a long-term WQG for $CaCl_2$ was not established. However, it is important to assess the protectiveness of the derived long-term WQG (NaCl based) to this chloride salt, since sodium and calcium chloride have been found to have similar toxicities, with slight greater toxicity for sodium (Section 3.1). From the comparison of short-term toxicities, the 5th percentile benchmark concentration for $CaCl_2$ was 703 mg Cl^- /L, 44 mg Cl^- /L higher than the derived guideline for NaCl (659 mg Cl^- /L). Although data limitations are reflected in the wide confidence limits for $CaCl_2$ (Table 3.1, BMDL), the current dataset and literature review suggests that the long-term WQG derived based on NaCl data should also provide protection to exposures from $CaCl_2$. To test this, all available long-term toxicity endpoints for CaCl2 and associated water hardness concentrations were plotted against the long-term WQG (NaCl).

Figure 6.2 shows that none of the available chloride effect concentrations from $CaCl_2$ toxicity assays (Appendix A) fell below the long-term WQG derived for NaCl. As a result, based on the current dataset, the long-term WQG will protect aquatic organisms from exposures to $CaCl_2$. This guideline, however, will not be protective to chloride exposures from the other main chloride salts, KCl and $MgCl_2$, which are comparatively more toxic than NaCl towards aquatic organisms (Section 3.1).

Table 6.1. Studies reporting endpoints near or below the long-term hardness adjusted WQG

Species	Common name	Salt	Endpoint	Endpoint criteria	Effect conc. (mg Cl^- /L)	Hardness (mg $CaCO_3$/L)	Citation
L. fasciola	Freshwater mussel	NaCl	24 h EC_{10}	Wavy-Rayed lampmussel	24	169	Bringolf *et al.* (2007)
E. torulosa rangiana	Freshwater mussel	NaCl	24 h EC_{10}	Northern Riffleshell	42	105	Gillis (2009)
E. torulosa rangiana	Freshwater mussel	NaCl	24 h EC_{20}	Northern Riffleshell	111	105	Gillis (2009)
L. siliquoidea	Freshwater mussel	NaCl	24 h EC_{30}	Glochidia survival	117	105	Gillis (2009)
E. torulosa rangiana	Freshwater mussel	NaCl	24 h EC_{30}	Northern Riffleshell	161	105	Gillis (2009)

Figure 6.2. Protectiveness of the long-term WQG



***Notes**: 1) All toxicity values, including those not utilized in the SSD derivation, are plotted; 2) Black line represents the long-term WQG concentration over a continuous hardness range, constrained to the hardness range used in deriving the long-term hardness adjustment equation (Figure 5.6.). 3) Effect concentration with no reported hardness data are plotted for context (black x) and were set to an arbitrary hardness of 1 mg $CaCO_3$/L.*

7 GUIDELINE SUMMARY

Of the four major chloride salts (NaCl, $CaCl_2$, KCl, $MgCl_2$), only sodium chloride met both short- and long-term toxicological and statistical requirements for Type A guideline derivation (SSD method) as per CCME (2007). Results obtained by comparing short-term SSDs confirmed literature results on the relative toxicity of salt pairs occurring in the order of KCl > $MgCl_2$ > NaCl ≈> $CaCl_2$. Based on evidence that the toxicity of KCl and $MgCl_2$ can be attributed to the corresponding cation (rather than chloride), these salts were not assessed further in this review which follows the rational of the CCME (2011).

The short-term (NaCl) data met all of the requirements for Type A guideline derivation with 56 species specific data points. The log-logistic model provided the best fit to the data and the derived short-term benchmark concentration was **659 mg/L**, in relatively close proximity to the value of 640 mg/L established by the CCME (2011). Using toxicity-hardness relationships from 8 species, a pooled slope of **0.294** was established and used to adjust the short-term SSD to a hardness of 50 mg $CaCO_3$ /L which produced a short-term benchmark of **478 mg/L**. From this analysis the hardness equation used for calculating benchmark concentrations was established (Table 7.1).

The long-term (NaCl) data met all of the requirements for Type A guideline derivation with 32 species specific data points. The log-logistic model provided the best fit to the data and the derived long-term WQG was **112 mg/L**, 8 mg/L lower than the 120 mg/L value established by the CCME (2011). Using toxicity-hardness relationships from 3 species, a pooled slope of **0.473** was established and used to adjust the long-term SSD to a hardness of 50 mg $CaCO_3$ /L which produced a long-term WQG of **108 mg/L**. From this analysis, the hardness equation used for calculating appropriate water quality guidelines was established (Table 7.1). Although a full Type A analysis was not possible for $CaCl_2$, the sodium chloride derived guidelines were found to be protective towards all appropriate $CaCl_2$ toxicity values available in the database (Section 7).

Table 7.1. Summary of chloride guidelines for the protection of aquatic life

	Short-term exposure (mg/L)	Long-term exposure (mg/L)
Hardness equation	***Benchmark =*** $10^{[0.294(\log(\text{hardness}))+2.179]}$	***WQG =*** $10^{[0.473(\log(\text{hardness}))+1.227]}$

Notes: *1) hardness measured as mg/L as $CaCO_3$; 2) the short-term hardness equation is applicable between the 5th and 95th percentile values of 42 to 563 mg $CaCO_3$ /L and should not be applied outside of this range; 3) the long-term hardness equation is applicable between the 5th and 95th percentile values of 20 to 699 mg $CaCO_3$ /L and should not be applied outside of this range.*

In the CCME (2011) guideline derivation for chloride, reasonable extremes of water hardness in Canada are presented as 5 to 240 mg $CaCO_3$ /L. However, in the context of Alberta and other provinces where water bodies generally contain harder water (Figure 5.2), an alternative range may be more appropriate. Table 7.2 is adapted from data presented in CCME (2011). The average hardness in Alberta surface waters was reported to be 126 mg $CaCO_3$ /L with 10th and 90th percentile values of 86 and 207 mg $CaCO_3$ /L, respectively (range of 23-602 mg $CaCO_3$ /L). These values agree with those reported by Mitchell (1990) who found an average hardness of 134 (range of 35-328) mg $CaCO_3$ /L from 100 lakes across Alberta. Applying the long-term WQG equation to the 10th and 90th percentiles of water hardness (Table 7.2), suggests that chloride guidelines could be expected to range between approximately 140 - 210 mg Cl^- /L for major surface waters in Alberta. It is important to note that the province's Canadian Shield region and some brown water lakes in northern Alberta are known to contain soft water with concentrations less than 10 mg $CaCO_3$ /L (Michell 1990). Alternatively, water hardness in sloughs and other small water bodies can reach concentration much higher than the 90th percentile shown in Table 7.2., highlighting the importance of the site-specific applications of the derived chloride guidelines.

Table 7.2. Water hardness summary for major surface waters across Canada

Province	Hardness (mg $CaCO_3$ /L)				
	Min	10th percentile	50th percentile	90th percentile	Max
Newfoundland and Labrador[4]	0.45	2.4	6.3	40	664
Nova Scotia[4]	0.25	1.2	2.1	4.6	94
New Brunswick[4]	0.62	2.2	9.7	66	831
P.E.I.[4]	0.17	33	54	110	459
Quebec[4]	2.9	9	38	112	1078
Ontario	5.8[1] 0.2[2]	93[1] 47[2]	226[1] 118[2]	318[1] 170[2]	1920[1] 1920[2]
Manitoba[4]	41	46	287	402	590
Saskatchewan[4]	25	145	300	531	702
Alberta[4]	**23**	**86**	**126**	**207**	**602**
British Columbia[3]	0.33	39	68	185	267
Yukon[4]	0.24	44	85	147	688
Northwest Territories[4]	42	99	134	214	357
Nunavut	NA	NA	NA	NA	NA

Notes: *1) adapted from CCME (2011); 2) NA = data was not available.*
[1]PWQMN data collected 2003 to 2007 (P.Desai, Ontario MOE, 2009, pers.comm.).
[2]Great Lakes - Great Lakes Connecting Channel data from Environmental Monitoring and Reporting Branch collected 1990 to 2007 (P.Desai, Ontario MOE, 2009, pers.comm.)
[3]British Columbia Federal-Provincial river trend sites, with data collected from 1979 to 2009 (T. Dessouki, British Columbia MOE, 2009, pers.comm.)
[4]C. Lochner, Water Quality Monitoring and Surveillance, Environment Canada, 2009, pers.comm

The derivation of the unadjusted short-term and long-term SSDs allows for comparisons with those derived by the CCME (2011). In terms of the short-term SSD, the 5th percentile benchmark concentration obtained was approximately 20 mg/L higher than that obtained by the CCME of 640 mg/L. This disparity might be explained by the different modeling approach (log-Normal model used by the CCME), the five additional species (*Hydra attenuate, Tricorythus sp., Pycnopsyche sp., Hyla chrysoscelis, Chironomus xanthus*) used in the current data set, or from the higher effect concentration used for the fatmucket clam (*L. siliquoidea*). The collated effect concentration used for this species was increased from 709 mg Cl^- /L (CCME 2011) to 892 mg Cl^- /L with the addition of data from Cope *et al.* (2008) and because of its low position on the SSD, may have resulted in a higher modeled benchmark concentration. Overall the 20 mg/L increase was not seen as a significant departure from the results obtained by the CCME (2011).

The unadjusted long-term WQG of 112 mg Cl^- /L is lower than the CCME derived guideline of 120 mg/L. In both derivations (CCME and herein), the Log-Logistic model was found to best fit the data. The more sensitive guideline is the result of new additions to the SSD dataset, along with revised effect concentrations for a number of species located relatively low on the curve. Three data points in particular are important, and likely the primary factors driving the greater sensitivity of the current model. One is a 96h EC_{10} endpoint of 96 mg Cl^- /L for the green algae *P. subcapitata* (Simmons 2012). This data point represents a departure from the CCME derivation where algae species were only represented high on the curve with effect concentrations > 6000 mg Cl^- /L (See Section 4.1.1 for further discussion). Two data points representing *C. dubia* and *L. minor* have also been reduced from 454 to 337 mg Cl^- /L and 1171 to 496 mg Cl^- /L respectively with the addition of data from Elphick *et al.* (2011a), Lasier and Harden (2010), and Simmons (2012).

8 KNOWLEDGE GAPS AND RESEARCH RECOMMENDATIONS

Knowledge gaps are discussed within the context of species for which hardness data are available, or not, for adjusting SSDs used to derive a hardness-based WQG. Particular focus is given to those species that are considered sensitive taxa, with effect concentrations that occurred below the 20th percentile of the two SSDs (short-term and long-term).

Research recommendations are provided for each of the major taxonomic groups, which take into account sensitivity to chloride, current gaps in knowledge of hardness-toxicity relationships, applicability to Alberta native species, and data currently available in the literature.

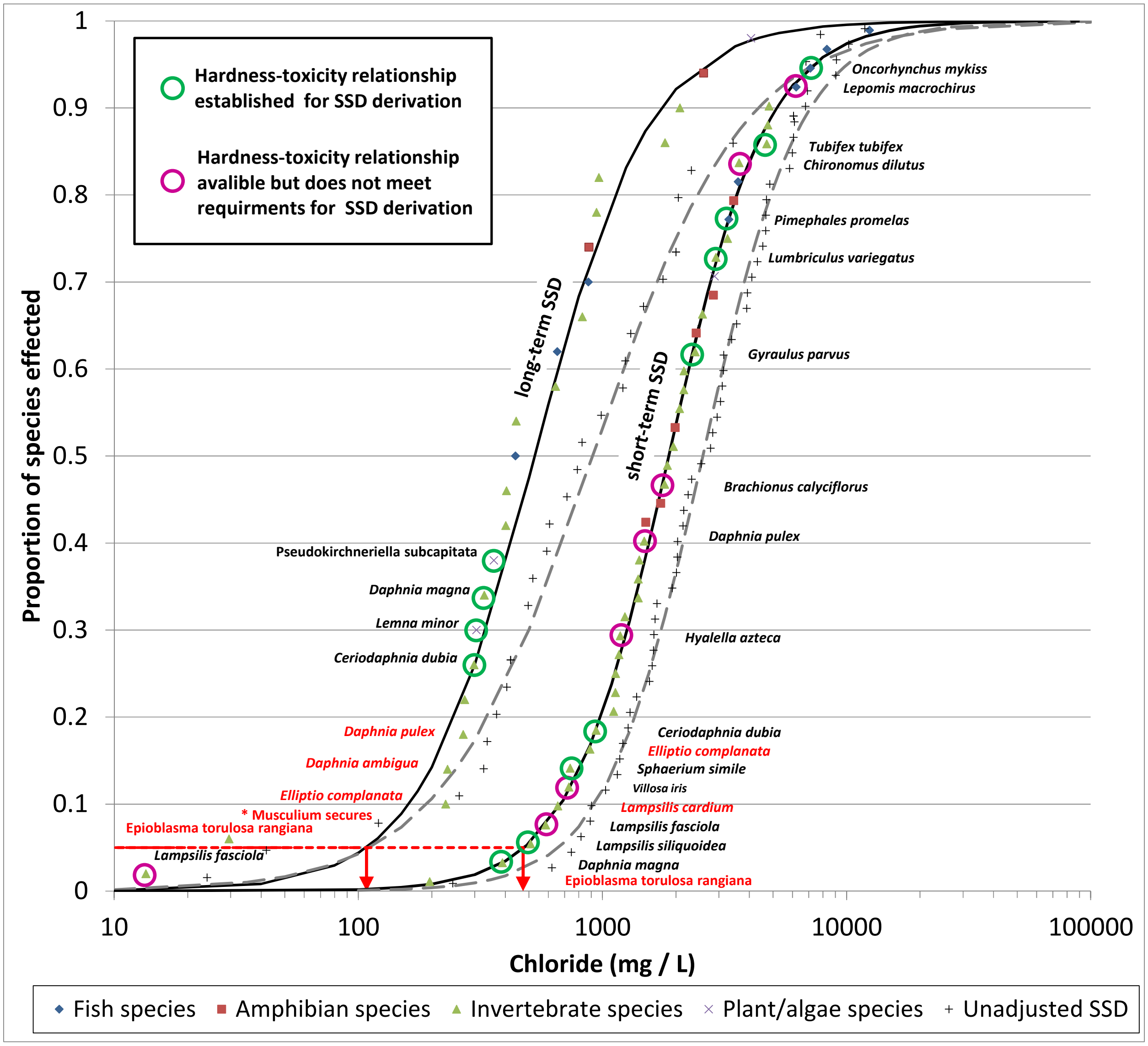
8.1 SUMMARY OF HARDNESS-TOXICITY DATA LIMITATIONS

In Figure 8.1 both the short- and long-term SSDs are presented. Data points circled in green represent species in which a hardness-toxicity relationship has been established, which was applied in SSD derivation. Purple circles represent those species where the ranges of hardness values available do not meet the minimum requirements for guideline derivation. More specifically, they do not meet the requirement that the highest hardness is at least three times the lowest and the highest is also at least 100 mg/L higher than the lowest (US EPA 2001; Stephan 1985). The absence of green or purple circles indicates that no hardness data is available (species below the 20th percentile with no available hardness data are indicated in red text).

