Equilibrium Environmental Inc.

FRESH WATER AQUATIC LIFE (FWAL) WORKING GROUP

DEVELOPMENT OF A LONG-TERM CHLORIDE WATER QUALITY GUIDELINE INCORPORATING HARDNESS-MODIFYING FACTORS

DRAFT

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

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I. ABSTRACT

Chloride (Cl^-) toxicity towards freshwater aquatic species as a function of water hardness was assessed to develop a long-term hardness-adjusted water quality guideline (WQG). In 2011, the Canadian Council of Ministers of the Environment (CCME) identified water hardness as an important factor modifying the toxicity of chloride towards aquatic life; however, a hardness-derived guideline was not established in 2011 due to data limitations. To address these limitations, a battery of toxicology experiments, funded by the Petroleum Technology Alliance Canada (PTAC), were completed to further elucidate the relationship between water hardness levels and chloride toxicity thresholds in sensitive species including representations from algae, amphibians, fish, mussels, and aquatic insects. Toxicity data generated from the experimental work were assessed and analyzed along with data published in peer reviewed literature since release of the CCME (2011) water quality guideline document. The CCME (2011) previously derived an aquatic life WQG for long-term chloride exposure of 120 mg/L.

Using results from the PTAC funded toxicity experiments, recently published literature studies, and guideline development methods used by CCME (2011) and United States Environmental Protection Agency (US EPA) (2001, 2010), in the absence of any hardness adjustment, a generally similar WQG of 125 mg/L was derived (compared to the CCME (2011) WQG of 120 mg/L). In order to derive a hardness-adjusted WQG, methods provided by US EPA, also used by CCME for cadmium and zinc WQGs, were implemented. An equation representing the change in WQG as a function of water hardness was derived, as follows:

$$\text{Chloride WQG} = \exp\,[0.38\,(\ln(\text{hardness})) + 3.18]$$

This equation was considered applicable to a hardness range of 5 to 350 mg $CaCO_3$/L. The corresponding chloride WQGs for this range were 44 mg Cl/L to 222 mg Cl/L. The lower WQG limit of 44 mg Cl/L was considered applicable to water with hardness values of 5 mg $CaCO_3$/L and less. The upper WQG limit of 222 mg Cl/L was considered applicable to water with hardness values of 350 mg $CaCO_3$/L and greater.

To ensure the resulting chloride guideline provided adequate protection for aquatic life exposure to other chloride salts such as $MgCl_2$ and $CaCl_2$, given the toxicity database was based primarily on NaCl data, an analysis of guideline protectiveness was completed. None of $MgCl_2$ and $CaCl_2$ toxicity values fell below guidelines. Comparative toxicity data from the major salt ions, present in all fresh waters, such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, and HCO_3^-, were also evaluated to assess differences in paired cation(s) on chloride toxicity.

Data from several studies indicates that the use of formulated laboratory water for toxicity testing was associated with greater toxicological effects at concentrations equivalent to those in 'wild' waters collected from the field. An approximately 2-fold greater sensitivity to formulated laboratory water compared to field water may be expected for daphnids, mayflies, freshwater mussels, and frogs (Gillis, 2011, Harless *et al.,* 2011, Johnson *et al.*, 2015, Nautilus, 2015). Since current WQGs were derived based on laboratory data, the greater sensitivity may serve as an additional safety factor.

II. EXECUTIVE SUMMARY

The Canadian Council of Ministers of the Environment (CCME, 2011) provided an updated Water Quality Guideline (WQG) for chloride (from 230 to 120 mg/L for the long-term exposure), applicable to freshwater aquatic life species. In the 2011 derivation, the CCME also identified water hardness as an important factor modifying the toxicity of chloride to aquatic life. As a result of limitations with the long-term dataset, a hardness-derived guideline was not established in 2011. Long-term hardness-toxicity data were either not available or did not meet the minimum data requirements for hardness-adjusted water quality guidelines at the time based on the methods of Stephen (1985).

Due to existing data limitations, the Petroleum Technology Alliance Canada (PTAC) commissioned a multi-year study, working with a third party consulting group Equilibrium Environmental Inc. (EEI) and associated research laboratories in Canada (Nautilus Environmental) and the United States (University of Georgia, Research Foundation Inc., Warnell School of Forestry and Natural Resources; Wisconsin State Laboratory of Hygiene, Environmental Health Division), in order to generate additional toxicity information for species sensitive to chloride toxicity, and data analysis on the topics described above, with a focus on water hardness.

The objectives of the present study were to:

1. identify relevant new data that has been published on chloride toxicity to aquatic life (since the CCME (2011) water quality guideline) and assess whether these data are of sufficient quality to be incorporated into a water quality guideline update;
2. summarize PTAC sponsored toxicity testing work that has been conducted from 2015 to 2019 on hardness influences towards chloride toxicity in algae, amphibians, fish, mussels, and aquatic insects (sensitive species from *Ephemenoptera*, *Plecoptera*, and *Tricoptera* (EPT) orders were selected);
3. provide a re-assessment of chloride water quality guidelines, post CCME (2011) analysis, given available new toxicology information;
4. provide an assessment of hardness influences on chloride toxicity and determine how, and to what extent, this may quantitatively affect a chloride water quality guideline; and,
5. summarize recent information on aspects of multi-ion toxicity.

An aquatic toxicological database for chloride was established using data from 167 long-term exposure studies, and 120 of them were considered acceptable. From these 88 studies, 21 represented the newest (2012-2019) additions to the CCME (2011) dataset. Besides effect concentrations, toxicity endpoints, and exposure durations, other experimental variables 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).

The first task scope was to incorporate newly published information, and data from the PTAC sponsored toxicity testing work, excluding the influence of hardness, into the WQG derivation process and determine whether the resulting WQG differed from the previously derived CCME (2011) WQG derived from a Species Sensitivity Distribution (SSD) approach and toxicity data for NaCl. The CCME (2011) dataset included the endpoints for 28 species. The current dataset includes the endpoints for 35 species, and it was evaluated for the best fit cumulative distribution function. In both derivations (CCME and herein), the Logistic model was found to best fit the data. The chloride long-term WQGs were based on CCME protocols for Type A (statistical - SSD derivation) data (CCME, 2007) and utilized endpoints for NaCl as chloride salt found to satisfy the requirements for Type A analysis.

The guideline derived from the unadjusted long-term SSD was 125 mg/L, which is 5 mg/L higher (4% relative percent difference) than the 120 mg/L value established previously by the CCME (2011). The differences may be due in part to the addition of new toxicological information to the dataset and/or minor differences in curve fitting to the data using the Logistic model.

To derive the long-term hardness-adjusted guidelines, chloride toxicity values from different studies were compared by converting them to a standardized hardness value. 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). The hardness-toxicity relationships were assessed with statistical analysis. Individual hardness-toxicity slopes were estimated for organisms where effect concentrations were available over a wide range of water hardness concentrations and similar experimental conditions. The pooled slope was analyzed to determine an overall coefficient of the hardness-toxicity relationship within the long-term dataset.

For some species, such as the water flea *Daphnia magna,* 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* Stephen *et al.,* 1985) or came from the different laboratories with different experimental conditions. The pooling of data from different laboratories for a particular species, where experiments were conducted at different hardness levels, may support an amelioration of chloride toxicity with increasing hardness (such is the case for *Daphnia magna*), however the potential influence of different experimental conditions between labs can not be quantified and the pooling of data from multiple studies/labs for one species was considered to be of insufficient rigor for including in the quantitative analysis of hardness and chloride toxicity.

The long-term hardness-adjusted (50 mg $CaCO_3$/L) chloride toxicity dataset was then assessed using the SSD, and the best fit cumulative distribution function was utilized for guideline derivation. The resulting SSD was built based on the data from 27 species modeled with a logistic function (8 out of 35 endpoints from an unadjusted SSD in a first step, had no reported hardness, and therefore, were not included in the hardness-adjusted model). The derived long-term WQG are presented as an exponential (natural log) function, which can be used to calculate a chloride WQG at a particular water hardness level. The guideline equation is shown below, along with the results chloride concentrations at various water hardness concentrations.

$$\textit{Long-term WQG} = \exp^{[0.38(\ln(\text{hardness})) + 3.18]}$$

Notes:

Hardness measured as mg/L as $CaCO_3$;
The long-term hardness equation is applicable from 5 to 350 mg $CaCO_3$ /L. For the hardness from 0 to 5 mg $CaCO_3$/L, the lower limit of 44 mg/L is applied. For the hardness >350 mg $CaCO_3$/L, the upper limit of 222 mg/L is applied.

Guidelines for the Protection of Fresh Water Aquatic Life at Various Hardness Values

Water hardness (mg/L as $CaCO_3$)	Long-term exposure (mg Cl^-/L)
Lower limit (0-5) *	*44*
Soft (50)	*106*
Moderately hard (150)	*161*
Hard (300)	*209*
Upper limit (350 and greater) **	*222*

Notes: *the long-term hardness equation can be used for direct calculation from 5 to 350 mg $CaCO_3$ /L.*
** 44 mg Cl-/L is the lower limit long-term WQG value that applies to waters with 5 mg $CaCO_3$/L and less.*
*** 222 mg Cl-/L is the upper limit long-term WQG value that applies to waters of 350 mg $CaCO_3$/L and greater.*

To ensure the resulting chloride guideline provides an adequate protection for $MgCl_2$ and $CaCl_2$ toxicity, given the toxicological dataset was primarily NaCl based, an analysis of protectiveness was conducted. Acceptable toxicity values, non-adjusted for hardness, were plotted against the hardness-adjusted guideline. No $MgCl_2$ or $CaCl_2$ toxicity values fell below the guidelines, indicating the hardness adjusted WQG derived from the NaCl toxicological dataset was also protective of aquatic life exposures to other chloride salts such as $MgCl_2$ and $CaCl_2$.

Comparative toxicity from the major salt ions, present in all fresh waters, such as Na+, K+, Ca^2, Mg^2, Cl-, SO_4^2-, and HCO_3^-, was evaluated additionally to the current guideline development. PTAC-funded studies by UGARF (2016b) and WSLH (2017) compared the long-term toxicity of the major salts to the freshwater mussel *Lampsilis siliquoidea*, and to the microalgae *Raphidocelis subcapitata*. Generally, for the freshwater mussels the salt-based toxicity values and the anion-based toxicity values had the similar order as observed in the literature review and as mentioned in CCME (2011), decreasing from KCl (the most toxic salt) to NaCl (the least toxic salt). For the microalgae, $CaSO_4$ was observed as the most toxic salt, whereas the least toxic effect was observed in $CaCl_2$ or NaCl.

III. BEST PRACTICES RECOMMENDATIONS / TANGIBLE PROJECT OUTCOME

Best Practices Recommendations are defined as "*a set of guidelines, ethics, or ideas that represent the most efficient or prudent course of action*". Best Practices herein are related to the derivation of WQG with and without hardness adjustment, as well as how the WQGs and associated adjustments can be implemented. Tangible Project Outcomes are defined as "*outcomes that the industry can take and use and put into practice*". These definitions were contractually set by PTAC. The two definitions overlap, but essentially encompass key aspects of the guideline derivation process as well as how the guidelines can be used or implemented.

i. Toxicological Database Criteria

The guidelines are built on the solid and thorough toxicological database, compiled from individual peer-reviewed articles, government guideline deviation documents and online databases, technical reports, and presentations by reputable researchers at major conferences and forums. The aquatic toxicological database was comprised of literature examining the toxicity of key chloride salts NaCl, $CaCl_2$, and $MgCl_2$ – the guidelines were derived primarily based on the extensive NaCl dataset with a protectiveness evaluation for the other two chloride salts Studies identified in the literature searches, and toxicity data collected and cited by CCME, as part of their guideline derivation process in 2011, were obtained and critically reviewed. Unpublished data generated from 3rd party researchers under contract to EEI, and funded via PTAC (WSLH, 2016, UGARF; 2016, Nautilus, 2016 and 2019), were included. Some critical grey literature (*e.g.*, presented by reputable researchers, but not published yet in a peer-reviewed journal) data were added for sensitive species, as anticipation of future publications that could influence the final guidelines. In total, 633 long-term data points were evaluated.