For the short-term SSD, the majority of sensitive species occurring below the 20th percentile, have hardness data available such that a hardness-toxicity relationship can be calculated, although two of these species, *Lampsilis fasciola* and *Villosa iris*, do not meet the minimum range requirements of the CCME (*sensu* Stephan *et al.* 1985). Three unionid bivalves (*E. torulosa rangiana*, *L. cardium*, *E. complanata*) represent the only sensitive organisms where no hardness-toxicity relationship has been established.

In contrast to the short-term SSD, long-term hardness-toxicity data is limited. Below the 20th percentile, only the most sensitive species, *L. fasciola*, has an established hardness-toxicity relationship; however it does not meet minimum range requirements for SSD derivation (*sensu* Stephan *et al.* 1985). Five sensitive species *E. tarulosa rangiana, M. secures, P. subcapitata, E. complanata, and D. Ambigua* have no hardness-toxicity relationship established. The sensitive endpoint available for the *M. secures* did not have any water hardness data associated with it and could not be included in the long-term adjusted SSD (see asterisk in Figure 8.1). On the long-term SSD, the three species used in the hardness-toxicity pooled slope calculation (*L. minor, C. dubia, D. magna*) occur between the 30th and 40th percentile.

Figure 8.1. Short- and long-term SSDs with associated hardness-toxicity relationships



***Notes**: 1) Species on the short- (solid line, right side) and long-term (solid line, left side) hardness adjusted (to 50 mg $CaCO_3$ /L) SSDs are circled in green if a hardness-toxicity relationship has been established which was applied in SSD derivation. Purple circles represent those species where the ranges of hardness values available do not meet the minimum requirements for guideline derivation (sensu Stephan et al. 1985). Sensitive species (occurring on the SSD below the 20th percentile) which lack any established hardness-toxicity relationship are indicated in red text; 2) grey hashed curve and black crosses represent the unadjusted short- (right) and long-term (left) SSDs (Figures 3.2 and 4.1).*

****Musculium secures:** this species was found to hold a sensitive position on the long-term unadjusted SSD but could not be included in the hardness adjusted SSD since no water hardness data was provided in the original paper (Mackie 1978).*

8.2 SUMMARY OF TAXA SPECIFIC RESEARCH RECOMMNDATIONS

Based on the findings presented in Figure 8.1, future research should focus on determining long-term hardness-toxicity relationships for the sensitive unionid mussels, fingernail clam, algae, and daphnid species, which occur below the 20th percentile of the SSD. Because of the apparent long-term data limitations, future research should target chronic endpoints for sensitive species. Table 8.1 provides a list of appropriate species for future research based on their sensitivity to chloride, the current gaps in knowledge of hardness-toxicity relationships, the applicability to Alberta native species, and data currently available in the literature. Section 8.2 to 8.8 describes the species mentioned in this table, in greater detail.

Table 8.1. Targeted species and toxicity assays for future research into chronic endpoints

Species	Long-term endpoint	Exposure duration (h)	Lifestage	Rational	Related literature	Priority level
			BIVALVES			
Lampsilis siliquoidea	EC_{10} (glochidia functional survival)	24	glochidia	1) sensitive unionid mussel; 2) native to Alberta; 3) establishment of long-term hardness-toxicity relationship	Cope *et al.* 2008; Wang & Ingersoll 2010; Bringolf *et al.* 2007; Gillis 2011	**1**
Sphaerium secures / simile	ECx (low-effect data)	≥168 (non-lethal); ≥504 (lethal)	juvenile	1) sensitive sphaeriid mussel; 2) native to Alberta; 3) establishment of long-term hardness-toxicity relationship	Soucek *et al.* 2011; Mackie 1978	**1**
Lampsilis fasciola	EC_{10} (glochidia functional survival)	24	glochidia	1) sensitive unionid mussel; 2) establishment of long-term hardness-toxicity relationship	Valenti et al 2007; Echols et al 2012; Bringolf et al 2007; Gillis 2011	**1**
OTHER SENSITIVE UNIONIDS - *Elliptio complanata, Lampsilis cardium*	EC_{10} (glochidia functional survival)	24	glochidia	1) sensitive unionid mussel; 2) establishment of long-term hardness-toxicity for unionids occurring low on the SSD (*i.e. Elliptio complanata, Lampsilis cardium*)	Valenti et al 2007; US EPA 2010; Soucek et al 2011; Waller et al 1996; Pandolfo et al 2012; Wurtz & Bridges 1961; Echols et al 2012; Mackie 1978; Cope *et al.* 2008; Wang & Ingersoll 2010; Bringolf et al 2007; Gillis 2011; Wang 2007	**2**
			BRANCHIOPODA			
Ceriodaphnia dubia	ECx (reproduction, low-effect data)	≥168	< 24 h old	1) standard test organism	CHRONIC DATA: Elphick et al 2011a; Lasier & Harden 2010; Cowgill & Milazzo 1990; Aragao & Pereira; Cooney et al 1992; Lasier et al 2006; Diamond et al 1992; Harmon et al 1992	**3**
			ALGAE			
Pseudokirchneriella subcapita	ECx (low-effect data)	≥24	N/A	1) highly sensitive algae species; 2) establishment of long-term hardness-toxicity relationship	Simmons 2012; Santos et al 2007; Geis et al 2000	**1**

Table 8.1. Targeted species and toxicity assays for future research into chronic endpoints

Species	Long-term endpoint	Exposure duration (h)	Lifestage	Rational	Related literature	Priority level
SPECIES BATTERY APPROACH	ECx (low-effect data)	≥24	N/A	1) Little data available for sensitive algae species	Swanson *et al.* (1991) provides a list of potential species	**2**
GASTROPODA						
Gyraulus parvus	ECx (low-effect data)	≥168 (non-lethal); ≥504 (lethal)	Most sensitive lifestage	1) establishment of long-term hardness-toxicity relationship; 2) gastropods are not represented in the long-term SSD; 3) native to Alberta	Soucek et al 2011; Birge et al 1985	**1**
Physa (or Physella) gyrina	ECx (low-effect data)	≥168 (non-lethal); ≥504 (lethal)	Most sensitive lifestage	1) establishment of long-term hardness-toxicity relationship; 2) gastropods are not represented in the long-term SSD; 3) native to Alberta	Soucek et al 2011; Birge et al 1985	**1**
AMPHIBIANS						
Ambystoma maculatum	ECx (low-effect data, preferably non-lethal)	≥504	Egg/embryo	1) poor representation of amphibians in long-term SSD; 2) establishment of long-term hardness-toxicity relationship; 3) genus is native to Alberta	Karraker & Ruthig; Collins 2010; Collins & Russell 2009; Karraker & Gibbs 2011	**1**
ACTINOPTERYGII						
Pimephales promelas	ECx (low-effect data, preferably non-lethal)	≥504	Egg/embryo	1) establishment of long-term hardness-toxicity relationship in conjunction with available data	CHRONIC DATA: Elphick et al 2011a; Beak 1999; Rescan 2007; Birge et al 1985; Diamond et al 1992; Pickering et al 1996	**1**
Oncorhynchus mykiss	ECx (embryo viability test, low-effect data)	≥504	Egg/embryo	1) evidence of sensitivity (BCME 2013); 2) establishment of long-term hardness-toxicity relationship	Elphick et al 2011a; Vosyliene 2006; Spehar 1986; Camargo & Tarazona 1991; Kosteck & Jones 1983; Beak 1999; Rescan 2007; Dow et al 2010	**2**
VASCULAR PLANTS						
Lemna minor	ECx (growth/biomass low-effect data)	Case-by-case	N/A	1) supplementing existing long-term hardness-toxicity data	Buckley et al 1996; Taraldsen & Norberg-King 1990; Simmons 2012; Keppeler 2009	**1**

8.2.1 Bivalvia

Previously, standard toxicity assays using daphnids were believed to represent the most sensitive receptors to chloride (IDNR 2009a; US EPA 1986). However, more recent work (Wang *et al.* 2013, CCME 2011, Gillis 2011, Bringolf *et al.* 2007) has demonstrated that freshwater bivalves (mussels and clams) may be more sensitive to chloride.

Recent research has been published that compares the sensitivity of six mussel and two snail species to commonly tested invertebrates (*Hyalella azteca*, *C. dubia* and *D. Magna*), through acute exposures to 10 chemicals representing different toxic modes of action (Wang *et al.* 2013). Preliminary results have shown that: 1) mussels from different tribes or families generally have similar sensitivity to the chemicals; 2) mussels were found to be sensitive to the majority toxicants when compared to the standard test organisms; and, 3) the current US EPA chloride Ambient Water Quality Criteria may not be protective of mussels.

As a result, toxicity testing of freshwater mussels and clams will be an important component of future research in order to ensure that water quality guidelines for chloride are protective of all aquatic organisms. As summarized by Clifford (2012) and Clarke (1973), there are two families of clams and mussels in Alberta:

1) Unionidae (large clams or mussels); and,
2) Sphaeriidae (fingernail clams).

Within the Unionidae Family, there are four genera (*Anodonta, Lampsilis, Anodontoides*, and, *Lasmigona*) comprised of six species in Alberta (bolded species are those that had relevant toxicity testing data considered by CCME):

- *Anodonta grandis* (including *A. g. simpsoniana*)
- *Anodonta kennerlyi*
- *Anodontoides ferussacianus*
- ***Lampsilis siliquoidea***
- *Lasmigona complanata*
- *Lasmigona compressa*

Lampsilis radiata

left to right: Lampsilis compressa; Lampsilis complanata

These species produce glochidia, which can be a sensitive lifestage of exposure to contaminants (CCME 2011, Wang *et al.* 2013). Glochidia are a larval stage of development and are released in a cloud formation from an adult when a fish host has been lured into close proximity. The glochidia attached to the host and go through a parasitic phase during which organ development occurs, and after which they detach from their host and settle into their new environment as juveniles (Clarke, 1973).

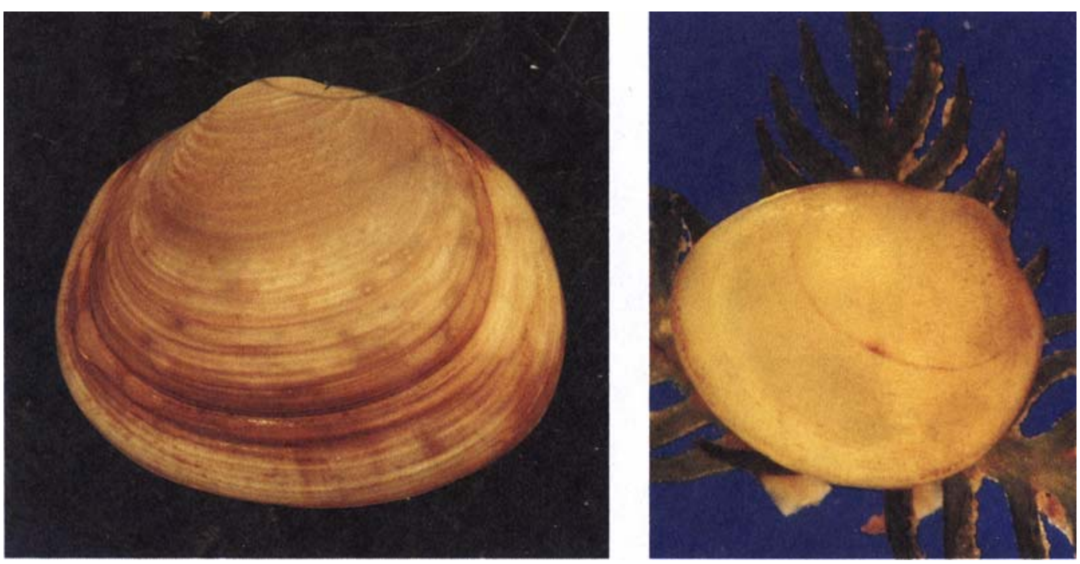
Toxicity testing data were available for one of the Alberta Unionidae species listed above (*Lampsilis siliquoidea* (Fatmucket mussel)), which were included by CCME in the development of a Canada-wide chloride water quality guideline. This species is considered COSEWIC Currently Stable and not of Special Concern or Endangered. An Effect Concentration 10% (EC_{10}) of 1,474 mg/L for the endpoint of glochidia survival was developed by CCME (2011) for this species, based on the data of Bringolf *et al.* (2007).