Studies were classified as primary or secondary (CCME, 2007), and unacceptable data were excluded. Data was deemed to be unacceptable when experimental controls were not reported, environmental conditions were inappropriate (*e.g.,* test temperatures were too high), the ionic composition of the test medium was unknown, the species tested was not appropriate for Canadian waters (*e.g.,* tropical species), an inadequate number of animals per exposure group was used, or high adverse effect levels were observed in the control group.

ii. <u>Best Practices Recommendations</u>

Should toxicity data be identified in the future that is sufficiently rigorous, with hardness data reported, the data should be evaluated for integration into the SSD. The figures within this report can be used to visually assess the potential influence of any new data on the SSD. An example of the datapoints that could potentially significantly alter the WQG would be a study where adverse effects occurred at relatively low chloride concentrations higher hardness levels, which would potentially shift the lower portion of the hardness-adjusted SSD curve.

iii. Hardness Adjustment

Water hardness has been identified as an ameliorating factor in aquatic guideline policy. To explore potential relationships between chloride toxicity and water hardness, long-term data was compiled for species, studied in the same laboratory with effect concentrations across a wide range of water hardness. Natural logarithms were used to characterize hardness-toxicity relationship following the methods outlined by Stephen *et al.* (1985), and in approaches utilized by the Canadian Environmental Protection Act (CEPA), CCME, and the US EPA in the hardness-adjusted guidelines for cadmium, cobalt, copper, lead, manganese, and nickel (CCME, 2017 and 2019; CEPA, 2020 and 2021; US EPA, 1998 and 2016). Effect concentrations were selected within a range, where the highest hardness is at least three times greater than the lowest one, and at least 100 mg/L higher than the lowest one (CCME, 2014, US EPA, 2001, Stephen *et al.*,1985). To avoid the potential introduction of variability due to differences in inter-laboratory experimental conditions, data points for each species were grouped and analysed by each laboratory independently.

Data points that met these criteria across comparable endpoints, exposure durations, life stages, and laboratory environment, were plotted together with natural log (hardness) and natural log (chloride effect concentration) as the independent (x-axis) and responding (y-axis) variables, respectively. The mathematical guideline calculations were completed using EasyFit 5.6 Professional software (Mathwave Technologies, 2015).

<u>**Best Practices Recommendations**</u>

WQGs are generally applied to surface water bodies inhabited by aquatic life. Measurements of hardness subsequently used to determine a hardness-adjusted chloride WQG should be taken from where the WQG is considered applicable (*i.e.*, the point of compliance). For example, hardness should be measured in a water sample taken from a surface water body for evaluating measured chloride concentrations in the water body. Most accredited laboratories are capable of measuring hardness in water samples.

In some jurisdictions (*e.g.*, Alberta), the WQGs are also implemented at the point of groundwater discharge into a surface water body (essentially the point of discharge into the hyporheic zone (mixing zone of groundwater and surface water)) when developing soil or groundwater remediation guidelines protective of aquatic life. In this instance, the hardness measurement(s) should be from groundwater considered representative of conditions at the point of discharge into the hyporheic zone of a surface water body. The proponent may want to consider the additional layers of protectiveness when developing Risk Management strategies.

iv. Assessment of Guideline Protectiveness

To assess the protectiveness of the long-term hardness-dependant guidelines, the acceptable chloride effect concentrations (log transformed and non-adjusted for hardness) were plotted against log hardness. The respective WQGs were plotted as the straight line. 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 provided a measure of the effectiveness of the guidelines towards Ca^{2+} chloride toxicity. In addition, a guideline protectiveness chart was used to assess the upcoming data from soft water aquatic organisms.

v. Guideline Application Range

The hardness equation for calculating long-term water quality guidelines is applicable between the 5 and 350 mg $CaCO_3$/L, and should not be applied outside of this range. Lower and upper hardness limits reflect the minimum and maximum hardness values beyond which WQG should not be extrapolated. The lower limit comes from the minimum values reported by the national survey of Canadian surface waters, conducted over the period 1975 to 1977 (Health Canada, 1979; CCME, 2011). The upper limit of 350 mg/L is linked to increased species sensitivity in extremely hard waters.

vi. Special Concern and Endangered Species Protectiveness/ Additional Safety Factor

To ensure protectiveness of the sensitive freshwater mussels *Epioblasma torulosa rangiana* and *Lampsilis fasciola*, the Protection Clause (CCME, 2007) can be applied. It means that in the areas where the COSEWIC special concern mussel (*L. fasciola*) or the COSEWIC endangered mussel (*E. torulosa rangiana*) are present, the WQGs are based on the lowest toxicity values observed (24 mg/L for the regions with the most sensitive broods of *L. fasciola*, and 42 mg/L for *E. torulosa rangiana*).

An approximately 2-fold greater sensitivity to laboratory waters may be expected for daphnids, freshwater mussels, and frogs. Since current guidelines were derived based on laboratory data, the greater sensitivity may serve as an additional safety factor, and make the guidelines more conservative.

Since chloride salts appear to be less toxic in natural waters compared to experiments with re-constituted laboratory waters, the natural water studies were excluded from the database. This approach led to more conservative WQGs that are applied to natural waters.

vii. Recommendations/ Outcomes

The current equation may be applied for direct calculation within a range between 5 and 350 mg $CaCO_3$/L, resulting in guidelines from 44 mg/L to 222 mg/L. For the very soft waters (0-5 mg $CaCO_3$/L), the lowest limit of 44 mg/L is applicable. This lowest limit covers the 90th percentile of the surface waters in Nova Scotia, with 4.6 mg/L of hardness. For the rest of Canada, the upper value covers the 90th percentile of the major surface waters, except Manitoba and Saskatchewan, where the median hardness was equal to 287 mg/L, and 300 mg/L, and 90th percentile was equal to 402 mg/L, and 531 mg/L, respectively (CCME, 2011). For these extremely hard waters (hardness >350 mg $CaCO_3$/L), the maximum of 222 mg/L chloride guidelines may be utilized, assuming a plateau of the ameliorating effect in extremely hard waters.

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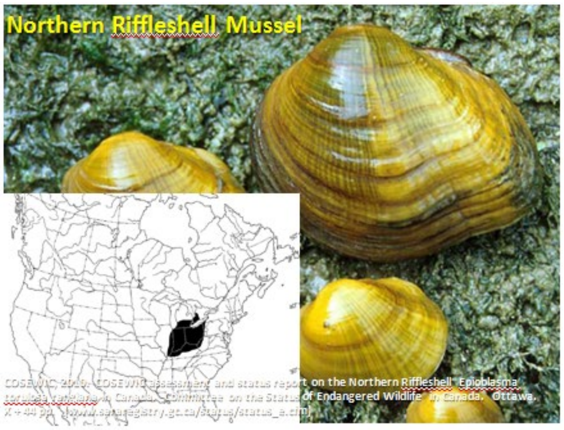
1 BACKGROUND

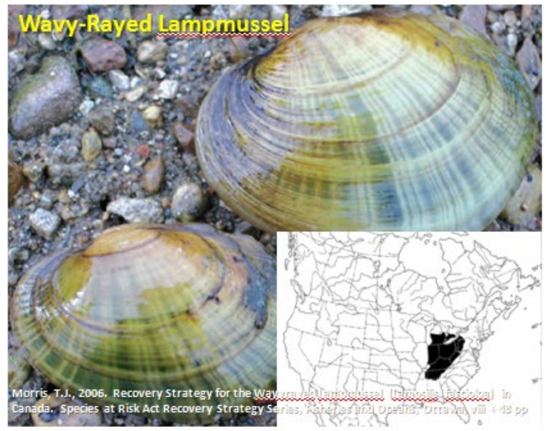
1.1 INTRODUCTION

Chloride is a detectable ion in aquatic ecosystems, entering solution naturally through the weathering of soils and parent geological material containing salts of sodium (NaCl), potassium (KCl), calcium ($CaCl_2$), and magnesium ($MgCl_2$). Chloride may enter aquatic ecosystems from anthropogenic sources such as road salts, oil and gas activities (*e.g.*, produced water spills), septic fields (*e.g.*, due to human waste and water softening salts), and livestock operations (*e.g.*, paddock runoff). In aquatic ecosystems, chloride is found within the water column and pore space, as it does not adsorb onto mineral surfaces (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 five million tonnes / year (Environment Canada and Health Canada (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%) (EC, 2004). The loading of chloride onto aquatic ecosystems in proximity to urban areas has been considered an important issue resulting in the Priority Substances List Assessment Report for Road Salts (EC, 2004) identifying chloride salts as toxic under subsections 64 (a) and (b) of the Canadian Environmental Protection Act (CEPA, 1999).

In 2011, the Canadian Council of Ministers of the Environment (CCME) updated the freshwater aquatic life water quality for chloride, based on a toxicological dataset that included results for two Committee on the Status of Endangered Wildlife in Canada (COSEWIC) species of Endangered or Special Concern. Results indicated the glochidia life stage of freshwater mussels can be particularly sensitive to adverse effects associated with chloride exposure. This in part resulted in the chloride water quality guideline decreasing from 230 to 120 mg/L by 2011 (CCME, 2011). It is noteworthy that the COSEWIC species at risk, *Lampsilis fasciola* and *Epioblasma torulosa rangiana*, demonstrated adverse effects in laboratory experiments with reconstituted lab water at chloride concentrations ranging from 24 to 42 mg/L, invoking the Protection Clause (CCME 2007) when these species are present in an aquatic system under investigation. Their habitat in Canada is focused in the Ontario Great Lakes region (shown in the map inserts below, black marked area of Canada and the United States). While these two species do not appear to be widely distributed throughout Canada, there are other species of the Family Unionidae (clams and mussels) encountered in various regions of Canada as are other Pelecypoda species that have been tested for chloride toxicity including *Lampsilis siliquoidea* (mussel), *Musculium securis* (clam), *Sphaerium tenue* (fingernail clam), and *Sphaerium simile* (fingernail clam). These species do not appear to be as sensitive as *Lampsilis fasciola* and *Epioblasma torulosa rangiana*, based on available toxicological data.





COSEWIC Special Concern: Wavy-rayed Lampmussel (Lampsilis fasciola).

The CCME (2011) identified a need for additional toxicity data investigating aspects of water hardness influence on chloride toxicity. Canadian surface water bodies can have variable water hardness and thus potential variability in toxicological response to chloride. Several acute studies indicate that elevated hardness ameliorates chloride toxicity. A single chronic study was available, which was considered an inadequate database for any adjustment to chloride toxicity based on hardness. The CCME (2011) indicated they would re-visit the chloride water quality guideline when sufficient studies are available and noted that jurisdictions can opt to derive site-specific hardness adjusted water quality guidelines.

CCME (2011) suggested the interpretation of a hardness effect on chloride toxicity may be complicated by a relatively large acute study involving multiple combinations of ion exposures, where the reduction of chloride toxicity may be related to a multi-ion effect rather than hardness. For one of the example data comparisons based on the work of Mount *et al.* (1997), no influence of hardness on the toxicity of chloride was found - 50% lethal concentrations were similar for NaCl and $CaCl_2$ (when expressed on a chloride concentration basis) regardless of differences in hardness level. Exact hardness values were not reported leaving it is difficult to discern the degree to which hardness could (or could not) have influenced toxicity, and thus the study conclusions in this regard are semi-quantitative. The second example from the same study involved an experiment where NaCl was added to KCl and the 48-hour 50% lethal concentration for *C. dubia* increased from 329 mg K/L to 458 mg K/L – the hardness levels were reportedly the same, although no supporting data (actual hardness measurements) were provided. This is not necessarily a hardness effect – the result could be the result of exposure to two different cations of considerably different toxic potencies towards *C. dubia* and interactions between the cations via a cross membrane antiporter, which are present in most animal species.

The CCME (2011) chloride guideline was not developed based on KCl salts, due in part to the relatively greater toxic potential compared to Ca and Na chloride salts. The complication of a multi-ion effect may result in different toxicity associated with the chloride associated cation. The multi-ion effect may have a lesser relevance for a database based on predominantly Ca and Mg toxicity studies for deriving a chloride water quality guideline, as was completed by the CCME (2011). A similar approach as per CCME (2011) was followed herein where the toxicological database focused on Ca and Mg chloride salts. Supporting work towards understanding the multi-ion effect was included in this document to foster the continued improvement of water quality guidelines across the country.

The variable chloride toxicity as a function of hardness (and potentially multi-ion composition) is an important issue for industries associated with anthropogenic chloride releases. If water hardness is typically determined to alter chloride toxicity for aquatic life species, then an update to the chloride water quality guideline, including aspects of hardness, will refine risk management strategies in the Canadian environment.

1.2 SCOPE OF WORK

Due to existing data limitations, the Petroleum Technology Alliance Canada (PTAC) commissioned a multi-year study, working with a third party consulting group Equilibrium Environmental Inc. (EEI) and associated research laboratories in Canada and the United States (University of Georgia, Research Foundation Inc., Warnell School of Forestry and Natural Resources; Wisconsin State Laboratory of Hygiene, Environmental Health Division; and Nautilus Environmental), in order to generate additional toxicity information and data analysis on the topics described above, with a focus on water hardness.

The key data limitation relates to long-term exposure scenarios studying influences of hardness on toxicity. In comparison, there is a more thorough database for short-term exposure scenarios, and the data limitations are relatively minor. The PTAC work focused on representative species from groups with limited data on hardness-influenced chloride toxicity (*i.e.*, algae, amphibians, fish), and sensitive species (*i.e.*, mussels, clams, and EPT index species). Few studies have been conducted on EPT (*Ephemeroptera* (mayflies), *Plecoptera* (stoneflies), and *Trichoptera* (caddisflies)) species that have been found more recently to be relatively sensitive to disturbance through anthropogenic pollution (Jacobus *et al.,* 2019; Morinière *et al.,* 2017).