Toxicity data were also available for two other species within the same genera (*Lampsilis fasciola* (Wavy-Rayed lampmussel, the Great Lake Special Concern mussel species) and *Lampsilis cardium* (Plain pocketbook)) – neither of these species has been identified in Alberta. These two species in addition to *Lampsilis siliquiodea* are mantle lure spawners that produce glochidia (CCME, 2011). Toxicity data for Plain pocketbook was not used in the development of a chronic chloride limit, although data for the Wavy-Rayed Lampmussel was utilized. A 24 hour EC_{10} of 24 mg/L for the endpoint of glochidia (larval lifestage) survival was developed by the CCME based on the study of Bringolf *et al.* (2007).

Although of a different genera, the Endangered Ontario Great Lake mussel species *Epioblasma torulosa rangiana* (Northern Riffleshell) produces glochidia, and it was a sensitive species in terms of chloride exposure. An EC_{10} of 42 mg/L for the glochidia survival endpoint was derived from the data of Gillis (2010) for the Northern Riffleshell and used by CCME (2011). This species is similarly not encountered in Alberta.

Within the Sphaeriidae Family, there are two genera (*Pisidium* and *Sphaerium*) comprised of 22 species in Alberta (bolded species are those that had relevant toxicity testing data considered by CCME):

- *Pisidium casertanum*
- *Pisidium compressum*
- *Pisidium conventus*
- *Pisidium fallax*
- *Pisidium ferrugineum*
- *Pisidium idahoense*
- *Pisidium lilljeborgi*
- *Pisidium milium*
- *Pisidium nitidum*
- *Pisidium punctatum*
- *Pisidium rotundatum*
- *Pisidium subtruncatum*
- *Pisidium variabile*
- *Pisidium ventricosum*
- *Pisidium walkeri*
- *Sphaerium lacustre*
- *Sphaerium nitidum*
- *Sphaerium rhomboideum*
- ***Sphaerium (Musculium) secures***
- ***Sphaerium simile***
- *Sphaerium striatinum*
- *Sphaerium transversum*



Left to Right: Sphaerium (Sphaeriidae); Pisidium (Sphaeriidae)

Two of the species above (*Sphaerium securis or Musculium securis* (Pond Fingernail clam) and *Sphaerium simile* (Grooved Fingernail clam)) had available toxicity testing data that was incorporated by CCME (2011) into the development of a Canada-wide chloride water quality guideline. The species of this Family do not produce glochidia and instead the juveniles develop within the gills of adults. The key difference between genera in the Family is based on the number of different lifestages contained at any one time on the adult gills. The toxicological endpoint considered by the CCME for the species *Sphaerium securis* was reduced natality. A 60 to 80 day LOEC of 121 mg/L was derived, although Mackie (1978) produced data that could potentially be used to derive an EC_{10}. This species is not considered Special Concern or Endangered.

The three species *Lampsilis siliquoidea*, *Sphaerium secures*, and *Sphaerium simile* should be considered in future toxicity testing as they are native to Alberta and have been investigated in the literature (Gillis 2011, Soucek *et al.* 2011, Wang and Ingersoll 2010, Bringolf *et al.* 2007, Cope *et al.* 2008, Mackie 1978). *Sphaerium (Musculium) secures* may be especially important as it was the third most sensitive species on the unadjusted long-term SSD. Unfortunately, the results from Mackie (1978) did not quantify the hardness of the test medium, therefore examining potential hardness-toxicity relationships should be a priority for future toxicity testing using this species. For the species *L. siliquoidea* and *S. simile*, hardness data was available for equivalent endpoints over a sufficient range to be incorporated into the short-term hardness-toxicity relationship. Though presented in LC/EC_{50} form, original data could potentially be used to derive EC_{10} endpoints with application to long-term hardness-toxicity relationships. Because of the sensitivity of the glochidia life-stage, Unionid mussels will be an important component of future research. The Unionid *L. siliquoidea* is well represented in the literature (Gillis 2011, Wang and Ingersoll 2010, Bringolf *et al.* 2007, Cope *et al.* 2008) but its representation on the long-term SSD was based on an adult stage endpoint, therefore chronic glochidia life-stage toxicity testing is a priority for this species. Opportunities to utilize the most sensitive mussel species, *L. fasciola*, though not native to Alberta would also be valuable. Although *L. fasciola* is listed as "special concern" by COSEWIC, it may not be imperilled in other jurisdictions where testing might be appropriate.

8.2.2 Branchipoda

Water fleas, in particular *C. dubia* and *D. magna*, have conventionally been used as standard test organisms in aquatic toxicity bioassays. Although recent work (Wang 2013, CME 2011, Gillis 2011, Bringolf *et al.* 2007) suggests that mussels and clams (particularly the glochidia lifestage) may be more sensitive to chloride, all species of water flea plotted under the 30th percentile in the current short- and long-term SSDs with *D. magna* as the second most sensitive species in the short-term SSD. Although data on this group is extensive, the use of either C*. dubia* or *D. magna* in potential future toxicity assays might be important as a reference organism providing comparative controls. Branchipoda has been given a low priority for future research (Table 8.1)

Although *D. ambigua* occurs below the 20th percentile on the long-term SSD, it has not been suggested for toxicity testing as it appears to be uncommonly used in chloride toxicity testing (only utilized by Harmon *et al.* 2003) and is a member of the same genus as *D. magna*, an extensively studied daphnid.

8.2.3 Algae

As discussed previously (Section 4.1.1) algae have not been traditionally used in standard bioassays and despite their important role as primary producers are relatively underrepresented in the aquatic ecotoxicology literature for chloride. Algae species range widely in there sensitivity to chloride salts. In the long-term SSD, algae represented some of the more tolerant species (*Chlorella minutissimo*, *C. zofingiensis, C. emersonii*), while *Pseudokirchneriella subcapitata* represented the 4th most sensitive, with an EC_{10} of (population growth) of 124 mg Cl^-/L based on recent data published by Simmons (2012). The difference in toxicological response between various species of algae to the same toxicant has been shown to be several orders of magnitude for metals, pesticides, surfactants, and effluents (Blanck *et al.* 1984, Stratton 1987, Wangberg and Blanck 1988; Swanson *et al.* 1991, Lewis 1995), and this may in part explain results observed for sodium chloride. Different strains and geographical races of algae have been shown to vary substantially in their toxicological responses (Lewis 1995). Due to this high variability, a species battery approach is suggested in which several unrelated species are exposed to chloride. Swanson *et al.* (1991) provides a list of potential species for toxicity assays using pesticides and recommends a representative of each of the following classes be included (bolded genus / species can be found in Appendix A):

1. Chlorophyseae (Green algae):
   Pseudokirchneriella subcapitata*, Scenedesmus quadricauda,* ***Scenedesmus obliquus****, Scenedesmus bijuga, Chlorella pyrenoidosa, Chlorella vulgaris, Chlorella emersonii,* ***Chlamydomonas reinhardtii****,* ***Chlamydomonas spp****.,* ***Chlamydomonas*** *dysosmos, Ankistrodesmus spp., Tetraedron minimum, Schroederia setigera, Oedogonium cardiacum, Spirogyra spp., Stigeoclonium spp., Stigeoclonium spp., Ulothrix spp., Kirchneriella subsolitaria, Elakatothrix spp., Chlorococcum hypnospermum, Sphaerocystis schroeteri, Oocystis lacustris, Coccomyxa subellipsoidea, Stichococcus bacillaris, Spongiochloris excentrica, Golenkinia sp., Pediastrum sp., Klebsormidium marinum, Raphidonema longiseta, Monoraphidium pussilum*
2. Cyanobacteria (Blue-green algae):
   Microcystis aeruginosa*,* ***Anabaena*** *flos-aquae,* ***Anabaena*** *cylindrical, Oscillatoria spp., Gloeocapsa alpicola, Anacystis nidulans, Aphanizomenon flos-aquae, Cylindrospermum sp., Aulosira fertillissima, Plectonema boryanum,* ***Synechococcus*** *leopoliensis*
3. Bacillariophyceae (Diatoms):
   Navicula *pelliculosa,* ***Navicula*** *incerta, Cyclotella menghiniana (& spp.), Nitzschia palea, Rhizosolenia sp., Diatom sp.*
4. Xanthophyceae (Yellow-green algae):
   Vaucheria geminate, Tribonema sp.
5. Chrysophyceae (Golden Brown algae):

Dinobyron sp.

6. Dinophyceae (Dinoflagellates):
   Glenodinium sp., Gymnodinium sp.
7. Cryptophyceae:
   Rhodomonas sp.
8. Rhodophyseae:
   Batrachospermum sp.

8.2.4 Gastropoda

Gastropods, like bivalves, are members of the phylum *mollusc*. They are represented by 2 species of aquatic snail (*Gyraulus parvus* and *Physa gyrina*) in the short-term SSD (Soucek *et al.* 2011; Birge *et al.* 1985) and have no representation in the long-term SSD. The work of Wurtz and Bridges (1961) established acute endpoints for three species of aquatic snail (*Helisoma campanulata*, *Gyraulus circumstriatus*, *Physa heterostropha*), however this data was excluded from this analysis since no control group survival was reported.

The relationship between water hardness and the amelioration of chloride toxicity remains unclear for gastropods (results of Soucek *et al.* (2011) contrasted against Wurtz and Bridges (1961); see Section 5.1). Ongoing research by Wang *et al.* (2013) is expected to establishing toxicological endpoints for two species of aquatic snail though no results have yet been published. Future research should aim to investigate hardness-toxicity relationships for species already represented in the literature, of which *Gyraulus parvus*, *Physa gyrina*, and *Gyraulus circumstriatus* are native to Alberta.

8.2.5 Amphibia

In general, the results from the short-term SSD suggest that early lifestage amphibians are more sensitive to chloride toxicity when compared to fish. Although amphibians appear to be less sensitive in the long-term dataset, two studies that could not be included in the long-term SSD demonstrated more sensitive endpoints over chronic time frames. A study by Karraker and Gibbs (2011) demonstrated an 18 day LOEC (egg biomass) for the Spotted Salamander (*Ambystoma maculatum*) of 145 mg Cl^- /L (study excluded from current analysis due to the use of road salt), while a LOEC (mortality) of 625 mg Cl^- /L was found for the wood frog (*Rana sylvatica*) over a 10 day exposure (Sanzo and Hecnar 2006; study excluded due to high control mortality).

Given that only mortality endpoints are available for amphibians in the current long-term SSD and that evidence (though unsuitable for incorporation following CCME methodology) suggests greater sensitivity under non-lethal exposures, future research should incorporate such non-lethal testing using the most sensitive amphibian species, the salamanders. The fact that the most sensitive amphibian in the short-term SSD (*Ambystoma maculatum*) is represented by an effect concentration lower than the most sensitive species on the long-term SSD (short-term SSD: *A. maculatum*, 1,178 mg/L; long-term SSD: *X. laevis*, 1,307 mg/L) highlights the need for further research examining the toxicity of chronic, non-lethal exposure to chloride. Although *A. maculatum* does not occur in Alberta, the only two species of salamander inhabiting the province are from the same genus, *Ambystoma* (the Long-toed Salamander (*A. macrodactylum*) and the Tiger Salamander (*A. tigrinium*)). Consequently, toxicity results using *A. maculatum* may have applicability to the entire salamander population in Alberta. The ameliorating effect of water hardness on the toxicity of chloride to amphibians is poorly understood. None of the reviewed articles in the database addressed this relationship and amphibians are not represented in either the short- or long-term hardness-toxicity pooled slope calculation. As such, toxicity assays designed to evaluate hardness amelioration of chloride toxicity for sensitive amphibian species and lifestages will be important.

8.2.6 Actinopterygii

The fathead minnow (*P. promelas*) was found to be the most sensitive fish species both on the short-term and long-term SSDs. Although a toxicity-hardness relationship was established for this species using short-term toxicity data, chronic data was not comparable due to differences in exposure durations and/or the endpoint criteria examined. Five studies in the database examined chronic endpoints for *P. promelas* over durations from 7 to 33 days (Elphick *et al.* 2011a, Beak 2009, Rescan 2007, Pickering *et al.* 1996, Birge *et al.* 1985). Utilizing this existing data in conjunction with toxicity testing would allow for the establishment of a long-term hardness-toxicity relationship.

A 7 day EC_{25} of 989 mg Cl^- /L for *O. Mykiss* (embryo viability) represented the only non-lethal endpoint for vertebrates in the long-term SSD (Beak 1999). British Columbia recently derived a provincial guideline for sulphate which incorporated a similar early lifestage toxicity test for *O. Mykiss* (BCME 2013). The 21 day LC_{10} eyed embryo to alevin lifestage toxicity assay was found to be the most sensitive endpoint and the basis for the derived guideline. Thus, incorporating an *O. Mykiss* embryo viability assay similar to previous work may be appropriate for future research.