In summary, the purpose of the PTAC commissioned work and this document was to:

1. identify relevant new data that has been published on chloride toxicity to aquatic life (since the CCME (2011) water quality guideline) and assess whether these data are of sufficient quality to be incorporated into a water quality guideline update;
2. summarize PTAC sponsored toxicity testing work that has been conducted from 2015 to 2019 on hardness influences towards chloride toxicity in algae, amphibians, fish, mussels, and a representative of the EPT species;
3. provide a re-assessment of chloride water quality guidelines given available new information without any interaction with water hardness;
4. provide an assessment of hardness influences on chloride toxicity and determine how, and to what extent, this may quantitatively affect a chloride water quality guideline; and,
5. summarize recent information on aspects of multi-ion toxicity.

2 LONG-TERM DATA PUBLISHED SINCE 2011

A literature search was conducted to identify papers published since 2011 (date of the last CCME chloride guideline release) on aspects of chloride toxicity, hardness amelioration, and multi-ion effects. Papers were assessed for relevance and whether they met minimum data quality requirements as per CCME (2007). Vetted toxicological datasets were subsequently incorporated into the derivation of short-term and long-term aquatic life guidelines for chloride.

CCME (2011) stated that for $MgCl_2$ and KCl, toxicity was due to the cations, whereas for $CaCl_2$ and NaCl, toxicity appeared more related to the anion. A review of recent literature including PTAC sponsored research on various ion combinations indicates that toxicity appears predominantly due to the cation for NaCl, KCl, $CaCl_2$, and $MgCl_2$. Between these ion pairs, more recent as well as historical data indicates that KCl is notably more toxic than the other cations for the majority of, but not all, species. The development of cation-specific aquatic life guidelines is somewhat unwieldy because most natural environments contain elevated and variable concentrations making it more difficult to distinguish natural from anthropogenic contributions towards total mass. This affords an important advantage to having a chloride guideline in that typically it is present in low concentrations in the natural environment and elevated concentrations are more frequently reflective of, and proportional to, anthropogenic inputs.

A comparison of the toxic potency between calcium (Ca^{2+}), magnesium (Mg^{2+}), and sodium (Na^+) based on historical and more recent published data indicates that they are in relatively close proximity compared to potassium (K^+). In some studies, it was found that $CaCl_2$ was more toxic than NaCl, whereas the opposite occurred for other species. Typically, $MgCl_2$ was found to be of equivalent general toxic potency to the other two salts. This concept is discussed further in Section 3.1. For the guideline derivation process herein, in instances where $CaCl_2$ or $MgCl_2$ was found to be more toxic than NaCl, those data were used preferentially over NaCl to ensure the resulting guideline was sufficiently conservative for exposures to any of these three chloride salts. Studies on KCl were not included in the guideline derivation as frequently it was more toxic by an order of magnitude or more, compared to the other three cation chloride salts.

2.1 DATABASE COMPILATION

The toxicological database was derived from individual peer-reviewed articles, government guideline deviation documents and online databases (*e.g.*, ECOTOX database, US EPA 2014a), technical reports, and presentations by reputable researchers at major conferences and forums. Toxicity data collected and cited by CCME, as part of their guideline derivation process in 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 was comprised of literature examining the toxicity of key chloride salts NaCl, $CaCl_2$, and $MgCl_2$. Studies examining non-chloride salts, whole effluents such as road salt (which can contain a number of trace metals, complicating the toxicological analysis), and natural waters with chloride impacts, were excluded as per CCME (2011). It should be noted that chloride salts appear to be less toxic in natural waters compared to experiments with re-constituted laboratory waters. As a result, the exclusion of studies with natural waters results in a more conservative guideline than what may be derived if natural water datasets were included.

In addition to effect concentrations, toxicity endpoints, and exposure durations, other experimental variables were incorporated into the dataset, including: 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). Data evaluated up to 2014 were presented in a previous report (EEI, 2014).

2.2 DATA QUALITY REQUIREMENTS

The guideline derivation process conducted herein paralleled that conducted by CCME (2011), incorporating a statistical species sensitivity distribution (SSD) method to determine a long-term exposure guideline. The protocols and minimum data requirements for this type of derivation are outlined in CCME (2007). The long-term WQGs are meant to protect all forms of aquatic life from any adverse effects over an indefinite exposure period (CCME, 2007, CCME, 2011).

To determine long-term SSDs, potential data points for incorporation are classified as primary or secondary (CCME, 2007), and unacceptable data are excluded. Data was deemed to be unacceptable when experimental controls were not reported, environmental conditions were inappropriate (*e.g.*, test temperatures were too high), the ionic composition of the test medium was unknown, the species tested was not appropriate for Canadian waters (*e.g.*, tropical species), an inadequate number of animals per exposure group was used, or high adverse effect levels were observed in the control group.

Long-term SSD data requirements were followed as outlined by the CCME (2007, 2011), shown in Table 2.1, with specific requirements for the multiple taxa. For the long-term guideline derivation, low- and no-effect toxicity values are preferred in the following order: EC/IC (inhibitory concentration) representing a no-effect threshold > EC/IC_{10} > $EC/IC_{11\text{-}25}$ > MATC (maximum acceptable toxicant concentration) > NOEC (no observable effect concentration) > LOEC (lowest observable effect concentration) > $EC/IC_{26\text{-}49}$ > nonlethal EC/IC_{50}. Applicable endpoints must be reported across long-term durations with additional requirements depending on the taxa examined (Table 2.1). If plants or algae are determined to be one of the most sensitive species, then additional data are required.

Table 2.1. Minimum Long-Term Data Requirements for SSD

Taxon	Number of Studies	Number of Species	Endpoint	Exposure duration (days)
Fish	3	3 (1 salmonid and 1 non-salmonid)	Low- and no-effect*	≥ 21 (larva: ≥ 7)
Invertebrates	3	3 (1 planktonic)	Low- and no-effect*	≥ 7(short-lived: ≥ 4) (lethal: ≥21)
Amphibians	None	None	Low- and no-effect*	≥ 21 (larva: ≥ 7)
** **Algae**	1 (or plant)	1 (or plant)	Low- and no-effect*	≥ 1
** **Plants**	1 (or algae)	1 (or algae)	Low- and no-effect*	Case-by-case

Notes:
Requirements are derived from CCME, 2007.
Toxicity data for amphibians is highly desirable but not required.
For semi-aquatic organisms, data must represent fully aquatic life stages.
**** Low- and no-effect data*** *preferred endpoints: EC/ICx representing a no-effect threshold > EC/IC_{10} > EC/IC_{11-25} > MATC > NOEC > LOEC > EC/IC_{26-49} > nonlethal EC/IC_{50}.*
***** Algae/Plants:*** *If a toxicity study indicates that a plant or algae species is among the most sensitive species in the dataset, then this substance is considered to be phytotoxic, and three studies on non-target freshwater plant or algal species are required.*

2.3 SPECIES SENSITIVITY DISTRIBUTION (SSD)

The long-term chloride toxicity SSD was calculated using data meeting the requirements outlined in Section 2.2. The long-term dataset for the three chloride salts (KCl, $CaCl_2$, and $MgCl_2$) did not meet the minimum requirements for Type A (SSD) guideline derivation (CCME, 2007). Due to data limitations, chloride toxicity data from NaCl endpoints was used in chronic SSD derivation, and the complete guideline derivation presented here is based on NaCl data. Similar data limitations occurred in the CCME (2011) derivation, where KCl and $MgCl_2$ toxicity values were excluded from the analysis. The rational for this approach was based on the 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) used to provide the rational for combining the datasets for these two salts. The work herein did not combine the two datasets. Instead, an analysis of protectiveness was conducted to determine whether the guideline derived based on NaCl toxicity data was protective for $CaCl_2$ toxicological endpoints available in the database (Section 8).

The methodology for the long-term SSD involved plotting chloride effect concentrations for each species against proportion of total species affected. A cumulative distribution function was applied, and the water quality guideline was defined as the chloride concentration corresponding to the 5th percentile of total species affected. Initially, the SSD curve was derived unadjusted for water hardness, as additional endpoints were available in the current dataset compared to the CCME (2011) to assess the extent to which the unadjusted SSD deviated from the CCME (2011) SSD. Due to this expanded dataset, it was expected that the long-term guidelines would potentially differ.

Protocols involve the plotting of one endpoint per species on an SSD, therefore multiple results for the same species were pre-processed following methods outlined by CCME (2011, 2007). The geometric mean of the effect concentrations was taken when multiple entries existed for the same species, endpoint, exposure duration, and life stage. 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. To reduce bias towards any particular study, the geometric 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).

When more than one effect concentration was available for a given species, with a different endpoint, exposure duration, and/or life stage, the geometric mean was not selected - instead, the most sensitive endpoint is selected, or professional judgment used to select a conservative representative species effect concentration.

Following this selection procedure, each data point was ranked according to sensitivity, and its position on the SSD was determined using the Hazen plotting position (HPP) 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

Calculated rank percentiles were plotted against the corresponding toxicological endpoint, and a typical sigmoid curve was produced. This approach is called Hazen plotting positions (HPP) and in this application it represents the rank percentile or HPP for the proportion of species affected in relation to toxicant exposure concentration.

Toxicological endpoint values were converted using log base 10 to improve assessment of the low end (more sensitive species) of the plot. Converted endpoints with corresponding HPP ranks were analyzed by EasyFit 5.6 Professional software (Mathwave Technologies, 2015) for the best distribution fit using cumulative distribution functions, where 65 various models were compared, evaluated, and ranked by Anderson-Darling, Kolmogorov-Smirnov, or Chi-Squared statistical procedures. The Anderson-Darling rank was preferred because of the greater sensitivity towards the distribution tails, and therefore, greater accuracy in predictions for more sensitive species.

The best-ranked model by Anderson-Darling analyses was the Logistic distribution. It was selected based on goodness-of-fit, model feasibility, and minimized scaled residuals. The Logistic cumulative density function was one of the six recommended models for deriving Canadian water quality guidelines (CCME, 2007). Using this model, a 5% Benchmark Dose (BMD), the concentration of chloride at which 5% of species are predicted to be affected was determined for the long-term dataset along with the lower and upper fiducial limits.

3 CHLORIDE SALTS CONSIDERED FOR GUIDELINE DEVELOPMENT

In alignment with CCME (2011), the current guideline development was focused on Cl - anion toxicity, associated primarily with the NaCl salt. CCME (2011) evaluated the approximate order of chloride salt toxicity to freshwater organisms as KCl > $MgCl_2$ > CaCl2 > NaCl, suggesting that the toxic effect of KCL and $MgCl_2$ was coming from cations, whereas NaCl and $CaCl_2$ toxicity was linked to anions. Based on these observations, CCME (2011) developed the chloride guidelines using $CaCl_2$ and NaCl salts, although the majority of the dataset involved NaCl and none of the most sensitive toxicological endpoints were due to $CaCl_2$. These salts were also mentioned as 'one of the most significant anthropogenic non-industrial sources of chloride to the aquatic environment, specifically in densely populated regions of Canada' (CCME, 2011).

Comparative toxicity from the major salt ions, present in all fresh waters, such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, and HCO_3^-, was evaluated additionally to the current guideline development. The PTAC-funded study by UGARF (2016b) compared the long-term toxicity of major salt ion pairs using the freshwater mussel *L. siliquoidea*. These experimental results, along with available published values, are discussed in detail in Section 9.1.

To ensure the resulting chloride guideline provided adequate protection for $MgCl_2$ and $CaCl_2$ salts, an analysis of protectiveness (Section 7) was applied to all toxicity endpoints available in the database for NaCl, $MgCl_2$, and $CaCl_2$. Acceptable toxicity values, non-adjusted for hardness, were plotted against the hardness-adjusted guideline – the harness-dependent guideline based on NaCl data was considered protective providing none of the $MgCl_2$ or $CaCl_2$ datapoints fell below the guideline derived from the NaCl dataset.

4 HARDNESS UNADJUSTED LONG-TERM TOXICOLOGICAL GUIDELINE

The following section outlines the results from toxicological analysis of the long-term dataset. The derivation of the long-term WQG for chloride is outlined using data for NaCl toxicity. A detailed summary of data utilized in the long-term SSD is provided with an overview of the derived guideline. Calculation methods were based on those published by CCME (2007) and Zajdlik and Associates Inc. (2005, 2006) on behalf of CCME. The methods used by CCME (2011) for developing chloride water quality guidelines were also incorporated.