8.2.7 Vascular Plants

Vascular plants are used less frequently than algae in toxicity assays for sodium chloride and other chloride salts. Where macrophytes (vascular plants) are used, duckweed (*Lemna spp.*) is the most commonly selected test organism due to their small size, ease of culture and rapid reproduction. It has been suggested that the limited use of macrophytes in toxicological research may be attributed to high levels of variability between replicates (Lewis 1995). Such variability can be seen in the present database where chronic 168 h EC_{50} (growth) values varied between 1,525 to 4,167 mg Cl^- /L (Buckley *et al.* 1996, Keppeler 2009, Simmons 2012; Appendix A). Furthermore, the 168h EC_{10} (growth - # live thalli) currently used in the long-term SSD (496 mg Cl^- /L; Simmons 2012) is less than half that of the 96 h MATC value used in the CCME derivation (1,171 mg Cl^- /L; Taraldsen and Norberg-King 1990). For future toxicity testing, utilizing a 168 h growth (# of live thalli) endpoint would be useful as it would allow comparisons with the sensitive data from Simmons (2012). Furthermore, since only two studies were utilized to establish a hardness-toxicity slope for *Lemna minor* (Simmons 2012 and Buckley *et al.* 1996), further testing at a range of water hardness would provide valuable hardness-toxicity slope data for *Lemna minor*.

8.3 SUMMARY OF CHLORIDE SALT RESEARCH RECOMMENDATIONS

Of the four major chloride salts (NaCl, $CaCl_2$, KCl, and $MgCl_2$), only sodium chloride was found to meet the requirements of both short- and long-term SSD derivation (CCME 2007; Table 2.1). Although all but magnesium chloride met the short-term requirements (Section 3.1), both a short- and long-term SSD is required for Type A (SSD) guideline derivation (CCME 2007). Table 8.2 summarizes the data limitations for long-term SSD derivation using $CaCl_2$, KCl, and $MgCl_2$ as well as limitations for short-term $MgCl_2$ SSD derivation. These findings, along with research recommendations are outlined in Section 8.3.1 to 8.3.3.

8.3.1 Calcium Chloride – Data Limitations and Research Recommendations

To establish Type A (SSD) guidelines for $CaCl_2$, additional long-term data is required. As outlined in Table 8.2, the requirements for fish and invertebrate taxa are not currently met. For fish, although acute data is available from seven studies, toxicity testing has not been conducted meeting the minimum exposer durations for long-term SSD data of ≥ 21 days (larva: ≥ 7 days). For invertebrates, adequate data is available for *D. magna* and *P. corneus*, however long-term toxicity results for one additional invertebrate species is required (requirement of three species from three studies).

Future research investigating chronic endpoints for an additional invertebrate species and potentially a battery approach targeting three fish species (1 of which must be a salmonid) would provide sufficient data to calculate a long-term SSD for $CaCl_2$. However, based on short-term toxicity findings in the literature and in the current dataset, $CaCl_2$ appears to have a similar toxicity towards aquatic organisms as NaCl (Section 3.1). Thus the derived guidelines herein (NaCl based) should provide adequate protection to aquatic organisms exposed to $CaCl_2$. This was demonstrated for both the short-term benchmark and the long-term WQG (Section 6).

8.3.2 Potassium Chloride – Data Limitations and Research Recommendations

To establish Type A (SSD) guidelines for KCl, additional long-term data is required. As outlined in Table 8.2, the requirements for invertebrate taxa are not currently met. Adequate long-term KCl exposure data is available for *M. transversum* and *D. magna* (Biesinger and Christensen 1972 and Anderson *et al.* 1978) but an additional species is a requirement for long-term SSD derivation. In terms of fish taxa, following protocols (CCME 2007), only a 168 h LOEC for *P. promelas* (Pickering *et al.* 1996) is currently available. However, minor exceptions to the protocols set out in by the CCME (2007) can be made if sufficient support is provided (*sensu* CCME 2011). In the current database, 168 h IC_{25} (growth) endpoints are available for the fry lifestage of *O. mykiss* and *S. fontinalis*, but do not meet the exposure duration requirement of 21 days (504 h) for test organism matured past the larval stage (larval duration requirement = 168 h). Including these data points would follow a similar allowance made by the CCME (2011) where a 192 h NOEC concentration of 607 mg Cl- /L was utilized for fish at the fingerling lifestage. Although NOEC values are generally less preferred and requirements (CCME 2007) are for exposure durations of ≥ 504 h for juvenile fish, the CCME (2011) task group chose to include this data point in their long-term SSD (Section 4.1.1).

Future research investigating chronic endpoints for an additional invertebrate species would provide sufficient data to calculate a long-term SSD for KCl (assuming that exceptions for fish taxa could be made as outlined above) and allow for short-term benchmark and long-term WQG derivation. Deriving such guidelines should be a high priority since KCl has been identified as the most toxic of the four major chloride salts (Section 3.1).

8.3.3 Magnesium Chloride – Data Limitations and Research Recommendations

$MgCl_2$ is the most data limited of all the major chloride salts. Currently, there is insufficient data to meet the requirements of short- or long-term SSD derivation. As outlined in table 8.2, the short-term requirements for invertebrates are met by LC50 endpoints for *C. dubia, D. hyalina, D. magna, C. abyssorum, and E. padanus* (Mount *et al.* 1997, Baudouin and Scoppa 1974, Biesinger and Christensen 1972), however only one shot-term data point is available for fish (*P. promelas* LC_{50}; Mount *et al.* 1997). No long-term requirements are currently met within the database.

Potassium chloride has been shown to be more toxic towards aquatic organisms compared to Magnesium chloride (Section 3.1). As a result establishing a short-term benchmark and long-term WQG for KCl would likely provide adequate protection to aquatic organisms exposed to $MgCl_2$. An approach similar to that utilized in herein (Section 6) could be utilized to demonstrate this protection.

Table 8.2. Summary of short- and long-term SSD requirements not met by calcium, potassium, and magnesium chloride salts

Requirement	Required durations (days)	Req. Met?	Rational	Related literature	Species
Calcium chloride ($CaCl_2$)					
Long-term SSD					
Fish - 3 studies on 3 different species; 1 salmonid, 1 non-salomnids	≥ 21 (larva: ≥ 7)	No	Minimum exposure duration requirements not met	-	-
Invertabrates - 3 studies on 3 different species; 1 planktonic crustacean, 2 others	≥ 7 (short-lived: ≥ 4) (lethal: ≥21)	No	Only 2 species from 2 studies	Biesinger&Christensen(1972), Mazuran_etal(1999)	*D. magna, P. corneus*
Plant/Algae - At least 1 study	≥ 1 (Algae), case-by-case (Plants)	Yes	2 species from 1 study	Simmons(2012)	*L. minor, P. subcapitata*
Potassium chloride (KCl)					
Long-term SSD					
Fish - 3 studies on 3 different species; 1 salmonid, 1 non-salomnids	≥ 21 (larva: ≥ 7)	No/Yes	- Scenario 1: 1 study and 1 species; - Scenario 2: 3 species from 2 studies if 168 h IC25 (growth) endpoints (Lazorchak&Smith 2007) are deemed acceptable for the fry lifestage of O. mykiss and S. fontinalis	Scenario 1: Pickering_etal(1996); Scenario 2: Pickering_etal(1996), Lazorchak&Smith(2007)	Scenario 1: *P. promelas*; Scenario 2: *P. promelas, O. mykiss, S. fontinalis*
Invertabrates - 3 studies on 3 different species; 1 planktonic crustacean, 2 others	≥ 7 (short-lived: ≥ 4) (lethal: ≥21)	No	2 species from 2 studies; Note: 240 h exposures from Trimble_etal(2010) for H. azteca and C. dilutus deemed unacceptable due to mortality endpoints (LC50s)	Biesinger&Christensen (1972), Anderson_etal (1978)	*M. transversum, D. magna*
Plant/Algae - At least 1 study	≥ 1 (Algae), case-by-case (Plants)	Yes	6 species from 2 studies	Simmons(2012), Dai_etal(2008)	*P. subcapitata, L. minor, Nostoc sp., M. aeruginosa, A. azotica, Synechococcus sp.*
Magnesium chloride ($MgCl_2$)					
Short-term SSD					
Fish - 3 studies on 3 different species; 1 salmonid, 1 non-salomnids	≤ 4	No	Only 1 species represented	Mount_etal(1997)	*P. promelas*
Invertabrates - 3 studies on 3 different species; 1 planktonic crustacean, 2 others	≤ 4	Yes	5 species from 3 studies	Mount_etal(1997), Baudouin&Scoppa(1974), Biesinger&Christensen(1972)	*C. dubia, D. hyalina, D. magna, C. abyssorum, E. padanus*
Long-term SSD					
Fish - 3 studies on 3 different species; 1 salmonid, 1 non-salomnids	≥ 21 (larva: ≥ 7)	No	No studies meeting requirements; Note: 672 h exposures from Birge_etal (1980) for O. mykiss deemed unacceptable due to mortality endpoints (LC50s)	-	-
Invertabrates - 3 studies on 3 different species; 1 planktonic crustacean, 2 others	≥ 7 (short-lived: ≥ 4) (lethal: ≥21)	No	Only 1 species represented	Biesinger&Christensen(1972)	*D. magna*
Plant/Algae - At least 1 study	≥ 1 (Algae), case-by-case (Plants)	No	No studies meeting requirements	-	-

8.4 TOXICITY TESTING WORK

Technical recommendations are provided for target chloride dose concentrations in addition to levels of hardness for dilution water. These recommendations provide a sufficient range of concentrations for toxicity endpoint calculation, along with hardness ranges that provide useful estimates of hardness-toxicity relationships.

8.4.1 Target Chloride Concentrations for Testing

The following chloride water concentrations (representing doses) would be considered for the toxicity testing work with all recommended species: 0, 25, 75, 225, 675, 2,025 and 3000 mg/L. This concentration range will provide resolution towards where an EC_{10} may be expected for guideline development and an upper bound of exposure producing more extensive effects for confirmation of toxicity (*e.g.*, EC_{50}). The experiments will be conducted in re-constituted water to allow for comparison with other toxicity testing data and the consistent control of parameters that may relate to toxicity. An appropriate number of animals per dose group should be considered to allow for reproducible results and statistical analysis.

8.4.2 Target Hardness Levels for Testing

For each species tested, selected chloride dose levels (n=3) will involve an assessment of chloride toxicity in the presence of varying water hardness levels. The following hardness levels are recommended (expressed as $CaCO_3$): 25 (Soft), 75 (Moderately Soft); 150 (Moderately Hard); 300 (Hard); and, 500 mg/L (Very Hard). Testing under water conditions of Extremely Hard (> 600 mg/L) was considered to be of lesser relevance given it is expected to represent a relatively minor portion of the water hardness environments encountered in Alberta. Where possible, testing should aim to identify upper boundaries where hardness derived amelioration is reduced, or possibly where hardness levels themselves become toxic to the test organism. This upper bound of amelioration effectiveness will be necessary in establishing a hardness range over which a guideline is applicable and may vary across species.

Due to the potential differences in toxicity modifying effects among calcium and magnesium cations (Leblanc and Surpenant 1984, Jackson *et al.* 2000, Welsh *et al.* 2000), differences in responses to chloride toxicity can be expected depending on the ratio of these ions. Furthermore, calcium to magnesium ratios recommended by the American Society for Testing and Materials (ASTM 2007) and the US EPA (2002) in standard reference water are different from ratios found in most natural surface waters (BCME 2013). Future research conduct toxicity assays in standard dilution water with Ca^{2+}:Mg^{2+} ratios similar to what is found in relevant natural surface waters. A similar approach is utilized by Soucek *et al.* (2011) where a calcium to magnesium ratio of 2.25 was suggested to be representative of natural conditions. This ratio is similar to Mitchell's (1990) assessment of fresh water lakes in Alberta in which an average Ca^{2+}:Mg^{2+} ratio of 1.9 was obtained and a USGS (2006) assessment of the Powder River drainage basin where ratios ranged from 1.9 to 3. Long-term water quality monitoring data is available from the Alberta Government (2014).

9 CLOSURE

Equilibrium Environmental Inc. has prepared this document for the exclusive use of the Petroleum Technology Alliance Canada (PTAC) and the organizations it represents via the AUPRF funding mechanism. This use is exclusive for the purpose of assisting in the decision making process for developing chloride aquatic life guidelines. Additional information may change any opinions presented herein.

No further warranty is made, either expressed or implied. Any uses which a third party makes of this document, or any reliance on decisions made based on it, are the responsibility of such third parties. Equilibrium Environmental Inc. accepts no duty or care to any other person or any liability or responsibility whatsoever, for any losses, expenses, damages, fines, penalties, or other harm that may be suffered or incurred by any other person as a result of the use of, reliance on, any decision made, or any action taken based on this document. Nothing in this document is intended to constitute or provide a legal opinion. The accuracy of investigation work and chemistry data collected by other parties cannot be verified and is not implied. Equilibrium Environmental Inc. believes information presented in this report is accurate but cannot guarantee or warrant its accuracy. Any questions regarding this document should be directed to Anthony Knafla at (403) 862 7758.