4.1 LONG-TERM SPECIES SENSITIVITY DISTRIBUTION (SSD)

In total, 557 chronic data points were obtained for NaCl across a wide range of aquatic organisms. Eighty-one of these were deemed unacceptable for guideline derivation for several reasons (see Section 2). The remaining 476 points could be classified as long-term data based on the requirements of the CCME (2007). When multiple toxicological endpoints were available from a study involving the same species, experimental duration, life stage, *etc.*, the most relevant and often sensitive endpoint was chosen. This approach reduced the pool to 91 points. From these 91 points, for studies involving different research groups that generated data for an identical endpoint for the same species under similar durations of exposure, life stage, etc., a representative geometric mean was calculated (see Section 2.3). The majority of data was from published literature sources. In addition, unpublished data generated from 3rd party researchers under contract to EEI, and funded via PTAC (WSLH, 2016, UGARF, 2016a and 2016b, Nautilus, 2016 and 2019), were included. Finally, some critical grey literature (*e.g.*, presented by reputable researchers, but not published yet in a peer-reviewed journal) data were included for sensitive species, as anticipation of future publications that could influence the final SSD. The final pool included 35 data points.

Thirty-five species made up the long-term SSD, with values ranging from a 42 mg Cl/L for the freshwater mussel *Epioblasma torulosa rangiana* to a 144 h LOEC of 7,820 mg Cl/L for the cyanobacteria (*Synechocystis sp.*; Table 4.1). A number of species had multiple data points, and the data were pooled to not over-represent a particular species on the SSD curve (Table 4.2). In two instances, the variability in endpoint (*e.g.*, EC_{10}) concentration was significant.

The first instance is data for *Lampsilis fasciola* where historically a low 24-hour EC_{10} of 24 mg Cl/L was available from Bringolf *et al.* (2007). Further research conducted by the same researcher in the same lab (UGARF, 2016a) revealed the EC_{10} of *L. fasciola* (glochidia survival) ranging from 212 mg/L to 964 mg/L of chloride. Communication between EEI and Dr. Bringolf indicated that time of brooding and natural water system from which brooding mothers were collected may have a significant influence on the response of a particular batch of *L. fasciola* glochidia. The most recent values (UGARF, 2016a) among with Bringolf *et al.* (2007) findings, were incorporated in the SSD presented herein, as geometric mean representing *L. fasciola*.

The second instance is data for *Pseudokirchneriella subcapitata*. A historical datapoint produced by Simmons (2012) had a 96 h EC_{10} of 96 mg Cl/L. The more recent published results from Geis and

Hemming (2014) and PTAC-sponsored research conducted by the Wisconsin State Laboratory of Hygiene (WSLH, 2016) produced EC_{10} values more than an order of magnitude higher, ranging from 756 mg/L to 1,529 mg/L of chloride. For the SSD, the geometric mean of all three data points was applied to represent *P. subcapitata.*

Four out of 35 endpoints (one fish and three invertebrates, namely, *Salmo trutta*, *Brachionus patulus*, *Physa*, and *Gammarus pseudolimnaeus*) represent NOEC, an endpoint considered to be less preferrable than a LOEC such as an EC_{10} or EC_{25}. All these NOECs were obtained for the less sensitive species, with corresponding Hazen plotting position (HPP) located in the mid/upper-mid part of the SSD curve. In this case, the use of a NOEC instead of LOEC for these species is not expected to significantly alter the calculated WQG.

Table 4.1. No/Low Effect Concentrations Used for the Long-Term SSD

Species	Common Name	Toxicological Endpoint	Biological Endpoint	No/Low Effect Concentration (mg Cl/L)	HPP	Source
Epioblasma torulosa rangiana	Freshwater mussel	24 h EC_{10}	Glochidia functional survival	42	0.014	Gillis, 2011
Musculium securis	Fingernail clam	1,440-1,920 h EC_{25}	Reproduction	88	0.043	Mackie, 1978
Lampsilis fasciola	Wavy-rayed Lampmussel	24 h EC_{10}	Survival	187	0.071	**see Table 4.2***
Centroptilum (Neocloeon) triangulifer	Mayfly	336 h IC_{25}	Survival	236	0.100	**see Table 4.2***
Daphnia ambigua	Water flea	240 h EC_{10}	Mortality and reproduction	259	0.129	Harmon *et al.,* 2003
Daphnia magna	Water flea	504 h EC16-IC25	Reproduction	294	0.157	**see Table 4.2***
Ceriodaphnia dubia	Water flea	168 h IC_{25}	Reproduction	340	0.186	**see Table 4.2***
Daphnia pulex	Water flea	504 h IC_{10}	Reproduction	368	0.214	Birge *et al.,* 1985
Elliptio complanata	Fatmucket clam	24 h EC_{10}	Survival	406	0.243	Bringolf *et al.,* 2007
Hyalella azteca	Amphipod	672 h EC_{25}	Growth, dry weight/ amphipod	420	0.271	Bartlett *et al.,* 2012
Raphidocelis (Pseudokirchneriella) subcapitata	Microalgae	96 h EC_{10}	Fluorescence and growth	502	0.300	**see Table 4.2***
Lemna minor	Common duckweed	168 h EC_{10}	Growth	496	0.329	Simmons, 2012
Chlorococcum humicola	Green algae	360 h EC_{10}	Growth/ biomass productivity	506	0.357	Singh *et al.,* 2018
Tubifex tubifex	Tubificid worm	672 h EC_{10}	Reproduction	519	0.386	Elphick *et al.,* 2011a
Pimephales promelas	Fathead minnow	816 h LC_{10}	Mortality	591	0.414	Birge *et al.,* 1985
Salmo trutta	Brown trout	192 h NOEC	Mortality	607	0.443	Camargo and Tarazona, 1991
Villosa delumbis	Freshwater mussel	24 h EC_{10}	Survival	716	0.471	Bringolf *et al*., 2007
Villosa constricta	Freshwater mussel	24 h EC_{10}	Survival	789	0.500	Bringolf *et al*., 2007
Lampsilis siliquoidea	Freshwater mussel	96 h EC_{10}	Survival and biomass	819	0.529	**see Table 4.2***
Lumbriculus variegatus	California blackworm	672 h IC_{25}	Biomass	825	0.557	Elphick *et al.,* 2011a
Oncorhynchus mykiss	Rainbow trout	168 h EC_{25}	Embryo viability	989	0.586	Beak, 1999
Brachionus patulus	Rotifer	336 h NOEC	Population increase	1,213	0.614	Peredo-Alvarez *et al.*, 2003
Brachionus calyciflorus	Rotifer	48 h IC_{10}	Reproduction	1,241	0.643	Elphick *et al.,* 2011a
Xenopus laevis	African clawed frog	168 h LC_{10}	Mortality	1,307	0.671	Beak, 1999
Chlorella vulgaris	Green algae	360 h EC_{10}	Growth/ biomass productivity	1,518	0.700	Singh *et al.,* 2018
Anodonta subangulata	Freshwater mussel	24 h EC_{10}	Glochidia functional survival	1,572	0.729	**see Table 4.2***
Salvinia natans	Floating fern	336 h LOEC	Growth	1,773	0.757	Jampeetong and Brix, 2009

Table 4.1. No/Low Effect Concentrations Used for the Long-Term SSD

Species	Common Name	Toxicological Endpoint	Biological Endpoint	No/Low Effect Concentration (mg Cl/L)	HPP	Source
Lithobates pipiens	Northern leopard frog	2,040-2,136 h EC_{20}	Biomass	1,828	0.786	Nautilus, 2016
Physa	Snail	1,440 h NOEC	Mortality	2,000	0.814	Williams *et al*., 1999
Gammarus pseudolimnaeus	Amphipod	1,440 h NOEC	Mortality	2,000	0.843	Williams *et al.,* 1999
Stenonema modestum	Mayfly	336 h MATC	Development	2,047	0.871	Diamond *et al*., 1992
Chironomus dilutus / tentans	Midge	480 h EC_{10}	Biomass	2,316	0.900	Elphick *et al.,* 2011a
Chlorella minutissimo	Green algae	672 h MATC	Growth	6,066	0.929	Kessler, 1974
Chlorella emersonii	Green alga	192-336 h MATC	Growth	6,824	0.957	Setter *et al*., 1982
Synechocystis sp.	Cyanobacteria	144 h LOEC	Physiological (Exopolysaccharide production)	7,820	0.986	Ozturk and Aslim, 2010

***HPP** -Hazen plotting position (Section 2.3)*
** Data shown is the geomean value taken from multiple studies. Intra-study comparisons use the most sensitive endpoint, whereas inter-study comparisons use geometric mean. Multiple study data are summarized in Table 4.2.*

Table 4.2. Multiple Study Data Used for the Long-Term SSD

Species	Common name	Toxicological Endpoint	Biological Endpoint	No/Low Effect Concentration (mg Cl/L)			Source
				Original Study Value	Intra-Lab Geometric Mean*	Geometric Mean	
Daphnia magna	Water Flea	504 h EC_{16}	Reproduction	205.2		294	Biesinger and Christensen, 1972
		504 h IC_{25}		421			Elphick *et al.,* 2011a
Ceriodaphnia dubia	Water Flea	168 h IC_{25}	Reproduction	454	292	340	Elphick *et al.,* 2011a
				117			
				264			
				146			
				580			
				521			
		168 h IC_{25}		147	284		Lasier and Hardin, 2010
				456			
				340			
		504 h EC_{10}		80	269		WSLH, 2016 *PTAC sponsored*
				236			
				279			
				492			
				540			
		168 h IC_{25}		600			Struewing *et al.,* 2015
Raphidocelis (P. subcapitata)	Microalgae	96 h EC_{10}	Growth	741	1,194	502	WSLH, 2016 *PTAC sponsored*
				1,139			
				1,152			
				1,460			
				1,338			
				1,529			
		96 h EC_{10}	Fluorescence	96			Simmons, 2012
		96 h EC_{10}	Fluorescence	1,270	1,101		Geis and Hemming, 2014
		96 h EC_{10}	Fluorescence	955			
Anodonta subangulata	Freshwater Mussel	EC_{10}	Survival	1,262		1,572	UGARF, 2016a *PTAC sponsored*
				877			
				1,429			
				2,245			
				2,701			
Lampsilis fasciola	Freshwater Mussel	24 h EC_{10}	Survival	24		187	Bringolf ,2007*
		24 h EC_{10}		247			UGARF, 2016a* *PTAC sponsored*
				212			
				964			
Lampsilis siliquoidea	Fatmucket	24 h EC_{10}	Survival	1,474	1,285	819	Bringolf, 2007*
		EC_{10}		730			UGARF, 2016a* *PTAC sponsored*
				681			
				2,877			
				1,662			
		672 h EC_{20} 2,016 h EC_{20}	Biomass	264	290		Wang *et al.,* 2018
				482			
		672 h EC_{20}		403			
				251			
		2,016 h EC_{20}		158			
Lithobates pipiens	Northern leopard frog	2,040 h IC_{20} 2,136 h IC_{20}		801		1,828	Nautilus, 2016 *PTAC sponsored*
				2,723			
				2,800			

Table 4.2. Multiple Study Data Used for the Long-Term SSD

Species	Common name	Toxicological Endpoint	Biological Endpoint	No/Low Effect Concentration (mg Cl/L)			Source
				Original Study Value	Intra-Lab Geometric Mean*	Geometric Mean	
Neocloeon (Centroptilum) triangulifer	Mayfly	864 h MATC	Survival	265		236	Soucek and Dickinson, 2015
	Mayfly	672 h LC_{20}	Survival	361	358		Nautilus, 2019 *PTAC sponsored*
				243			
				373			
				502			
		336 h EC_{25}		139			

Intra-lab geometric mean was taken to collate multiple data points for the same species from the same author/laboratory prior to taking geometric mean for the multiple studies from the different authors/ laboratories. This ensured that no one study had a greater influence on the derived SSD value.
**Bringolf et al., 2007 and UGARF, 2016a studies were conducted by the same author in the same laboratory.*

4.2 EVALUATION OF LONG-TERM TOXICOLOGICAL DATA

In some instances, it was necessary to apply professional judgement for the selection of appropriate toxicological data considered representative for a particular species. This was due to several factors including:

- differences in study strength and quality for a similar toxicological endpoint and species;
- differences in source of water/ ion composition used for a similar species (*e.g.,* field water versus reconstituted water, or sea salt used);
- differences between the species origin (*e.g.,* field-collected mussels from different places);
- age difference between similar tested organisms;
- differences in sensitivity as well as data quality for various endpoints for the same species (*e.g.*, growth versus reproductive success); and,
- quantitative representation of an endpoint for the same species such as the availability of an EC_{10} from one study and a NOEC from a separate study.

Key professional judgements that were made for various endpoints were organized by group. Evaluated groups included fish, invertebrates, amphibians, and photosynthetic organisms.

Long-Term Toxicity to Fish

Chronic endpoints were available for eight fish species, and three of them 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.,* 1985)), 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 Canadian WQG derivation, the CCME (2011) took the same approach, using the same LC_{10} mortality endpoint from Birge *et al.* (1985) for *P. promelas* (Rescan (2007) data were 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 life stage. Although NOEC values are less preferred, and CCME (2007) requires the exposure duration of ≥ 504 h for juvenile fish, the CCME (2011) included the data point in the long-term SSD. The current work followed the CCME (2011) approach and included the brown trout NOEC concentration of 607 mg Cl/L in the 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 2,040-2,136 h EC_{20} (biomass) for the northern leopard frog (*L. pipiens*) from Nautilus (2016), PTAC-sponsored research. 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 were utilized in CCME (2011). The current work follows the CCME (2011) approach including the African clawed frog LC_{10} value, and replaces the severe endpoint for the northern leopard frog by IC_{20} of 1,828 mg/L (biomass) derived from the three studies conducted by Nautilus (2016).

As part of the PTAC funded research, Nautilus (2016) evaluated toxicity data for northern leopard frog (tadpoles) and found the species to be moderately sensitive to insensitive in terms of response to chloride toxicity, which varied as a function of water hardness. The IC_{20} values for biomass ranged from 476 mg Cl/L to 2,800 mg Cl/L depending on water hardness and test day (28, 56, or 85-89 days).

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 made up of bivalve, mayfly, and water flea taxa. Across the SSD, bivalves are represented by eight species of freshwater mussels and clams. The sensitivity of these animals to chloride makes them especially vulnerable, particularly the glochidia life stage, a larval form taking the two most sensitive positions on the long-term SSD in CCME (2011). 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 rangiana*) were 24 (Bringolf *et al*., 2007) and 42 mg Cl/L (Gillis, 2011), respectively.

Four bivalve data (*E. torulosa rangiana, E. complanata, V. delumbis, V. constricta*) in the current long-term dataset are 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 life stage, 24 h EC_{10} values (glochidia survival) are considered long-term endpoints.

For *L. fasciola*, the historical value of 24 mg/L (Bringolf *et al.,* 2007) was replaced by the geometric mean of 187 mg/L, derived from the historical 2007 data and three more recent values from the study conducted by the same author in the same laboratory (UGARF, 2016). As part of the PTAC-funded research, UGARF (2016) evaluated chloride toxicity data for *L. fasciola*, and observed the EC_{10} (survival) values ranging from 247 to 964 mg/L, varied with water hardness.

In the CCME (2011) long-term SSD dataset, the *M. securis* (fingernail clam) had LOEC (natality) value of 121 mg/L. This value was replaced by EC_{25} (natality) value of 88 mg/L, calculated from the raw data presented in Mackie (1978), using the log probit model (BMDS calculation is shown in Appendix C).

For *L. siliquoidea* (fatmucket), CCME (2011) used the endpoint of 1,474 mg/L, based on the single study by Bringolf (2007). This value was replaced by the geometric mean of 819 mg/L, derived from the multiple PTAC-funded study by UGARF (2016a), and from Wang *et al.* (2018). The UGARF (2016a) study tested glochidia functional survival with the static test and 24 h exposure duration, using four various hardness levels, from 42 to 298 mg/L. The EC_{10} ranged from 730 to 2,877 mg/L. Wang *et al.* (2018) evaluated sublethal chloride toxicity using the juvenile fatmucket (10 to 74 days old) with the flow-through test and exposure duration of four and twelve weeks. The EC_{20} (biomass, dry weight) observed by Wang *et al.* (2018) ranged from 158 to 482 mg/L, with water hardness of approximately 100 mg/L.

PTAC-funded research for *A. subangulata* (freshwater mussel) conducted by UGARF (2016a) added the EC_{10} (survival) endpoint of 1,572 mg/L for this species. This value represents a geometric mean from five values, ranging from 877 mg/L to 2,701 mg/L, depending on water hardness.

Water fleas represent another sensitive species of invertebrate. The water flea long-term endpoints fell in close proximity to one another on the SSD, with 168-504 h $EC/IC_{10\text{-}25}$ ranging from 259 mg/L (*D. ambigua;* Harmon *et al.,* 2003) to 368 mg/L (*D. pulex*; Birge *et al.,* 1985). These values correspond to the values utilized in the CCME (2011) long-term SSD derivation with the exception of *C. dubia*. Whereas 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 uses the geometric mean of 340 mg/L from the IC_{25} and IC_{10} (reproduction) endpoints based on the multiple studies. These studies include Elphick *et al.,* 2011, Lasier and Hardin, 2010, Struewing *et al.,* 2015, and PTAC-funded research by WSLH (2016).

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 IC_{10} of 2,316 mg Cl/L (Elphick *et al.,* 2011a).

The geometric mean of 236 mg/L was added among the most sensitive species to a long-term SSD curve for the mayfly species *Centriptilum (Neocloeon) triangulifer*. The values used for the geomean are coming from Soucek and Dickinson (2015), Struewing *et al.* (2015), and Nautilus (2019). Soucek and Dickinson (2015) observed the MATC of 265 mg/L with a full-life test (approximately 35 days), whereas Struewing *et al.* (2015) found the EC_{25} of 139 mg/L for the 14-d survival test. PTAC-funded research conducted by Nautilus (2016) involved several 28-d experiments with four water hardness levels, with LC_{20} ranging from 243 to 502 mg/L depending on water hardness.

Long-term toxicity to photosynthetic organisms

Two aquatic macrophytes, five algae species and cyanobacteria 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), and a 336 h LOEC (growth) of 1,773 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 NaCl and other chloride salts. Where macrophytes (vascular plants) are used, duckweed (*Lemna* spp.) is the most commonly selected test organism due to the 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 168 h EC_{10} (growth – numbers of 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 1,500 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, algae species *C. minutissimo, C. zolingiensis, and C. emersonii* fell at the upper end of the in the long-term SSD. Since these species are from the same genus, and toxicological endpoints for *C. minutissimo* and *C. zolingiensis* was equal to 6,066 mg/L for both species, the current SSD does not include *C. zolingiensis.*

Two green algae species, *Chlorococcum humicola* and *Chlorella vulgaris*, studied by Singh *et al.* (2018) for 360 h exposure, were added to the current dataset. The EC_{10} (growth) values of 506 mg/L and 1,518 mg/L, respectively, were calculated by BMDS software from the raw data provided by Singh *et al*. (2018).

The geometric mean of 502 mg/L (EC_{10}, growth and fluorescence) was added for the microalgae *Raphidocelis (Pseudokirchneriella) subcapitata*, based on Simmons *et al.* (2012), Geis and Hemming (2014), and PTAC-funded research by WSLH (2016). When Simmons *et al.* (2012) found the species to be particularly sensitive to chloride, with EC_{10} of 92 mg/L, other studies observed the EC_{10} values ranging from 741 mg/L to 1,529 mg/L, depending on water hardness.

4.3 DERIVATION OF THE LONG-TERM SSD AND WATER QUALITY GUIDELINE

The SSD chloride concentrations (Table 4.1) were plotted against their corresponding HPP values. Next, the toxicological endpoint values were converted to the base 10 logs, for an improved assessment of more sensitive toxicological values on the the lower end of the plotting positions. Converted endpoints with corresponding HPP ranks were analyzed by EasyFit 5.6 Professional software (Mathwave Technologies, 2015) for the best distribution fit using cumulative distribution function, where 61 various models were compared, evaluated, and ranked by Anderson-Darling, Kolmogorov-Smirnov, or Chi-Squared statistical procedures. The Anderson-Darling rank was preferred because of the highest sensitivity towards the tails, and therefore, higher accuracy in predictions for more sensitive species.

The best-ranked model by Anderson-Darling analyses was the Logistic distribution. It was selected based on goodness-of-fit, model feasibility, and minimized scaled residuals. The equation of the model is:

$$y = 1/(1 + \exp(-z)) \quad \textbf{(Eq. 4.1)}$$

where $z = (x - \mu)/\delta$

Where y is the proportion of species affected, and x is the base 10 log of the chloride concentration. The continuous scale parameter δ and continuous location parameter μ were calculated by EasyFit as 0.27 and 2.90, respectively. Note that the Logistic model provided the best fit and was applied in CCME (2011).

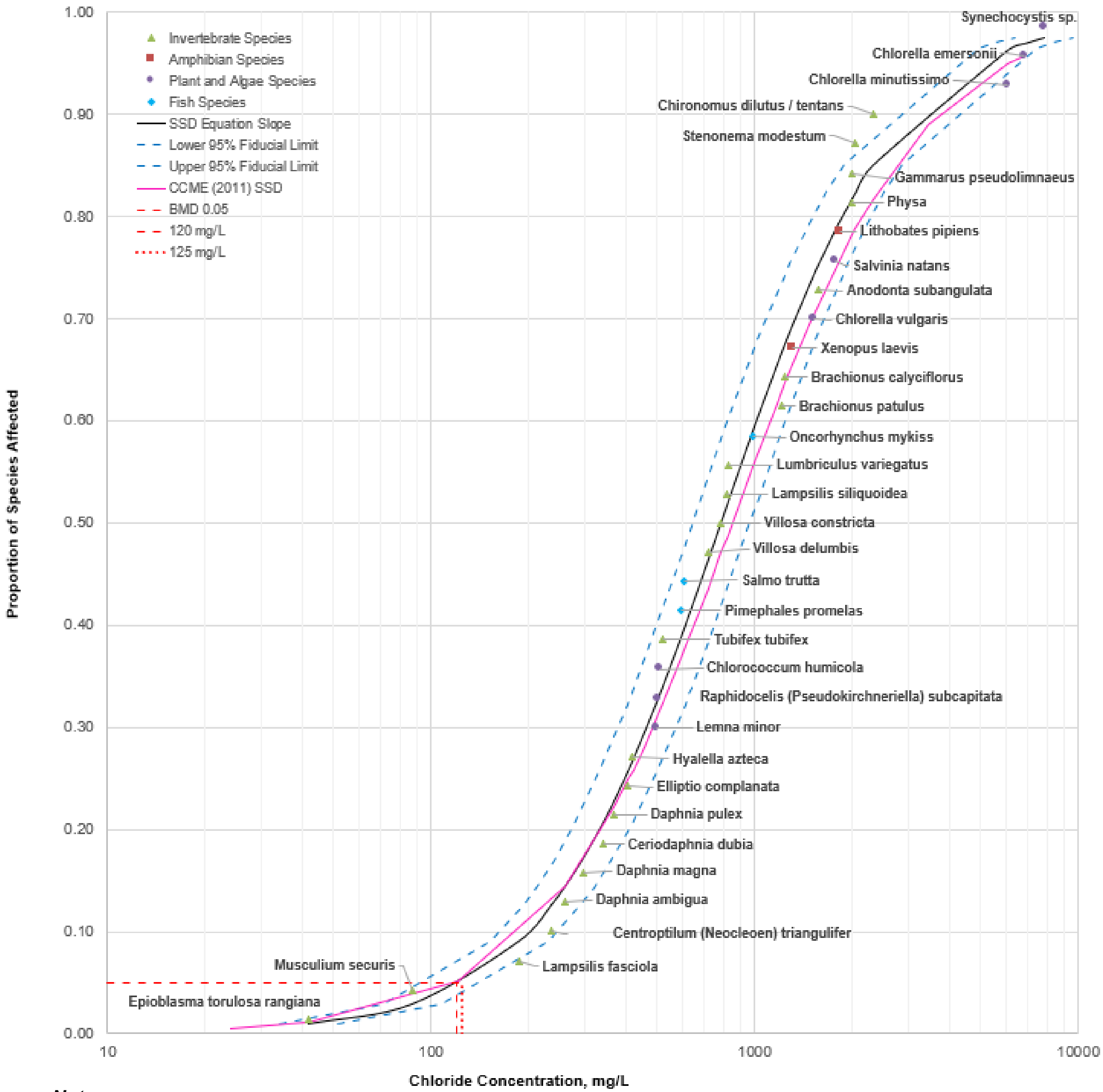
Figure 4.1 shows the long-term SSD made up of the no- and low-effect endpoints and corresponding HPP values (Table 4.1), along with the fitted Logistic function (Eq. 4.1). The long-term WQG, defined as the 5^{th} percentile along the derived Logistic function was 125 mg/L chloride with a lower fiducial limit of 101 mg/L chloride.

Compared to CCME (2011), the long-term WQG concentration derived here is similar. The lower 10^{th} percentile data points are the primary factors driving the greater sensitivity of the current model. The CCME (2011) model had three data points within the lowest 10^{th} percentile in the following ascending order: *L. fasciola, E. torulosa rangiana*, and *M. securis*, with all of them representing freshwater mussels.