EQUILIBRIUM ENVIRONMENTAL INC.

Ian McIvor, M.Sc.
Aquatic Biologist / Environmental Scientist

Anthony L. Knafla, M.Sc., DABT
Risk Assessment Specialist / Toxicologist

10 REFERENCES

Abegg R (1949) Some implications from the changes in the mucous covering of fish subjected to salt solutions. Proceedings of the Louisiana Academy of Science 12:7-10

Academy of Natural Sciences (1960) The Sensitivity of Aquatic Life to Certain Chemicals Commonly Found in Industrial Wastes. Final Rep.No.RG-3965(C2R1), U.S.Public Health Service Grant, Acad.of Nat.Sci., Philadelphia, PA:89 p. Cited In: McKee & Wolf (1963).

Adelman I, Smith L (1976) Standard test fish development: part I, fathead minnows (Pimephales promelas) and goldfish (Carassius auratus) as standard fish in bioassays and their reaction to potential reference toxicants., EPA/600/3-76/061a, Duluth: US EPA.

Adelman IR, Smith LL, Siesennop GD (1976) Acute toxicity of sodium-chloride, pentachlorophenol, guthion, and hexavalent chromium to fathead minnows (pimephales-promelas) and goldfish (carassius-.auratus). Journal of the Fisheries Research Board of Canada 33:203-208.

Alberta Government (2011) Water softening factsheet. Agdex 716(D32). Weblink (Accessed on 06/11/2013): http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex9354

Alberta Government (2014) Surface water quality data. Weblink (accessed on 28/08/2014): http://esrd.alberta.ca/water/reports-data/surface-water-quality-data/default.aspx

Al-Daham NK, Bhatti MN (1977) Salinity Tolerance of Gambusia affinis (Baird & Girard) and Heteropneustes fossilis (Bloch). Journal of Fisheries Biology 11:309-313.

Aldenberg T, Jaworska JS, Traas TP 2002. Normal species sensitivity distributions and probabilistic ecological risk assessment. In Species Sensitivity Distributions in Ecotoxicology. Posthuma, L., Suter, G. W. I., and Traas, T. (ed.). Boca Raton, FL, CRC Press LLC.

Anderson BG (1946) The toxicity thresholds of various sodium salts determined by the use of Daphnia magna. Sewage Works Journal 18:82-87.

Anderson BG (1948) The apparent thresholds of toxicity to Daphnia magna for chlorides of various metals when added to Lake Erie water. Transactions of the American Fisheries Society 78:96-113.

Anderson BG, Andrews TF, Chandler DC, Jahoda WJ (1948) The Evaluation of Aquatic Invertebrates as Assay Organisms for the Determination of the Toxicity of Industrial Wastes. Final Report 51, American Petroleum Institute Project, Ohio State University, Columbus, OH. Cited in: McKee & Wolf (1963), CCME (2011).

Anderson KB, Sparks RE, Paparo AA (1978) Rapid Assessment of Water Quality, Using the Fingernail Clam, Musculium transversum. WRC Res.Rep.No.133, Project No.B-097-ILL, Univ.of Illinois, Urbana, IL:115 p.

ANZECC (Australian and New Zealand Environment and Conservation Council) (2000) Australian and New Zealand guidelines for fresh and marine water quality. volume 1 - the guidelines.

Aragao MA, Pereira EV (2003) Sensitivity of Ceriodaphnia dubia of different ages to sodium chloride. Bulletin of Environmental Contamination and Toxicology 70:1247-1250. Cited In: CCME (2011).

Arambasic MB, Bjelic S, Subakov G (1995) Acute toxicity of heavy-metals (copper, lead, zinc), phenol and sodium on allium-cepa l, lepidium-sativum l and daphnia-magna st - comparative investigations and the practical applications. Water Research 29:497-503.

Arenzon A, Pinto RF, Colombo P, Raya-Rodriguez MT (2003) Assessment of the freshwater annual fish Cynopoecilus melanotaenia as a toxicity test organism using three reference substances. Environmental Toxicology and Chemistry 22:2188-2190.

ASTM (American Society for Testing and Materials) (2007) Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians. E729 – 96.

ASTM (American Society for Testing and Materials) (2013) Standard guide for conducting laboratory toxicity tests with freshwater mussels. E2455 – 06.

Bagchi SN, Das PK, Banerjee S, Saggu M, Bagchi D (2012) A Bentazone-Resistant Mutant of Cyanobacterium, Synechococcus elongatus PCC7942 Adapts Different Strategies to Counteract on Bromoxynil- and Salt-Mediated Oxidative Stress. Physiology Molecular Biology of Plants 18:115-123.

Bartlett AJ, Rochfort Q, Brown LR, Marsalek J (2012) Causes of toxicity to Hyalella azteca in a stormwater management facility receiving highway runoff and snowmelt. Part II: Salts, nutrients, and water quality. Science of the Total Environment 414:238-247.

Bartolome MC, D'Ors A, Sanchez-Fortun S (2009) Toxic effects induced by salt stress on selected freshwater prokaryotic and eukaryotic microalgal species. Ecotoxicology 18:174-179.

Baudouin MF, Scoppa P (1974) Acute toxicity of various metals to freshwater zooplankton. Bulletin of Environmental Contamination and Toxicology 12:745-751.

BCME (British Columbia Ministry of Environment) (2013) Ambient water quality guidelines for sulphate.

Beak (Beak International Inc.) (1999) Ecotoxicology test results. Unpublished report for M.S. Evans. Environment Canada, National Water Research Institute. Cited In: CCME (2011).

Beux LF, Filho EZ (2007) Survival and the growth of pintado (Pseudoplatystoma corruscans) post-larvae on different salinities. Brazilian Archives of Biology and Technology 50:821-829.

Biesinger KE, Christensen GM (1972) Effects of various metals on survival, growth, reproduction, and metabolism of Daphnia magna. Journal of the Fisheries Research Board of Canada 29:1691-1700.

Birge WJ, Black JA, Westerman AG, Hudson JE (1980) Aquatic toxicity tests on inorganic elements occurring in oil shale. In: C Gale (Ed) Oil Shale Symposium: Sampling, Analysis and Quality Assurance, March 1979; EPA-600/9- 80-022, U S EPA, Cincinnati, OH:519-534.

Birge WJ, Black JA, Westerman AG, Short TM, Taylor SB, Bruser DM, Wallingford ED (1985) Recommendations on numerical values for regulating iron and chloride concentrations for the purpose of protecting warm water species of aquatic life in the Commonwealth of Kentucky. Memorandum of agreement No 5429. Kentucky Natural Resources and Environmental Protection Cabinet, Lexington, K.Y.

Black HH (1950) Federal industrial pollution studies. Sewage and Industrial Wastes 22:1049-1053.

Blanck H, Wallin G, Wangberg S (1984) Species dependent variation in algal sensitivity to compounds. Ecotox. Environ. Safety 8:339-351.

Brand AB, Snodgrass JW, Gallagher MT, Casey RE, Van Meter R (2010) Lethal and Sublethal Effects of Embryonic and Larval Exposure of Hyla versicolor to Stormwater Pond Sediments. Archives of Environmental Contamination and Toxicology 58:325-331.

Bright DA, Addison J (2002) Derivation of matrix soil standards for salt under the British Columbia Contaminated Sites Regulation. Prepared for BC Ministry of Water, Land and Air Protection, BC Ministry of Transportation and Highways, BC Buildings Corp, and the Canadian Association of Petroleum Producers. Royal Roads University, Victoria, BC.

Brix KV, Grosell M (2005) Report on the effects of total dissolved solids on Arctic Grayling and Dolly Varden fertilization success. Prepared for Teck Cominco, Red Dog Mine, Kotzebue, AK.

Bringolf RB, Barnhardt MC, Cope WG (2013) Determining the appropriate duration of toxicity tests with glochidia of native freshwater mussels. USEPA Region V.

Bringolf RB, Cope WG, Eads CB, Lazaro PR, Barnhart MC, Shea D (2007) Acute and chronic toxicity of technical-grade pesticides to glochidia and juveniles of freshwater mussels (Unionidae). Environmental Toxicology and Chemistry 26:2086-2093.

Brown MG, Dobbs EK, Snodgrass JW, Ownby DR (2012) Ameliorative effects of sodium chloride on acute copper toxicity among Cope's gray tree frog (Hyla chrysoscelis) and green frog (Rana clamitans) embryos. Environmental Toxicology and Chemistry 31:836-842.

Buchmann K, Felsing A, H.C. S (1992) Effects of metrifonate, sodium chloride and bithionol on an European population of the gill parasitic Monogeneans Pseudodatylogrus spp. and the host. Bulletin of the European Association of Fish Pathologists 12:57-60.

Buckley JA, Rustagi KP, Laughlin JD (1996) Response of Lemna minor to sodium chloride and a statistical analysis of continuous measurements for EC50 and 95% confidence limits calculation. Bulletin of Environmental Contamination and Toxicology 57:1003-1008.

Burton GA, NorbergKing T, Ingersoll CG, Benoit DA, Ankley GT, Winger PV, Kubitz J, Lazorchak JM, Smith ME, Greer E, Dwyer FJ, Call DJ, Day KE, Kennedy P, Stinson M (1996) Interlaboratory study of precision: Hyalella azteca and Chironomus tentans freshwater sediment toxicity assays. Environmental Toxicology and Chemistry 15:1335-1343.

Cairns JCJ, Scheier A (1958) The relationship of bluegill sunfish body size to its tolerance for some common chemicals. Proceedings of the 13th Industrial Waste Conference. P. 243-252.

Calleja MC, Persoone G, Geladi P (1994) Comparative acute toxicity of the first 50 multicenter evaluation of in-vitro cytotoxicity chemicals to aquatic non-vertebrates. Archives of Environmental Contamination and Toxicology 26:69-78.

Camargo JA, Tarazona JV (1990) Acute toxicity to fresh-water benthic macroinvertebrates of fluoride-ion (f-) in soft-water. Bulletin of Environmental Contamination and Toxicology 45:883-887.

Camargo JA, Tarazona JV (1991) Short-term toxicity of fluoride-ion (f-) in soft-water to rainbow-trout and brown trout. Chemosphere 22:605-611.

Castillo G, Schafer L (2000) Evaluation of a bioassay battery for water toxicity testing: A Chilean experience. Environmental Toxicology 15:331-337.

CCME (Canadian Council of Ministers of the Environment) (2007) A protocol for the derivation of water quality guidelines for the protection of aquatic life 2007. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, 1999, Winnipeg.

CCME (Canadian Council of Ministers of the Environment) (2011) Canadian Water Quality Guidelines: Chloride Ion. Scientific Criteria Document. Canadian Council of Ministers of the Environment, Winnipeg. PN 1460, ISBN 978-1-896997-77-3 PDF.

CCME (Canadian Council of Ministers of the Environment) (2014) Canadian Water Quality Guidelines: Cadmium. Scientific Criteria Document. Canadian Council of Ministers of the Environment, Winnipeg.

Chang IH, Cheng KT, Huang PC, Lin YY, Cheng LJ, Cheng TS (2012) Oxidative stress in greater duckweed (Spirodela polyrhiza) caused by long-term NaCl exposure. Acta Physiologiae Plantarum 34:1165-1176.

Chinathamby K, Reina RD, Bailey PCE, Lees BK (2006) Effects of salinity on the survival, growth and development of tadpoles of the brown tree frog, Litoria ewingii. Australian Journal of Zoology 54:97-105.

Christy MT, Dickman RD (2002) Effects of salinity on tadpoles of the green and golden bell frog (Litoria aurea). Amphibia-Reptilia 23:1-11.

Clarke AH (1973) The freshwater molluscs of the Canadian Interior Basin. Malacologia 13:1-509.

Clemens TA, Jones WA (1954) Toxicity of brime water from oil wells. American Fisheries Society 84:97-109.

Clifford HF (2012) Aquatic Invertebrates of Alberta. Pelecypoda (Clams). Department of Biological Sciences, University of Alberta.

Collins S (2010) Toxicity of Deicing Salt Components to Early Amphibian Life Stages. M.S. Thesis, Saint Mary's University, Canada: 109 p.

Collins SJ, Russell RW (2009) Toxicity of road salt to Nova Scotia amphibians. Environmental Pollution 157:320-324.

Conrad AU, Fleming RJ, Crane M (1999) Laboratory and field response of Chironomus riparius to a pyrethroid insecticide. Water Research 33:1603-1610.

Cooney JD, Degraeve GM, Moore EL, Lenoble BJ, Pollock TL, Smith GJ (1992) Effects of environmental and experimental-design factors on culturing and toxicity testing of Ceriodaphnia dubia. Environmental Toxicology and Chemistry 11:839-850.

Cope GW, Bringolf RB, Buchwalter DB, Newton TJ, Ingersoll CG, al. e (2008) Differential exposure, duration, and sensitivity of unionoidean bivalve life stages to environmental contaminants. Journal of the North American Benthological Society 27:451-462.

Cowgill UM, Milazzo DP (1990) The sensitivity of 2 cladocerans to water-quality variables - salinity and hardness. Archiv Fur Hydrobiologie 120:185-196. Cited In: CCME (2011)

Cressman CPI, Williams PL (1994) Reference toxicants for toxicity testing using Caenorhabditis elegans in aquatic media. In: Dwyer, F.J., Doane T.R., Hunman M.L. (Eds), Environmental Toxicology and Risk Assessment: Modeling and Risk Assessment. In: Dwyer FJ, Doane TR, Hunman ML, (Eds) (eds), 6th Volume. ASTM STP 1317.