The current model included additional sensitive endpoints such as *C.triangulifer* (mayfly), and refined the *L. fasciola* endpoint with a larger dataset. The *M. securis* raw data was evaluated, and the final endpoint was re-calculated to switch from LOEC to the more representative EC_{25} (the BMDS calculation result is shown in Appendix C). This contribution resulted in more elaborated SSD lower tail, and placed the 10^{th} percentile species in the following ascending order: *E. torulosa rangiana, M. securis, L. fasciola, and C. triangulifer.*

Other additions included important microalgae species, sensitive mayfly *S. modestum* (Soucek and Dickinson, 2015), and two freshwater mussel species (*L. siliquoidea and A. subangulata).* Data points representing *C. dubia* and *L. minor* were reduced from 454 to 340 mg Cl/L and from 1,171 to 496 mg Cl/L, respectively. These changes increased the SSD sensitivity.

Figure 4.1. Long-Term SSD: No- and Low-Effect Toxicity Data for NaCl



***Notes**:*
SSD derived by plotting no- and low- effect concentrations (preferred endpoints: EC/IC representing a no-effect threshold > EC/IC$_{10}$ > EC/IC$_{11-25}$ > MATC > NOEC > LOEC > EC/IC$_{26-49}$ > nonlethal EC/IC$_{50}$) for 35 species of aquatic organism against their corresponding Hazen plotting position (HPP);
Red dashed line indicates the 5th percentile (y-axis) and the resulting long-tern water quality guidelines derived by the CCME (x-axis at hashed line) of 120 mg/L, and red dotted line derived in this report (x-axis at red arrow) of 125 mg Cl/L.

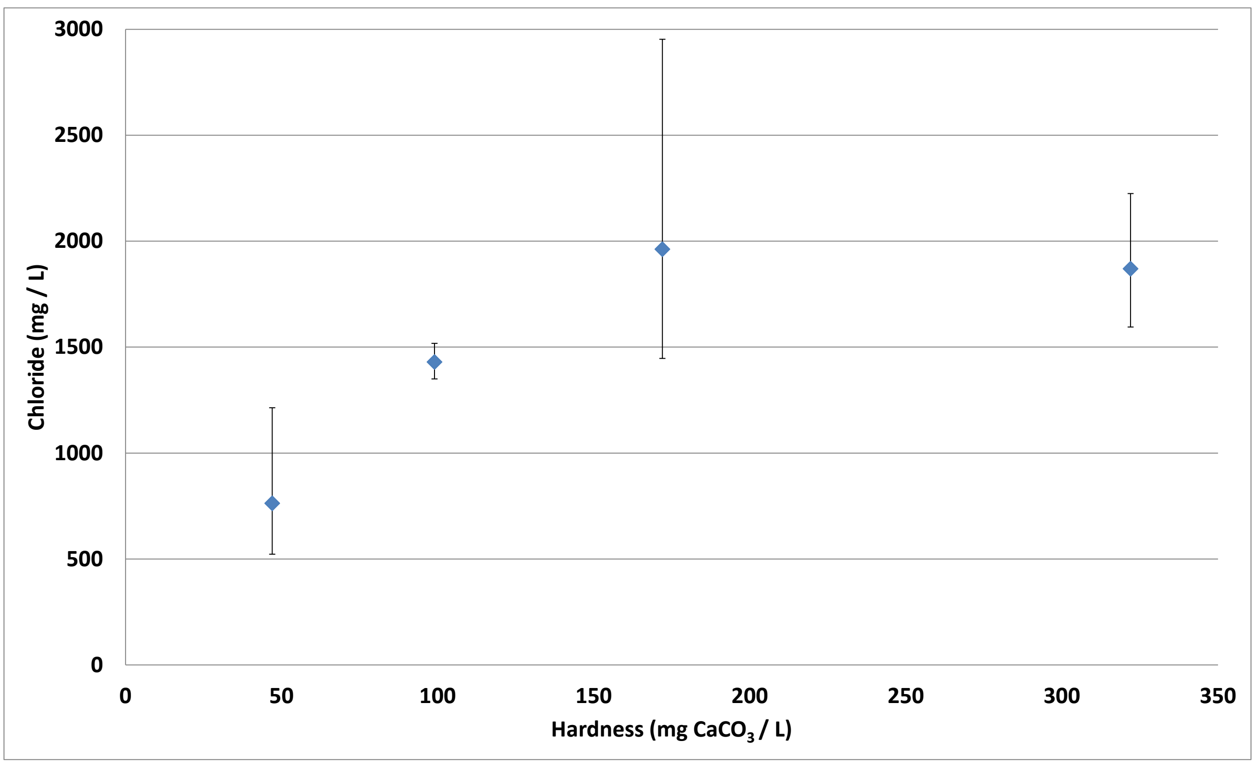
5 HARDNESS MODIFICATION METHODS AND DATABASE

This section begins with an overview of the concept of water hardness along with research that has been conducted to identify the relationship between water hardness and chloride toxicity. It examines hardness relationships for the long-term (Section 5.3) dataset. Subsections outline the derivation of long-term pooled hardness-toxicity slopes along with the associated SSD, adjusted to 50 mg $CaCO_3$/L hardness. Finally, the equation to derive long-term WQGs based on water hardness concentrations is presented.

5.1 BACKGROUND AND THEORETICAL CONSIDERATIONS

Hardness has been identified as an important variable regarding the toxicity of chloride salts towards aquatic organisms. Gillis (2011) studied the effects of water hardness on *L. siliquoidea* (fatmucket mussel), a species found in Alberta. The 24-hour EC_{50} values in soft (47 mg $CaCO_3$/L), moderately hard (99 mg/L $CaCO_3$), hard (172 mg $CaCO_3$/L), and very hard (322 mg $CaCO_3$/L) 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 *L. siliquoidea* (Gillis 2011)



Note: *chart shows 24 h EC_{50} (glochidia survival) values and 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. Similar relationships have been observed for metals such as zinc and cadmium, although the mechanism may be distinct for chloride salts (CCME 2011).

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 refer to chronic toxicity endpoints and the hardness relationship that meet 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). Table 5.1 outlines the functions utilized the derived chronic guidelines for metals and ions, including cadmium (Cd), cobalt (Co), chromium (Cr) III, copper (Cu), lead (Pb), manganese (Mn), nickel (Ni), zinc (Zn), and sulphate (SO_4^{2-}).

Table 5.1. Summary of Hardness-Dependent WQGs for Metals and Ions

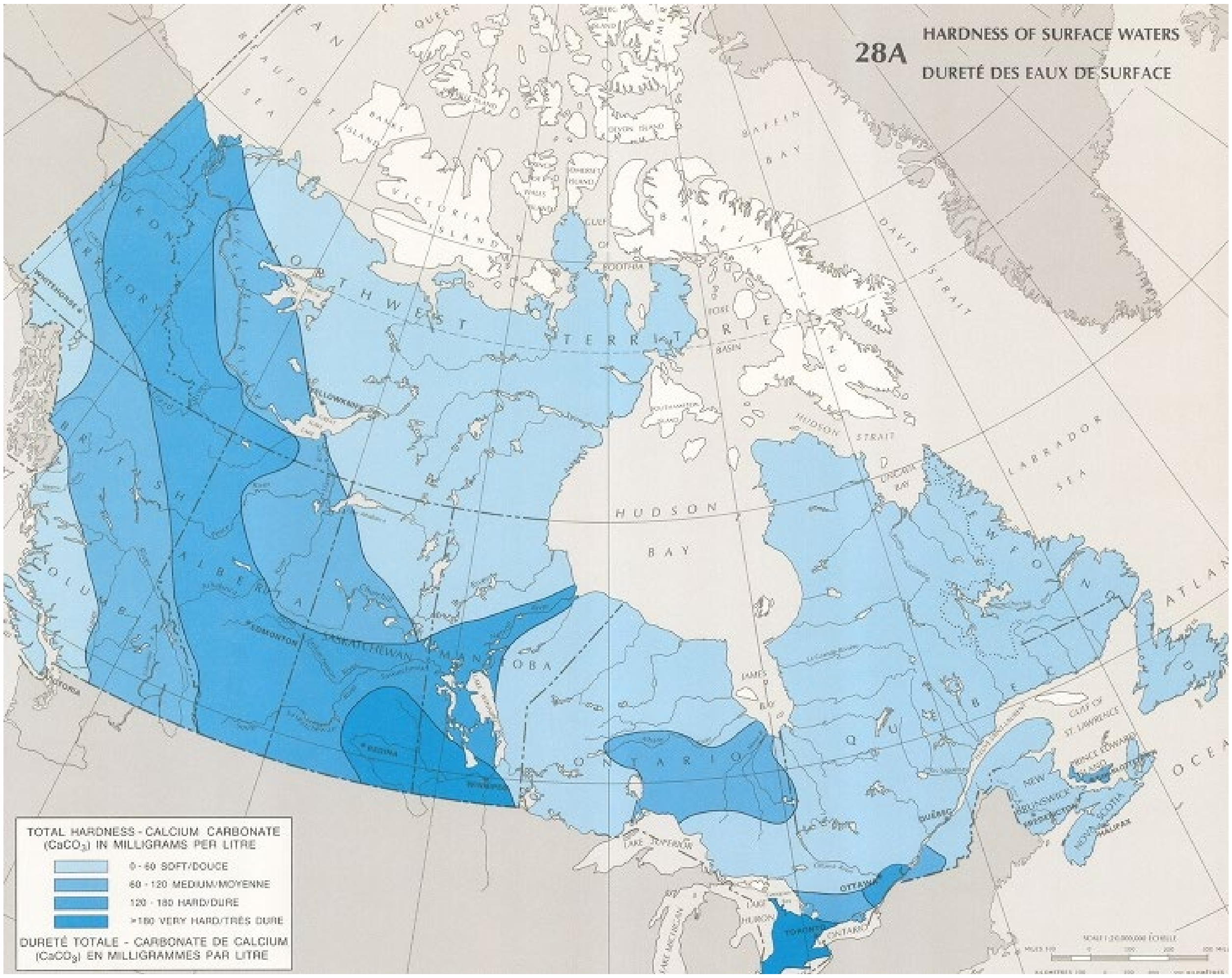
Metal	slope	intercept	Freshwater conversion factor (FC)	Guidelines (µg /L) at hardness of 100 mg/L	Source
Cadmium	0.83	-2.46	*na*	0.16	CCME, 2014
	0.9789	-3.866	1.136672 - [(ln hardness) (0.041838)]	0.72	US EPA, 2016
Cobalt	0.414	-1.887	*na*	1.0	CEPA, 2017
Chromium III	0.819	0.685	0.86	74	US EPA, 1995
Copper	0.979123	-8.64497	*na*	16 (acute)	AEP, 1996
Lead*	0.214	0.4152	*na*	2.84	CEPA, 2020
Manganese	0.878	4.76	*na*	3,600 (acute)	CCME, 2019
Nickel	0.846	0.058	0.997	52	US EPA 1995
Silver	1.72	-6.59	0.85 (acute)	3.2 (acute)	US EPA, 2020
Sulphate**	*na*	*na*	*na*	309,000	BC MOE, 2013
Zinc	0.75	7.5	*na*	15	BC MOE, 1999

All guidelines are long-term (chronic), if not specified
** Lead WQGs are based on hardness-and dissolved organic carbon (DOC) values; DOC concentration of 0.5 mg/L is assumed*
***Sulphate WQGs are derived for the water hardness categories; the SO_4^{2-} value 309 mg/L is given for the moderately soft/ hard to hard water (76-180 mg $CaCO_3$/ L)*

Several regulatory agencies have previously developed major salt 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 $287.8 \times (\text{hardness})^{0.205797}(SO_4^{2-})^{-0.07452}$; and $177.87 \times (\text{hardness})^{0.205797}(SO_4^{2-})^{-0.07452}$, respectively (IDNR 2009b).

Water hardness has also been identified as an ameliorating factor in aquatic guideline policy under the National Water Quality Management Strategy involving Australian and New Zealand governments that recognized greater water "softness" in freshwater ecosystems dominated by chloride and sodium can pose a greater risk to biota – this relates to 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: $124 \times \ln(\text{hardness}) - 128$) 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, the hardness incorporation is an important stage of the research on the toxicity of chloride towards sensitive aquatic organisms. Better understanding of the hardness-dependent toxicity can be used to improve the accuracy of guidelines developed on a provincial or national scale.

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, Ca^{2+} and Mg^{2+} occur at much higher concentrations compared to other polyvalent cations; hence, water hardness can be measured based on these two divalent cations. Hardness is generally expressed as total hardness in $CaCO_3$ equivalents using the following formula (Hiscock, 2005):

$$\textbf{Total hardness (mg } CaCO_3\textbf{/L)} = 2.5[Ca^{2+}] + 4.1[Mg^{2+}] \qquad \textit{(Eq. 5.1)}$$

Numerous studies have shown an inverse relationship between water hardness and chloride toxicity. Naumann (1934), working with *D. 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 Ca^{2+} concentrations from 3 to 100 mg/L. Examining various reconstituted water formulations and NaCl toxicity, Lasier *et al.* (2006) demonstrated reduced *C. dubia* reproductive success in lower hardness concentrations. For the chloride concentration of 565 mg/L, reproduction was reduced by 21 % after water hardness was lowered from 100 to 45 mg $CaCO_3$/L (Lasier *et al*., 2006).