Crisinel A, Delaunay L, Rossel D, Tarradellas J, Meyer H, Saiah H, Vogel P, Delisle C, Blaise C (1994) Cyst-based ecotoxicological tests using anostracans - comparison of 2 species of streptocephalus. Environmental Toxicology and Water Quality 9:317-326.

Crowther RA, Hynes HBN (1977) Effect of road deicing salt on drift of stream benthos. Environmental Pollution 14:113-126.

Dai GZ, Deblois CP, Liu SW, Juneau P, Qiu BS (2008) Differential sensitivity of five cyanobacterial strains to ammonium toxicity and its inhibitory mechanism on the photosynthesis of rice-field cyanobacterium Ge-Xian-Mi (Nostoc). Aquatic Toxicology 89:113-121.

Davenport J, Huat KK (1997) Salinity tolerance and preference in the frog Rana rugulosa Wiegmann. Herpetological Journal 7:114-115.

Davies TD, Hall KJ (2007) Importance of calcium in modifying the acute toxicity of sodium sulphate to Hyalella azteca and Daphnia magna. Environmental Toxicology and Chemistry 26:1243-1247.

De Beers (De Beers Canada Inc.) (2013) Development of total dissolved solids chronic effects benchmark for aquatic life in Snap Lake.

Degraeve GM, Cooney JD, Marsh BH, Pollock TL, Reichenbach NG (1992) Variability in the performance of the 7-d Ceriodaphnia dubia survival and reproduction test - an intralaboratory and interlaboratory study. Environmental Toxicology and Chemistry 11:851-866.

Dejong LED (1965) Tolerance of Chlorella vulgaris for metallic and non-metallic ions. Antonie Van Leeuwenhoek Journal of Microbiology and Serology 31:301.

Deniz F, Saygideger SD, Karaman S (2011) Response to Copper and Sodium Chloride Excess in Spirulina sp (Cyanobacteria). Bulletin of Environmental Contamination and Toxicology 87:11-15.

Denoel M, Bichot M, Ficetola GF, Delcourt J, Ylieff M, Kestemont P, Poncin P (2010) Cumulative effects of road de-icing salt on amphibian behavior. Aquatic Toxicology 99:275-280.

Diamond JM, Winchester EL, Mackler DG, Gruber D (1992) Use of the mayfly Stenonema modestum (heptageniidae) in subacute toxicity assessments. Environmental Toxicology and Chemistry 11:415-425.

Doe KG (2010) Email to M. Nowierski November 2. Results of a 108 day exposure of the Northern leopard frog (Rana pipiens) to NaCl. Moncton Aquatic Toxicity Laboratory Environment Canada.

Dougherty CK, Smith GR (2006) Acute effects of road de-icers on the tadpoles of three anurans. Applied Herpetology. 3: 87-93.

Dow S, Pitts K, Saucedo P, Murray K (2010) Summary of Zequanox biobox trials at DeCew II generation facility, St. Catharine's Ontario, Canada. Prepared for Niagara Plant Group, Ontario Power Generation. December 13, 2010.

Dowden BF, Bennett HJ (1965) Toxicity of selected chemicals to certain animals. Journal of The Water Pollution Control Federation 1308-1316.

Durand-Hoffman ME (1995) Analysis of Physiological and toxicological effects of potassium on Dreissena polymorpha and toxicological effects on fish. MSc. Thesis, Ohio State University, Columbus, OH.

Dwyer FJ, Burch SA, Ingersoll CG, Hunn JB (1992) Toxicity of trace-element and salinity mixtures to striped bass (morone-saxatilis) and Daphnia magna. Environmental Toxicology and Chemistry 11:513-520.

Echols BS, Currie RJ, Cherry DS (2010) Preliminary results of laboratory toxicity tests with the mayfly, Isonychia bicolor (Ephemeroptera: Isonychiidae) for development as a standard test organism for evaluating streams in the Appalachian coalfields of Virginia and West Virginia. Environmental Monitoring and Assessment 169:487-500.

Echols BS, Currie RJ, Valenti TW, Cherry DS (2012) An Evaluation of a Point Source Brine Discharge into a Riverine System and Implications for TDS Limitations. Human and Ecological Risk Assessment 18:588-607.

EC & HC (Environment Canada and Health Canada) (2001). Priority Substances List Assessment Report: Road Salts. Ottawa, Ontario.

Eddy F (1975) The effect of calcium on gill potentials and on sodium and chloride fluxes in the goldfish, Carassius auratus. J. Comp. Physiol 96:131-142.

Edmister JO, Gray JW (1948) The Toxicity Thresholds for Three Chlorides and Three Acids to the Fry of Whitefish (Coregonus clupeaformis) and Yellow Pickerel (Stizostedion v. vitreum). The Progressive Fish-Culturalist 10:105-106.

Eisentraeger A, Dott W, Klein J, Hahn S (2003) Comparative studies on algal toxicity testing using fluorometric microplate and Erlenmeyer flask growth-inhibition assays. Ecotoxicology and Environmental Safety 54:346-354.

Ellis MM (1937) Detection and measurement of stream pollution (related principally to fish life). U.S. Department of Commerce, Bull. U.S. Bur. Fish 18:263-437.

Elphick JRF, Bergh KD, Bailey HC (2011a) Chronic toxicity of chloride to freshwater species effects of hardness and implications for water quality guidelines. Environmental Toxicology and Chemistry 30:239-246.

Elphick JR, Davies M, Gilron G, Canaria EC, Lo B, Bailey HC (2011b) An aquatic toxicological evaluation of sulfate the case for considering hardness as a modifying factor in setting water quality guidelines. Environmental Toxicology and Chemistry 30:247-253.

Environment Canada. 2004. Code of Practice for the Environmental Management of Road Salts. EPS1/CC/5.

Environ (Environ International Corporation) (2009) Chloride toxicity test results. Prepared for: Iowa Water Pollution Control Association. Project Number: #20-22235A. Cited In: CCME (2011).

Evans M, Frick C (2001) The effects of road salt on aquatic ecosystems. NWRI Contribution No. 02-308. Environment Canada, Saskatoon, SK.

Fisher JC, Belden JB, Bidwell JR (2010) Can site-specific heuristic toxicity models predict the toxicity of produced water? Chemosphere 80:542-547.

Fowler J (1931) The relations of numbers of animals to survival in toxic concentrations of electrolytes. Physiol. Zool 4:212-245. Cited in: Anderson 1948, CCME 2011.

Freitas EC, Rocha O (2011) Acute Toxicity Tests with the Tropical Cladoceran Pseudosida ramosa: The Importance of Using Native Species as Test Organisms. Archives of Environmental Contamination and Toxicology 60:241-249.

Gardner KM, Royer TV (2010) Effect of Road Salt Application on Seasonal Chloride Concentrations and Toxicity in South-Central Indiana Streams. Journal of Environmental Quality 39:1036-1042.

Garibay R, Hall S (2007) Evaluation of chloride water quality criteria protectivness of Upper Santa Clara River aquatic life: an emphasis on threatened and endangered species. The Advent Group, Brentwood, TN.

Garrey WE (1916) The resistance of fresh water fish to changes of osmotic and chemical conditions. American Journal of Physiology 39:313-329.

Geis S, Fleming K, Korthals E, Searle G, Reynolds L, Karner D (2000) Modifications to the algal growth inhibition test for use as a regulatory assay. Environmental toxicology and chemistry 19:36-41.

Geis S, Hemming J (2014) Email to I. McIvor September 12. Calculated effect concentrations from Results of a 96 hour exposure of the green algae Raphidocelis (Pseudokirchneriella) subcapitata (formerly Selenastrum capricornutum) to NaCl in dilution waters of 85 versus 170 mg CaCO3/L. Wisconsin Laboratory of Hygiene.

Gillis PL (2011) Assessing the toxicity of sodium chloride to the glochidia of freshwater mussels: Implications for salinization of surface waters. Environmental Pollution 159:1702-1708.

Gilron G, Gransden SG, Lynn DH, Broadfoot J, Scroggins R (1999) A behavioral toxicity test using the ciliated protozoan Tetrahymena thermophila. I. Method description. Environmental Toxicology and Chemistry 18:1813-1816.

Goetsch PA, Palmer CG (1997) Salinity tolerances of selected macroinvertebrates of the Sabie River, Kruger National Park, South Africa. Archives of Environmental Contamination and Toxicology 32:32-41.

Gorai M, Ennajeh M, Khemira H, Neffati M (2010) Combined effect of NaCl-salinity and hypoxia on growth, photosynthesis, water relations and solute accumulation in Phragmites australis plants. Flora 205:462-470.

Gorai M, Ennajeh M, Khemira H, Neffati M (2011) Influence of NaCl-salinity on growth, photosynthesis, water relations and solute accumulation in Phragmites australis. Acta Physiologiae Plantarum 33:963-971.

Grizzle JM, Mauldin JC (1995) Effect of calcium on acute toxicity of sodium chloride to larval and newly transformed newly striped bass. Journal of Aquatic Animal Health 7:298-303.

Hamilton RW, Buttner JK, Brunetti RG (1975) Lethal levels of sodium-chloride and potassium-chloride for an oligochaete, Oligochaeta naididae a chironomid midge, Diptera chironomidae and a caddisfly trichoptera, Hydroptilidae of Lake Michigan. Environmental Entomology 4:1003-1006.

Harless ML, Huckins CJ, Grant JB, Pypker TG (2011) Effects of six chemical deicers on larval wood frogs (Rana sylvatica). Environmental Toxicology and Chemistry 30:1637-1641.

Harmon SM, Specht WL, Chandler GT (2003) A comparison of the daphnids Ceriodaphnia dubia and Daphnia ambigua for their utilization in routine toxicity testing in the Southeastern United States. Archives of Environmental Contamination and Toxicology 45:79-85.

Hinton MJ, Eversole AG (1978) Toxicity of ten commonly used chemicals to American eels. Agricultural Experiment Station Technical Contribution 1595. Texas A&M University., College Station, TX.

Hinton MJ, Eversole AG (1979) Toxicity of ten chemicals commonly used in aquaculture to the black eel stage of American eel. Proceedings of the World Mariculture Society 10:554-560.

Hiscock K (2005) Hydrogeology: principles and practice. Blackwell Science Ltd., p. 199.

Hoke RA, Gala WR, Drake JB, Giesy JP, Flegler S (1992) BIcarbonate as a potential confounding factor in cladoceran toxicity assessments of pore water from contaminated sediments. Canadian Journal of Fisheries and Aquatic Sciences 49:1633-1640.

Hughes JS (1973) Acute Toxicity of Thirty Chemicals to Striped Bass (Morone saxatilis). La Dep. Wildl. Fish. V318.

Hutchinson EG (1933) Experimental Studies in Ecologie. I. The Magnesium Tolerance of Daphniidae and its Ecological Significance. Internationale Revue der gesamten Hydrobiologie und Hydrographie 28:90-108.

IDNR (Iowa Department of Natural Resources) (2009a) Water Quality Standards Review: Chloride, Sulfate and Total Dissolved Solids. Iowa Department of Natural Resources.

IDNR (Iowa Department of Natural Resources) (2009b) Proposed Chloride Criteria Update.

Iwao T (1936) Comprehensive investigation of the toxicity of various metals. Jiken Yakubutsugaku Zassi (Jour. Exp. Pharnacol.) (Japanese) 10:357-380. Cited in CCME 2011.

Jackman P (2010) Email to M. Nowierski December 9. Reference toxicant data for NaCl tested on various aquatic organisms. Moncton Aquatic Toxicity Laboratory, Environment Canada. Cited In: CCME(2011).

Jackson BP, Lasier P, Miller W, Winger P (2000) Effects of calcium, magnesium and sodium on alleviating cadmium toxicity to Hyalella azteca., Book 64. Bulletin of Environmental Contamination and Toxicology.

Jacobsen T (1995) Acute toxicity of 16 water-soluble chemicals to the fungus geotrichum-candidum measured by reduction in glucose-uptake. Toxicology in Vitro 9:169-173.

Jampeetong A, Brix H (2009) Effects of NaCl salinity on growth, morphology, photosynthesis and proline accumulation of Salvinia natans. Aquatic Botany 91:181-186.

Jones JRE (1940) A further study of the relation between toxicity and solution pressure, with Polycelis nigra as test animal. Journal of Experimental Biology 17:408-415.

Jones JRE (1941) A study of the relative toxicity of anions, with Polycelis nigra as test animal. Journal of Experimental Biology 18:170-181.

Karraker NE (2007) Are embryonic and larval green frogs (rana clamitans) insensitive to road deicing salt? Herpetological Conservation and Biology 2:35-41.

Karraker NE, Gibbs JP (2011) Road deicing salt irreversibly disrupts osmoregulation of salamander egg clutches. Environmental Pollution 159:833-835.

Karraker NE, Gibbs JP, Vonesh JR (2008) Impacts of road deicing salt on the demography of vernal pool-breeding amphibians. Ecological Applications 18:724-734.

Karraker NE, Ruthig GR (2009) Effect of road deicing salt on the susceptibility of amphibian embryos to infection by water molds. Environmental Research 109:40-45.