The exact mechanism by which Ca^{2+} and Mg^{2+} ameliorate chloride toxicity, may vary between species, and differ in the magnitude and direction of effect. Several researchers have shown that Ca^{2+} is more important than Mg^{2+} in ameliorating toxicity (Leblanc and Surpenant,1984; Jackson *et al.*, 2000; Welsh *et al.*, 2000). One plausible rationale is that Ca^{2+} 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+} cations are known to reduce the toxicity of metals by competing for binding sites on the surface of cell membranes (Paquin *et al.,* 2002). The same mechanism has been suggested for the hardness amelioration of NaCl (and $NaSO_4$) toxicity (Soucek *et al.,* 2011; Davies and Hall, 2007; Elphick *et al.,* 2011a).

In 1997, Mount *et al.* tested the toxicity of twelve 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 results, the amelioration of chloride (NaCl, KCl, $CaCl_2$, and $MgCl_2$) and other major ion toxicity was linked to a multi-ion effect rather than the hardness itself (*i.e.,* specifically Ca^{2+} and Mg^{2+} ions). 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 the study were not designed to evaluate the influence of water hardness on chloride toxicity, and the study did not involve a range of hardness values, using the moderately hard reconstituted water as the test medium. Thus, no conclusions about the ameliorating effect (or not) of water hardness on chloride toxicity was drawn from Mount *et al.* (1997). Elphick *et al.* (2011a) suggested that in addition to the observed ameliorating influence of water hardness, it may represent a proxy for higher overall ionic strength or more balanced ionic ratios for major ions.

What the Mount *et al.* (1997) results demonstrate is that major ion toxicity is ameliorated by a multiple ion effect in complex solutions containing multiple salts. The study is unique, since 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: Mount *et al.* (1997) used over 2,900 ion solutions.

5.2 HARDNESS ADJUSTMENT – GUIDELINE DERIVATION

To explore potential relationships between chloride toxicity and water hardness, long-term data was compiled for species, studied in the same laboratory with effect concentrations across a wide range of water hardness. Natural logarithms were used to characterize hardness-toxicity relationship following the methods outlined by Stephen *et al.* (1985), and in approach utilized by the CCME and the U.S. Environmental Protection Agency (US EPA) in the hardness-adjusted guidelines for cadmium (CCME, 2014; US EPA, 2001). It is the preferred method to derive hardness-adjusted guidelines for chloride (CCME, 2011). To include the species data, effect concentrations are required to be within a range, where the highest hardness is at least three times greater than the lowest one, and at least 100 mg/L higher than the lowest one (CCME, 2014; US EPA, 2001; Stephen *et al.*,1985). To avoid the potential interference from inter-laboratory experimental conditions, data points for each species were grouped by laboratory. Data points that met these criteria across comparable endpoints, exposure durations, life stages, and laboratory environment, were plotted together with natural log (hardness) and natural log (chloride effect concentration) as the independent (x-axis) and responding (y-axis) variables, respectively.

The slope evaluation and statistical analysis was performed using the degradation model. The stability test with lower confidential limit of 95% was applied.

The relatively low hardness concentration of 50 mg/L (measured as $CaCO_3$ equivalents) was chosen as a starting point, from which subsequent guidelines in harder water could be calculated. The equation to derive the long-term hardness-adjusted effect concentrations was as follows:

$$\mathbf{LT}\boldsymbol{EC}_{x(50\ mg\ CaCO_3/L)} = \mathbf{exp}^{\{ ([\ln(50) - \ln(hardness)] \times LTPS) + \ln(LTECx) \}} \qquad \textit{(Eq. 5.2)}$$

where:

LT*EC*$_{x(50\ mg\ CaCO3\ /L)}$: a given long-term effect concentration normalized to 50 mg $CaCO_3$/L
LT*ECx:* a given long-term effect concentration
LTPS: the pooled slope derived from long-term data
hardness (permanent) measured as $CaCO_3$ equivalents

The long-term hardness-adjusted SSD was derived for the 50 mg/L hardness-adjusted data, and the 5th percentile long-term guideline was calculated as outlined in Section 2.3.

Using the 5th percentile chloride concentration (representing the y-coordinate on the fitted line), a hardness of 50 mg/L (representing the x-coordinate on the fitted line), and the pooled slope value (m), the equation could be solved for the y-intercept. If the equation ln(y) = m × ln(x) + b is rearranged to solve for b (the y-intercept), the *b = ln([Chloride* $^{5th\%}$*]) – ((pooled slope) × ln(hardness))*. Therefore, the resulting equation to derive the long-term WQG concentrations is:

$$\textbf{Long-term WQG} = \mathbf{exp}^{\{ LTPS\ (\log[hardness]) - LTb \}} \qquad \textit{(Eq.5.3)}$$

where:

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 long-term hardness-dependant guidelines, the acceptable chloride effect concentrations (log transformed and non-adjusted for hardness) were plotted against log hardness. The respective WQGs were plotted as the straight line. 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 provided a measure of the effectiveness of the guidelines towards Ca^{2+}

chloride toxicity. The assessment of protectiveness is shown in Section 8 and is based on methods utilized by the CCME (2014).

5.3 MULTIPLE HARDNESS LEVELS AND CHLORIDE TOXICITY

Table 5.2 summarizes studies that directly tested the toxicity-modifying effects of water hardness on chloride salt toxicity over chronic timeframes. In these studies, eight species of aquatic organisms were exposed to NaCl salts in eleven individual investigations of hardness-toxicity relationships. Of these eleven investigations eight were used to derive the quantitative ameliorating effect of hardness. The studies outlined in Table 5.2 are examined in more detail in the following discussion.

Table 5.3 combines the datapoints from different studies for the four species (*D. magna*, *L. minor*, *O. mykiss*, and *P. subcapitata*), where distinct water hardness ameliorating trend was observed. These studies were not included in the final dataset due to potential inter-laboratory variability interference in hardness amelioration interpretation, but they illustrate the importance of multiple hardness level investigations for these particular species.

Table 5.2. Long-term Studies with Multiple Hardness Level

Species	Tox. Endpoint	Biol. Endpoint	Exposure duration (h)	LT (SSD)	Data Quality	Effect Concentration (mg Cl/L)	Hardness (mg $CaCO_3$/L)	Alkalinity (mg $CaCO_3$/L)	Life Stage	Source
Anodonta subangulata (Freshwater mussel)	EC_{10}	Glochidia functional survival	24	LT	P/S	1,262	42	42	Glochidia	UGARF, 2016a
	EC_{10}	Glochidia functional survival	24	LT	P/S	877	78	76	Glochidia	
	EC_{10}	Glochidia functional survival	24	LT	P/S	1,429	150	144	Glochidia	
	EC_{10}	Glochidia functional survival	24	LT	P/S	2,246	310	278	Glochidia	
	EC_{10}	Glochidia functional survival	24	LT	P/S	2,701	548	376	Glochidia	
Centroptilum (Neocloeon) triangulifer (Mayfly)	LC_{20}	Mortality	672	LT	P/S	361	42	54	<48 hr old	Nautilus, 2019
	EC_{25}	*Emergence*	*672*	*LT*	*P/S*	*330*	*42*	*54*	*<48 hr old*	
	IC_{25}	Dry weight	672	LT	P/S	>326	42	54	<48 hr old	
	LC_{20}	Mortality	672	LT	P/S	243	84	85	<48 hr old	
	EC_{25}	*Emergence*	*672*	*LT*	*P/S*	*192*	*84*	*85*	*<48 hr old*	
	IC_{25}	Dry weight	672	LT	P/S	>640	84	85	<48 hr old	
	LC_{20}	Mortality	672	LT	P/S	373	168	135	<48 hr old	
	EC_{25}	*Emergence*	*672*	*LT*	*P/S*	*159*	*168*	*135*	*<48 hr old*	
	IC_{25}	Dry weight	672	LT	P/S	>642	168	135	<48 hr old	
	LC_{20}	Mortality	672	LT	P/S	502	292	164	<48 hr old	
	EC_{25}	*Emergence*	*672*	*LT*	*P/S*	*146*	*292*	*164*	*<48 hr old*	
	IC_{25}	Dry weight	672	LT	P/S	>344	292	164	<48 hr old	
Ceriodaphnia dubia (Water flea)	IC_{25}	Reproduction	168	LT	P/S	117	10	N/S	<24 h old	Elphick *et al.*, 2011a
	IC_{25}	Reproduction	168	LT	P/S	264	20	N/S	<24 h old	
	IC_{25}	Reproduction	168	LT	P/S	146	40	N/S	<24 h old	
	IC_{25}	Reproduction	168	LT	P/S	454	80	57-64	<24 h old	
	IC_{25}	Reproduction	168	LT	P/S	580	160	N/S	<24 h old	
	IC_{25}	Reproduction	168	LT	P/S	521	320	N/S	<24 h old	
	LC_{50}	Reproduction	168	LT	P/S	161	10	N/S	<24 h old	
	LC_{50}	Reproduction	168	LT	P/S	301	20	N/S	<24 h old	

Table 5.2. Long-term Studies with Multiple Hardness Level

Species	Tox. Endpoint	Biol. Endpoint	Exposure duration (h)	LT (SSD)	Data Quality	Effect Concentration (mg Cl/L)	Hardness (mg $CaCO_3$/L)	Alkalinity (mg $CaCO_3$/L)	Life Stage	Source
Ceriodaphnia dubia (Water flea)	LC_{50}	Reproduction	168	LT	P/S	481	40	57-64	<24 h old	Elphick *et al.,* 2011a
	LC_{50}	Reproduction	168	LT	P/S	697	80	N/S	<24 h old	
	LC_{50}	Reproduction	168	LT	P/S	895	160	N/S	<24 h old	
	LC_{50}	Reproduction	168	LT	P/S	700	320	N/S	<24 h old	
	LC_{50}	Mortality	168	LT	Chronic	132	10	N/S	<24 h old	
	LC_{50}	Mortality	168	LT	Chronic	316	20	N/S	<24 h old	
	LC_{50}	Mortality	168	LT	Chronic	540	40	N/S	<24 h old	
	LC_{50}	Mortality	168	LT	Chronic	1,134	80	N/S	<24 h old	
	LC_{50}	Mortality	168	LT	Chronic	1,240	160	N/S	<24 h old	
	LC_{50}	Mortality	168	LT	Chronic	1,303	320	N/S	<24 h old	
	IC_{50}	Reproduction	144-168	LT	P/S	342	39	45	<24 h old	Lasier and Hardin, 2010
	IC_{25}	Reproduction	144-168	LT	P/S	147	39	45	<24 h old	
	IC_{50}	Reproduction	144-168	LT	P/S	653	85	66	<24 h old	
	IC_{25}	Reproduction	144-168	LT	P/S	456	85	66	<24 h old	
	IC_{50}	Reproduction	144-168	LT	P/S	563	39	101	<24 h old	
	IC_{25}	Reproduction	144-168	LT	P/S	340	39	101	<24 h old	
	EC_{13}	Reproduction (live neonates / adult)	144-168	LT	P/S	342	100	69	<24 h old	Lasier *et al.,* 2006
	EC_{22}	Reproduction (live neonates / adult)	168	LT	P/S	342	45	99	<24 h old	
	EC_{35}	Reproduction (live neonates / adult)	168	LT	P/S	342	46	44	<24 h old	
	EC_{17}	Reproduction (live neonates / adult)	168	LT	P/S	342	99	96	<24 h old	
	EC_{33}	Reproduction (live neonates / adult)	168	LT	P/S	565	100	69	<24 h old	
	EC_{54}	Reproduction (live neonates / adult)	168	LT	P/S	565	45	99	<24 h old	
	EC_{59}	Reproduction (live neonates / adult)	168	LT	P/S	565	46	44	<24 h old	
	EC_{44}	Reproduction (live neonates / adult)	168	LT	P/S	565	99	96	<24 h old	
	IC_{10}	Reproduction	144	LT	P/S	80	11	10	<24 h old	WSLH, 2016