Kearney BD, Byrne PG, Reina RD (2012) Larval Tolerance to Salinity in Three Species of Australian Anuran: An Indication of Saline Specialisation in Litoria aurea. Plos One 7(issue 8).

Keppeler EC (2009) Toxicity of Sodium Chloride and Methyl Parathion on the Macrophyte Lemna minor (Linnaeus, 1753) with Respect to Frond Number and Chlorophyll. Biotemas 22:27-33.

Kersey K (1981) Laboratory and field studies on the effects of road deicing salt on steam invertebrates SIC-9. R.J. Mackay, ed. University of Toronto, Toronto. Cited In: Evans & Frick (2001), CCME (2011).

Kessler E (1974) Physiological and biochemical contributions to taxonomy of genus Chlorella .9. salt tolerance as a taxonomic character. Archives of Microbiology 100:51-56. Cited In: CCME (2011).

Khangarot BS (1991) Toxicity of metals to a fresh-water tubificid worm, Tubifex tubifex (muller). Bulletin of Environmental Contamination and Toxicology 46:906-912.

Khangarot BS, Ray PK (1989) Investigation of correlation between physicochemical properties of metals and their toxicity to the water flea Daphnia magna straus. Ecotoxicology and Environmental Safety 18:109-120.

Khanna N, Cressman CP, Tatara CP, Williams PL (1997) Tolerance of the nematode Caenorhabditis elegans to pH, salinity, and hardness in aquatic media. Archives of Environmental Contamination and Toxicology 32:110-114.

Komarudin O, Shariff M, Shaharom F (1992) Toxicity of Sodium Chloride to Clarias batrachus (Linnaeus) and Its Effectiveness as a Chemotherapeutant for Quadricanthus kobiensis Ha Ky, 1968 Infections. In: M.Shariff, R.P.Subasinghe, and J.R.Arthur (Eds.), Proceedings of the First Symposium on Diseases in Aquaculture 1, Nov.26-29, 1990, Bali, Indonesia:425-430. Cited In: USEPA 2014.

Kostecki PT, Jones JJ (1983) The effect of osmotic and ion-osmotic stresses on the mortality of rainbow-trout (Salmo gairdneri). Comparative Biochemistry and Physiology a-Physiology 74:773-775.

Kszos LA, Winter JD, Storch TA (1990) Toxicity of chautauqua lake bridge runoff to young-of-the-year sunfish (Lepomis macrochirus). Bulletin of Environmental Contamination and Toxicology 45:923-930.

Lange M, Gebauer W, Markl J, Nagel R (1995) Comparison of testing acute toxicity an embryo of zebrafish, Brachydanio rerio and rtg-2 cytotoxicity as possible alternatives to the acute fish test. Chemosphere 30:2087-2102.

Lasier PJ, Hardin IR (2010) Observed and predicted reproduction of Ceriodaphnia dubia exposed to chloride, sulfate, and bicarbonate. Environmental Toxicology and Chemistry 29:347-358.

Lasier PJ, Winger PV, Hardin IR (2006) Effects of hardness and alkalinity in culture and test waters on reproduction of Ceriodaphnia dubia. Environmental Toxicology and Chemistry 25:2781-2786.

Lasier PJ, Winger PV, Reinert RE (1997) Toxicity of alkalinity to Hyalella azteca. Bulletin of Environmental Contamination and Toxicology 59:807-814.

Lazorchak JM, Smith ME (2007) Rainbow trout (Oncorhynchus mykiss) and brook trout (Salvelinus fontinalis) 7-day survival and growth test method. Archives of Environmental Contamination and Toxicology 53:397-405.

Leblanc G, Surprenant D (1984) The influence of mineral salts on fecundity of the water flea (Daphnia magna) and the implications on toxicity testing of industrial wastewater. Hydrobiologia 108:25-31.

LeClerc E, Devlaminck F (1950) Toxicological experiments with some products utilized in tanneries (estimated) Reprint from Centre Belge d'Etude et de Documentsdes Eaux 541. Cited In: McKee & Wolf (1963), Evans and Frick (2001), CCME (2011).

Lewis M (1995) Use of freshwater plants for phytotoxicity testing: a review. Environ. Pollut. 87:319-336.

Licht P, Feder ME, Bledsoe S (1975) Salinity tolerance and osmoregulation in the salamander Batrachoseps. Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology 102:123-134.

Lilius H, Hastbacka T, Isomaa B (1995) A comparison of the toxicity of 30 reference chemicals to daphnia-magna and Daphnia pulex. Environmental Toxicology and Chemistry 14:2085-2088.

Lilius H, Isomaa B, Holmstrom T (1994) A comparison of the toxicity of 50 reference chemicals to freshly isolated rainbow trout hepatocytes and Daphnia magna. Aquatic Toxicology 30:47-60.

Lowell RB, Culp JM, Wrona FJ (1995) Toxicity testing with artificial streams - effects of differences in current velocity. Environmental Toxicology and Chemistry 14:1209-1217.

Mackie GL (1978) Effects of pollutants on natality of Musculium securis (Bivalvia pisidiidae). Nautilus 92:25-33.

Magondu EW, Rasowo J, Oyoo-Okoth E, Charo-Karisa H (2011) Evaluation of sodium chloride (NaCl) for potential prophylactic treatment and its short-term toxicity to African catfish Clarias gariepinus (Burchell 1822) yolk-sac and swim-up fry. Aquaculture 319:307-310.

Mayer, T., Snodgrass, W.J., and Morin, D. 1999. Spatial Characterization of the Occurrence of Road Salts and Their Environmental Concentrations as Chlorides in Canadian Surface Waters and Benthic Sediments. Water Quality Research Journal of Canada 34: 545-574.

Mazuran N, Hrsak V, Tomic M, Papes D (1999) Effects of CaCl2 and CaBr2 on the fecundity of Planorbarius corneus L. Chemosphere 38:2345-2355.

McNulty EW, Dwyer FJ, Ellersieck MR, Greer EI, Ingersoll CG, Rabeni CF (1999) Evaluation of ability of reference toxicity tests to identify stress in laboratory populations of the amphipod Hyalella azteca. Environmental Toxicology and Chemistry 18:544-548.

Meyer JS, Sanchez DA, Brookman JA, McWhorter DB, Bergman HL (1985) CHEMISTRY AND AQUATIC TOXICITY OF RAW OIL-SHALE LEACHATES FROM PICEANCE BASIN, COLORADO. Environmental Toxicology and Chemistry 4:559-572.

Mitchell P, Prepas Ee (1990) Atlas of Alberta Lakes. University of Alberta Press, Edmonton.

Mohammed AA, Shafea AA (1992) Growth and some metabolic-activities of scenedesmus-obliquus cultivated under different NaCl concentrations. Biologia Plantarum 34:423-430.

Moser C, Bell G (2011) Genetic correlation in relation to differences in dosage of a stressor. Journal of Evolutionary Biology 24:219-223.

Mosslacher F (2000) Sensitivity of groundwater and surface water crustaceans to chemical pollutants and hypoxia: implications for pollution management. Archiv Fur Hydrobiologie 149:51-66.

Mount DR, Drottar KR, Gulley DD, Fillo JP, Oneil PE (1992) Use of laboratory toxicity data for evaluating the environmental acceptability of produced water discharge to surface waters. Produced Water: Technological / Environmental Issues and Solutions 46:175-185.

Mount DR, Gulley DD, Evans JJ (1993) Salinity/Toxicity Relationships To Predict the Acute Toxicity of Produced Waters to Freshwater Organisms. Society of Petroleum Engineers SPE 26007: 605-614.

Mount DR, Gulley DD, Hockett JR, Garrison TD, Evans JM (1997) Statistical models to predict the toxicity of major ions to Ceriodaphnia dubia, Daphnia magna and Pimephales promelas (fathead minnows). Environmental Toxicology and Chemistry 16:2009-2019.

Naumann E (1934) Uber die Beeinflussung con Daphnia magna durch einege Salze und Salzmischungen. K. fysiogr. Sallsk. Lund Forh 4:11-12. Cited In: CCME 2011, Anderson 1948.

Newman MC, Aplin MS (1992) Enhancing toxicity data interpretation and prediction of ecological risk with survival-time modeling - an illustration using sodium-chloride toxicity to mosquitofish (Gambusia holbrooki). Aquatic Toxicology 23:85-96.

Newman MC, Ownby DR, Mézin LCA, Powell DC, Christensen TRL, Lerberg SB, Anderson B-A, Padma TV (2002) Species sensitivity distributions in ecological risk assessment: Distributional assumptions, alternate bootstrap techniques, and estimation of adequate number of species. In: Posthuma, L., G.W. Sutter II and T.P. Traas (Eds.). Species Sensitivity Distributions in Ecotoxicology. Lewis Publishers, New York, NY.

Novelli A, Vieira BH, Cordeiro D, Cappelini LTD, Vieira EM, Espindola ELG (2012) Lethal effects of abamectin on the aquatic organisms Daphnia similis, Chironomus xanthus and Danio rerio. Chemosphere 86:36-40.

NRCAN (Natural Resources Canada) (1978) Hydrological atlas of Canada: water quality of surface waters - hardness.

Oshima S (1931) On the toxic action of dissolved salts and their ions upon young eels (Anguilla japonica). Jour. Imperial Fisheries Exp. Sta. (Japanese) 2:139-193.

Ozturk S, Aslim B (2010) Modification of exopolysaccharide composition and production by three cyanobacterial isolates under salt stress. Environmental Science and Pollution Research 17:595-602.

Padhye AD, Ghate HV (1992) Sodium chloride and potassium chloride tolerance of different stages of the frog, Microhyla ornata. Herpetological Journal 2:18-23.

Palmer CG, Muller WJ, Gordon AK, Scherman PA, Davies-Coleman HD, Pakhomova L, de Kock E (2004) The development of a toxicity database using freshwater macro invertebrates, and its application to the protection of South African water resources. South African Journal of Science 100:643-650.

Pandolfo TJ, Cope WG, Young GB, Jones JW, Hua D, Lingenfelser SF (2012) Acute effects of road salts and associated cyanide compounds on the early life stages of the unionid mussel Villosa iris. Environmental Toxicology and Chemistry 31:1801-1806.

Paquin P, Gorsuch J, Apte S, Batley G, Bowles K, et al. (2002) The biotic ligand model: a historical overview. Comparative Biochemistry and Physiology C 133:3-35.

Parker DL, Kumar HD, Rai LC, Singh JB (1997) Potassium salts inhibit growth of the cyanobacteria Microcystis spp. in pond water and defined media: Implications for control of microcystin-producing aquatic blooms. Applied and Environmental Microbiology 63:2324-2329.

Patrick R, Cairns JJ, Scheier A (1968) The relative sensitivity of diatoms, snails and fish to twenty common constituents of industrial wastes. The progressive Fish-Culturist 30:137-140. Cited In: CCME 2011.

Penttinen S, Kostamo A, Kukkonen J (1998) Combined effects of dissolved organic material and water hardness on toxicity of cadmium to Daphnia magna. Environ. Toxicol. Chem 17:2498-2503.

Peredo-Alvarez VM, Sarma SSS, Nandini S (2003) Combined effect of concentrations of algal food (Chlorella vulgaris) and salt (sodium chloride) on the population growth of Brachionus calyciflorus and Brachionus patulus (Rotifera). Revista De Biologia Tropical 51:399-407.

Perrucci S, Cecchini S, Pretti C, Cognetti AMV, Macchioni G, Flamini G, Cioni PL (1995) In-vitro antimycotic activity of some natural-products against Saprolegnia ferax. Phytotherapy Research 9:147-149.

Pic P, Maetz J (1981) Role of external calcium in sodium and chloride transport in the gills of seawater-adapted Mugil capito. J. Comp. Physiol B 141:511-521.

Pickering QH, Lazorchak JM, Winks KL (1996) Subchronic sensitivity of one-, four-, and seven-day-old fathead minnow (Pimephales promelas) larvae to five toxicants. Environmental Toxicology and Chemistry 15:353-359.

Potts W, Fleming W (1970) The effects of prolactin and divalent ions on the permeability to water of Fundulus kansae. J. Exp. Biol 53:317-324.

Powers EB (1917) The goldfish (Carassius carassius) as a test animals in the study of toxicity. ILL Biol. Monogr 4:1-73. Cited In: Doudoroff & Katz 1953, Evans & Frick 2001, CCME 2011.

Reynoso GT, Degamboa BA (1982) Salt tolerance in the fresh-water algae Chlamydomonas reinhardii - effect of proline and taurine. Comparative Biochemistry and Physiology a-Physiology 73:95-99.

Roback SS (1965) Environmental Requirements of Trichoptera. Cited In: CM Tarzwell (Ed), Biological Problems in Water Pollution, Trans 3rd Seminar, Aug 13-17, 1962, Tech Rep

999-WP-25. U.S. Public Health Service, R A Taft Sanitary Engineering Center, Cincinnati, OH.

Robertson J (1941) The function and metabolism of calcium in the invertebrata. Biol. Rev. 16:106-133.

Robison AL (2011) Influence of Predation-Based Chemical Cues on Contaminant Sensitivity in Fathead Minnows (Pimephales promelas) and Daphnia pulex. M.S Thesis: Oklahoma State University.

Rosicky P, Kouril J, Prikryl I (1987) The Effect of the Length of Short-Term Exposure to NaCl on the Tolerance of Early Common Carp and Silver Carp Fry to NaCl Baths. Bul Vyzk Ustav Ryb Hydrobiol Vodnany 23:8-13.

Santos M, Vicensotti J, Monteiro R (2007) Sensitivity of four test organisms (Chironomus xanthus, Daphnia magna, Pseudokirchneriella subcapitata) to NaCl: an alternative reference toxicant. J. Braz. Soc. Ecotoxicol 2:229-236.

Sanzo D, Hecnar SJ (2006) Effects of road de-icing salt (NaCl) on larval wood frogs (Rana sylvatica). Environmental Pollution 140:247-256.

Schiewer U (1984) The ecophysiology of salt adaptation in freshwater blue-green algae. Limnologica 15:555-558.

Rescan (Rescan Environmental Services) (2007) Ekati Diamond Mine Proposed Discharge Criterion for the Sable Kimberlite Pipe Development. Toxicity Testing for Chloride: Appendix A- Data Report., Yellowknife, NWT and Vancouver, B.C.

Setter TL, Greenway H, Kuo J (1982) Inhibition of cell-division by high external nacl concentrations in synchronized cultures of Chlorella emersonii. Australian Journal of Plant Physiology 9:179-196.

Seymour DT, Verbeek AG, Hrudey SE, Fedorak PM (1997) Acute toxicity and aqueous solubility of some condensed thiophenes and their microbial metabolites. Environmental Toxicology and Chemistry 16:658-665.

Shiogiri NS, Carraschi SP, Cubo P, Schiavetti BL, Cruz CD, Pitelli RA (2010) Ecotoxicity of Glyphosate and Aterbane BR Surfactant on Guaru (Phalloceros caudimaculatus). Acta Scientiarum. Biological Sciences 32:285-289.

Silva LVF, Golombieski JI, Baldisserotto B (2003) Incubation of silver catfish, Rhamdia quelen (Pimelodidae), eggs at different calcium and magnesium concentrations. Aquaculture 228:279-287.

Simmons JA (2012) Toxicity of major cations and anions (Na+, K+, Ca2+, Cl-, and SO42-) to a macrophyte and an alga. Environmental Toxicology and Chemistry 31:1370-1374.

Singh DP, Kshatriya K (2002) Characterization of salinity-tolerant mutant of Anabaena doliolum exhibiting multiple stress tolerance. Current Microbiology 45:165-170.

Smith ME, Lazorchak JM, Herrin LE, BrewerSwartz S, Thoeny WT (1997) A reformulated, reconstituted water for testing the freshwater amphipod, Hyalella azteca. Environmental Toxicology and Chemistry 16:1229-1233.

Soucek DJ (2007) Comparison of hardness- and chloride-regulated acute effects of sodium sulfate on two freshwater crustaceans. Environmental Toxicology and Chemistry 26:773-779.

Soucek DJ, Kennedy AJ (2005) Effects of hardness, chloride, and acclimation on the acute toxicity of sulfate to freshwater invertebrates. Environmental Toxicology and Chemistry 24:1204-1210.

Soucek DJ, Linton TK, Tarr CD, Dickinson A, Wickramanayake N, Delos CG, Cruz LA (2011) Influence of water hardness and sulfate on the acute toxicity of chloride to sensitive freshwater invertebrates. Environmental Toxicology and Chemistry 30:930-938.

Spehar RL (1986) Criteria Document Data. Memorandum to D.J. Call. September 16. Cited In: Iowa Chloride Criteria Development Reference List May 2009.

Stangenberg M (1975) The Influence of the Chemical Composition of Water on the Pike Perch (Lucioperca lucioperca L.) Fry From the Lake Gopio. Limnologica 9:421-426. Cited In: CCME 2011.

Stanley RA (1974) Toxicity of heavy metals and salts to Eurasian milfoil (Myriophyllum spicatum L.). Archives of Environmental Contamination and Toxicology 2:331-341.

Stekoll MS, Smoker WW, Failor-Rounds BJ, Wang IA, Joyce VJ (2009) Response of the early developmental stages of hatchery reared salmonids to major ions in a simulated mine effluent. Aquaculture 298:172-181.

Stephan CE, Mount DI, Hansen DJ, Gentile JH, Chapman GA, Brungs WA (1985) Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. U.S. Environmental Protection Agency. PB85-227049.

Stom DI, Zubareva LD (1994) Comparative resistance of Daphnia and Epischura to toxic substances in acute exposure. Hydrobiol Journal 30:35-38. Cited In: CCME 2011.

Stratton GW (1987) The effects of pesticides and heavy metals towards phototrophic microorganisms. In: Reviews in Environmental Toxicology 3, Hodgson E (eds.). Elsevier, NY, pp. 71-147.

Sutcliffe DW (1961) Studies on the salt and water balance in caddis larvae (Trichoptera): II. osmotic and ionic regulation of body fluids in Limnephilus stigma Curtis and Anabolia nervosa leach. Journal of Experimental Biology 38:521-530.

Swanson SM, Rickard CP, Freemark KE, Mac-Quarrie P (1991) Testing for pesticide toxicity to aquatic plants: Recommendations for test species. In Plants for Toxicity Assessment, ASTM STP 1115, ed. J. W. Gorsuch 8~ W. R. Lower, W. Wang & M. A. Lewis. American Society for Testing and Materials, PA, pp. 77-97.

Taraldsen JE, Norbergking TJ (1990) New method for determining effluent toxicity using duckweed (Lemna minor). Environmental Toxicology and Chemistry 9:761-767.

Tatara CP, Newman MC, McCloskey JT, Williams PL (1997) Predicting relative metal toxicity with ion characteristics: Caenorhabditis elegans LC50. Aquatic Toxicology 39:279-290.

Teeter JW (1965) Effects of sodium chloride on the sago pondweed. Journal of Wildlife Management 129:388-845.

Thapar R, Srivastava AK, Bhargava P, Mishra Y, Rai LC (2008) Impact of different abiotic stresses on growth, photosynthetic electron transport chain, nutrient uptake and enzyme activities of Cu-acclimated Anabaena doliolum. Journal of Plant Physiology 165:306-316.

Thornton KW, Sauer JR (1972) Physiological effects of NaCl on Chironomus attenuatus (Diptera - Chironomidae). Annals of the Entomological Society of America 65:872-875.

Threader RW, Houston AH (1983) Use of NaCl as a reference toxicant for goldfish, Carassius auratus. Canadian Journal of Fisheries and Aquatic Sciences 40:89-92.

Tietge JE, Hockett JR, Evans JM (1997) Major ion toxicity of six produced waters to three freshwater species: Application of ion toxicity models and tie procedures. Environmental Toxicology and Chemistry 16:2002-2008.

Trama FB (1954) The acute toxicity of some common salts of sodium, potassium, and calcium to the common bluegill (Lepomis macrochirus). Proceedings of the Academy of Natural Sciences of Philadelphia 196:185.

Trimble AJ, Belden JB, Mueting SA, Lydy MJ (2010) Determining modifications to bifenthrin toxicity and sediment binding affinity from varying potassium chloride concentrations in overlying water. Chemosphere 80:53-59.

US DCA (United States District Court of Alaska) Case No. 3:11-cv-00200-TMB: Plaintiffs, Native village of Point Hope, Alaska community action on toxics and the Northern Alaska Environmental Centre v. Defendants, U.S. Environmental Protection Agency and Intervener-defendants, Nana Regional Corporation Inc. and Teck Alaska Inc.

US EPA (United States Environmental Protection Agency) (1991) Methods for Aquatic Toxicity Identification Evaluations. Phase I Toxicity Characterization Procedures. EPA/600/6-91/003. EPA.

US EPA (United States Environmental Protection Agency) (2001) 2001 update of ambient water quality criteria for cadmium. U.S. EPA EPA-822-R-01-001.

US EPA (United States Environmental Protection Agency) (2002) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. U.S. EPA EPA-821-R-02-012.

US EPA (United States Environmental Protection Agency) (2010) Final report on acute and chronic toxicity of nitrate, nitrite, boron, manganese, fluoride, chloride and sulfate to several aquatic animal species. Book EPA-905-R-10-002. U.S. Environmental Protection Agency, Office of Science and Technology, Health and Ecological Criteria Division, Region 5 Water Division.

US EPA (United States Environmental Protection Agency) (2013a) US EPA Benchmark Dose Software (BMDS) 2.4 (version 1.8).

US EPA (United States Environmental Protection Agency) (2013b) Aquatic life ambient water quality criteria for ammonia - freshwater. Book EPA 822-R-13-001. USEPA Office of Water, Washington, DC.

US EPA (United States Environmental Protection Agency) (2013c) Environmental water quality information

US EPA (United States Environmental Protection Agency) (2014a) ECOTOX User Guide: ECOTOXicology Database System. Version 4.0.

US EPA (United States Environmental Protection Agency) (2014b) National Recommended Water Quality Criteria – Summary Table. Weblink (Accessed on 09/11/2014): http://water.epa.gov/scitech/swguidance/standards/criteria/current/index.cfm

USGS (United States Geological Survey) (2006) Water-quality characteristics, including sodium-absorption ratios, for four sites in the Powder River drainage basin, Wyoming and Montana, water years 2001-2004. USGS Report 2006-5113.

Valenti TW, Cherry DS, Neves RJ, Locke BA, J.J. S (2007) Case study: Sensitivity of mussel Glochidia and regulatory test organisms to mercury and a reference toxicant. In: Freshwater Bivalve Ecotoxixology, Farris JL, Van Hassel JH (eds). CRC Press.

van Horn WM, Anderson JB, Katz M (1949) The effect of kraft pulp mill wastes on some aquatic organisms. Transactions of the American Fisheries Society 79:55-63. Cited In: CCME 2011

Viertel B (1999) Salt tolerance of Rana temporaria: spawning site selection and survival during embryonic development (Amphibia, Anura). Amphibia-Reptilia 20:161-171.

Vosyliene MZ, Baltrenas P, Kazlauskiene A (2006) Toxicity of Road Maintenance Salts to Rainbow Trout Oncorhynchus mykiss. Ekologija 2:15-20.

Wallen IE, Greer WC, Lasater R (1957) Toxicity to Gambusia affinis of certain pure chemicals in turbid waters. Sewage and Industrial Wastes 29:695-711.

Waller DL, Fisher SW, Dabrowska H (1996) Prevention of zebra mussel infestation and dispersal during aquaculture operations. Progressive Fish-Culturist 58:77-84.

Waller DL, Rach JJ, Cope WG, Marking LL, Fisher SW, Dabrowska H (1993) Toxicity of candidate molluscicides to zebra mussels (Dreissena polymorpha) and selected non-target organisms. Journal of Great Lakes Research 19:695-702.

Wang N (2007) Email to C. Stephan. September 11. Cited In: Iowa Chloride Criteria Development Reference List. May 2009. Cited in CCME 2011.

Wang N, Ingersoll C, Ivey C, Brumbaugh B, Hammer E, al. e (2013) Acute sensitivity of freshwater mussels to select chemicals with differing toxic modes of action. Presentation at SETAC (Society of Environmental Toxicology and Chemistry).

Wang N., C.J. I (2010) Email to M. Nowierski December 20. Reference toxicity test data for NaCl and various aquatic invertebrates. United States Geological Survey. Cited In: CCME (2011).

Wangberg S, Blanck H (1988) Multivariate patterns of algal sensitivity to chemicals in relation to phylogeny. Ecotoxicol. Environ. Safety 16:72-82.

Welsh P, Lipton J, Chapman G, Podrabsky T (2000) Relative importance of calcium and magnesium in hardness-based modification of copper toxicity. Environmental Toxicology and Chemistry 19:1624-1631.

Wiebe AH, Burr JG, Faubion HE (1934) The problem of stream pollution in Texas with special reference to salt water from oil fields. Cited In: Evans and Frick, 2001. Transactions of the American Fisheries Society 64:81-86. Cited In: Doudoroff & Katz 1953, Evans & Frick 2001, CCME 2011.

Wilcox DA, Andrus RE (1987) The role of Sphagnum fimbriatum in secondary succession in a road salt impacted bog. Canadian Journal of Botany-Revue Canadienne De Botanique 65:2270-2275.

Wilcox DA (1984) The effects of NaCl deicing salts on Sphagnum recurvum P. Beauv. Environmental and Experimental Botany 24:295-304.

Williams DD, Williams NE, Cao Y (1999) Road salt contamination of groundwater in a major metropolitan area and development of a biological index to monitor its impact. Water Research 34:127-138.

Wisconsin State Laboratory of Hygiene (WISLOH) (2007) Tables of Data (provided to C. Stephan, US EPA). Development Reference List May 2009. Cited In: Iowa Chloride Criteria Development Reference List May 2009. Cited In: CCME 2011.

Wurtz C.B. BCH (1961) Preliminary results from macroinvertebrate bioassays. Proceedings of the Pennsylvania Academy of Science 35:51-56.

Yarzhombek AA, Mikulin AE, Zhdanova AN (1991) Toxicity of Some Substances to Fish in Relation to Form of Exposure. Journal of Ichthyology 31:99-106.

Young R (1923) Resistance of fish and alkalinity. American Journal of Physiology 63:373. Cited in Evans & Frick, CCME 2011.

Zuccarelli MD, Ingermann RL (2007) Calcium-induced quiescence of sperm motility in the bluegill (Lepomis macrochirus). Journal of Experimental Zoology Part a-Ecological Genetics and Physiology 307A:590-599.