Table 5.2. Long-term Studies with Multiple Hardness Level

Species	Tox. Endpoint	Biol. Endpoint	Exposure duration (h)	LT (SSD)	Data Quality	Effect Concentration (mg Cl/L)	Hardness (mg $CaCO_3$/L)	Alkalinity (mg $CaCO_3$/L)	Life Stage	Source
Ceriodaphnia dubia (Water flea)	IC_{10}	Reproduction	168	LT	P/S	236	44	30	<24 h old	WSLH, 2016
	IC_{10}	Reproduction	168	LT	P/S	279	89	59	<24 h old	
	IC_{10}	Reproduction	168	LT	P/S	492	173	114	<24 h old	
	IC_{10}	Reproduction	168	LT	P/S	540	350	224	<24 h old	
Lampsilis fasciola (Wavy-rayed lampmussel)	EC_{10}	Glochidia functional survival	24	LT	P/S	247	44	42	Glochidia	UGARF, 2016a
	EC_{10}	Glochidia functional survival	24	LT	P/S	212	80	80	Glochidia	
	EC_{10}	Glochidia functional survival	24	LT	P/S	964	324	292	Glochidia	
Lampsilis siliquoidea (Fatmucket clam)	EC_{10}	Glochidia functional survival	24	LT	P/S	730	42	41	Glochidia	
	EC_{10}	Glochidia functional survival	24	LT	P/S	681	82	80	Glochidia	
	EC_{10}	Glochidia functional survival	24	LT	P/S	2,877	168	162	Glochidia	
	EC_{10}	Glochidia functional survival	24	LT	P/S	1,662	298	280	Glochidia	
Lithobates pipiens (Northern leopard frog)	EC_{20}	Biomass	168	LT	P/S	801	8	9.1	Tadpole	Nautilus, 2016
	LC_{20}	Mortality	2040	LT	P/S	2,471	8	9.1	Tadpole	
	EC_{20}	Dry weight	2040	LT	P/S	2,282	8	9.1	Tadpole	
	EC_{20}	Length, total	2040	LT	P/S	4,164	8	9.1	Tadpole	
	EC_{20}	Length, snout to vent	2040	LT	P/S	4,274	8	9.1	Tadpole	
	EC_{20}	Biomass	2136	LT	P/S	2,723	90	73.5	Tadpole	
	LC_{20}	Mortality	2136	LT	P/S	2,609	90	73.5	Tadpole	
	EC_{20}	Dry weight	2136	LT	P/S	4,180	90	73.5	Tadpole	
	EC_{20}	Length, total	2136	LT	P/S	2,740	90	73.5	Tadpole	
	EC_{20}	Length, snout to vent	2136	LT	P/S	4,168	90	73.5	Tadpole	
	EC_{20}	Biomass	2136	LT	P/S	2,800	300	207.5	Tadpole	
	LC_{20}	Mortality	2136	LT	P/S	2,600	300	207.5	Tadpole	
	EC_{20}	Dry weight	2136	LT	P/S	4,321	300	207.5	Tadpole	

Table 5.2. Long-term Studies with Multiple Hardness Level

Species	Tox. Endpoint	Biol. Endpoint	Exposure duration (h)	LT (SSD)	Data Quality	Effect Concentration (mg Cl/L)	Hardness (mg $CaCO_3$/L)	Alkalinity (mg $CaCO_3$/L)	Life Stage	Source
	EC_{20}	Length, total	2136	LT	P/S	4,168	300	207.5	Tadpole	
	EC_{20}	Length, snout to vent	2136	LT	P/S	4,234	300	207.5	Tadpole	
Pseudokirchneriella subcapitata (green algae)	IC_{50}	Population fluorescence	96	LT	P/S	2,149	85	N/S	N/S	Geis and Hemming, 2014
	IC_{50}	Population fluorescence	96	LT	P/S	1,986	170	N/S	N/S	
	IC_{10}	Population fluorescence	96	LT	P/S	1,270	85	N/S	N/S	
	IC_{10}	Population fluorescence	96	LT	P/S	955	170	N/S	N/S	
	IC_{10}	Growth (cell density)	96	LT	P/S	756	11	18	4-7 days	WSLH, 2016
	IC_{10}	Growth (cell density)	96	LT	P/S	1,139	44	38	4-7 days	
	IC_{10}	Growth (cell density)	96	LT	P/S	1,152	89	66	4-7 days	
	IC_{10}	Growth (cell density)	96	LT	P/S	1,460	173	121	4-7 days	
	IC_{10}	Growth (cell density)	96	LT	P/S	1,338	350	231	4-7 days	
	IC_{10}	Growth (cell density)	96	LT	P/S	1,529	576	336	4-7 days	
Pimephales promelas (Fathead minnow)	LC_{10}	Mortality	168	LT	U	1,638	40	38	N/S	UGARF, 2016a
	LC_{10}	Mortality	168	LT	U	2,912	80	74	N/S	
	LC_{10}	Mortality	168	LT	U	2,669	164	158	N/S	
	LC_{10}	Mortality	168	LT	U	1,432	198	188	N/S	

LT (SSD): *indicates that the data point meets the requirements for long-term SSD data. 'Chronic' indicates that the data is classified as chronic, but does not meet the specific requirements for SSD derivation.*
P/S: *indicates that toxicity data is classified as primary or secondary and can be used in guideline derivation*
Grey highlight indicates studies not included in the final dataset for the slope calculation

Table 5.3. Hardness Ameliorating Effect from Select Long-Term Studies

Species	Tox. Endpoint	Biol. Endpoint	Exposure duration (h)	LT (SSD)	Data Quality	Effect Concentration (mg Cl/L)	Hardness (mg $CaCO_3$/L)	Alkalinity (mg $CaCO_3$/L)	Life Stage	Source
Daphnia magna (Water flea)	IC_{50}	Reproduction	504	LT	P/S	1,573	45	42	12-24 h old	Biesinger and Christensen, 1972
	IC_{50}	Reproduction	504	LT	P/S	1,037	85	60	<24 h old	Elphick *et al.*, 2011a
	EC_{50}	Reproduction (mean brood size)	240	LT	P/S	2,451	170	N/S	N/S	Cowgill and Milazzo, 1990
	EC_{50}	Reproduction (Total progeny)	240	LT	P/S	2,597	170	N/S	N/S	
	EC_{50}	Growth (dry weight)	240	LT	P/S	2,614	170	N/S	N/S	
	EC_{50}	Reproduction (mean number of broods)	240	LT	P/S	3,504	170	N/S	N/S	
Lemna minor (Common duckweed)	EC_{50}	Growth (# live thalli)	168	LT	P/S	496	140	N/S	N/S	Simmons, 2012
	EC_{50}	Growth inhibition	168	LT	P/S	3,033	699	N/S	Adult	Buckley *et al.*, 1996
	EC_{50}	Growth inhibition	168	LT	P/S	2,960	699	N/S	Adult	
	EC_{50}	Growth inhibition	168	LT	P/S	3,270	699	N/S	Adult	
	EC_{50}	Growth inhibition	168	LT	P/S	3,336	699	N/S	Adult	
Oncorhynchus mykiss (Rainbow trout)	NOEC	Mortality	1296	LT	P/S	1,104	58 (40-76)	36-60	Fertilized gametes	Rescan, 2007
	NOEC	Mortality	192	LT	P/S	485	23	32	Fingerling (1440 h old)	Camargo and Tarazona, 1991
Pseudokirchneriella subcapitata (Green algae)	EC_{50}	Population fluorescence	96	LT	P/S	674	15	N/S	N/S	Simmons, 2012
	EC_{50}	Population growth (log cell density)	96	LT	P/S	780	15	N/S	N/S	
	IC_{50}	Population fluorescence	96	LT	P/S	1,213	170	N/S	N/S	Geis *et al.*, 2000
	IC_{50}	Population fluorescence	96	LT	P/S	1,820	170	N/S	N/S	
	IC_{50}	Population fluorescence	96	LT	P/S	2,149	85	N/S	N/S	Geis and Hemming, 2014*
	IC_{50}	Population fluorescence	96	LT	P/S	1,986	170	N/S	N/S	

**Also listed in Table 5.2 as a part of the multiple hardness level studies;*
Note, that data listed in this table were not used for the final slope calculation due to potential inter-lab variabilities

Elphick et al. 2011a

Elphick *et al.* (2011a) used reproductive (IC_{25} and IC_{50} values) and mortality (LC_{50}) endpoints to examine hardness effects over durations of 168 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.

Lasier and Hardin 2010 and Lasier et al. 2006

Conducting the *C. dubia* three-brood reproduction test, Lasier and Hardin (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). An additional ameliorating effect was observed for alkalinity, as IC_{25} and IC_{50} values were increased in low-hardness moderate-alkalinity water compared to exposures in low-hardness low-alkalinity water. Overall, the Lasier and Hardin (2010) studies demonstrated the substantial ameliorating effect of hardness on chloride toxicity (NaCl exposures). These results corroborate earlier work by Lasier *et al.* (2006) where the same 168 h three-brood test was performed on *C. dubia*. Two solutions with static chloride concentrations of 342 and 565 mg/L were used with the varied 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.

Although both studies observed the hardness-toxicity relationships, none of them met the CCME (2011) requirements for the hardness range. Thus, Lasier and Hardin (2010) and Lasier *et al.* (2006) values were not used quantitatively in determining the ameliorating effect of hardness.

Geis and Hemming 2014

The *P. subcapitata* 96 hours sodium chloride exposure test was conducted by Geis and Hemming (2014) from Wisconsin Laboratory State of Hygiene (WLSH). Tests were completed with two hardness levels, 85 mg/L and 170 mg/L, with corresponding IC_{50} (population fluorescence) of 2,149 and 1,986 mg Cl/L, respectively. An ameliorative effect of water hardness was not observed. More recent research, conducted by WSLH as a part of PTAC-funded project, are discussed below. Geis and Hemming (2014) data was not included in the final dataset, since it did not meet the CCME (2011) requirements for the hardness range.

UGARF 2016a

In 2016, under subcontract to EEI and funded by PTAC, The University of Georgia Research Foundation Inc. (UGARF) examined the ameliorative effect of hardness on sodium chloride toxicity over a chloride concentration range of 0 to 6,066 mg/L and a hardness range of approximately 40 to 310 mg/L (soft to very hard). Some tests were run at extremely hard water (approximately 450 to 500 mg $CaCO_3$/L); however, at this level, toxicity can occur, and it was challenging to consistently dissolve different salts that make up standard US EPA hard water solutions. The following species were tested for key toxicological endpoints:

1. *A. subangulata* (freshwater mussel)
2. *L. fasciola* (wavy-rayed lampmussel)
3. *L. siliquoidea* (freshwater mussel)
4. *P. promelas* (fathead minnow)

Freshwater mussel tests were conducted for the most vulnerable life stage, glochidia, where glochidia functional survival was evaluated by 24 hours sodium chloride exposure tests, under static conditions. The substantial hardness effect was observed in *A. subangulata*, where EC_{10} consequently increased from 877 to 2,701 mg Cl/L at 78 to 548 mg/L hardness interval. *L. fasciola* showed similar substantial dose-response difference with EC_{10} of 212 mg/L at 80 mg/L hardness level, and 964 mg/L response at 324 mg/L hardness. Similar pattern was observed in *L. siliquoidea*, where EC_{10} consequently increased from 681 mg/L at 82 mg/L to 1,662 mg/L at 298 mg/L. Interestingly, no ameliorating hardness effect was observed between the very soft and soft water treatment (40-80 mg/L hardness interval) for three species.

P. promelas testing required the relatively large volumes of water, with difficulties achieving the targeted higher hardness levels of approximately 300 and 550 mg/L. The measured hardness levels were correspondingly 198 and 272 mg/L suggesting partial dissolution of hardness salts. Results for the range of soft to hard and soft to very hard water were evaluated separately. An analysis of 7-day LC_{50} values up to a hardness of 160 mg/L resulted in a weak relationship between decreasing chloride toxicity and increasing hardness (slope = 0.005; r^2 of 0.095). At two higher hardness levels where suspected partial dissolution occurred, this trend did not appear, and the entire direction become negative (slope = 0.01; r^2 of 0.43). Due to conflicting results and concerns regarding experimental setup as indicated by the toxicity lab researcher (Bringolf, R., *pers. comm.*), the dataset for *P. promelas* was not used quantitatively in determining the ameliorating effect of hardness.

WSLH 2016

Under subcontract to EEI and funded by PTAC, WSLH (2016) conducted a sodium chloride toxicity study at six different hardness levels, ranging from the very soft water (10 mg/L) to extremely hard water (576 mg/L).

The following species were tested for key toxicological endpoints:

1. *C. dubia* (water flea)
2. *R. (Pseudokirchneriella) subcapitata* (microalgae)

C. dubia tests were 168 hours static-renewal chronic tests assessing mortality and reproduction. *P. subcapitata* static tests lasted 96 hours and assessed growth. The extremely hard water values were not included in the *C. dubia* final dataset, due to potential toxicity interference. For *C. dubia*, IC_{10} consequently increased from 80 to 540 mg/L on a water hardness interval from 11 to 350 mg/L. For *P. subcapitata,* IC_{10} an overall increase was observed from 756 mg Cl/L at 11 mg/L hardness (very soft water) to 1,529 mg Cl/L at 576 mg/L (extremely hard water); however, the difference between soft, moderately hard, hard, and very hard water (44, 89, 173, 350 mg/L) was not consistent. The corresponding IC_{10} of 1,139, 1,152, 1,460, and 1,338 mg Cl/L did not show substantial hardness-ameliorating pattern.

Nautilus 2016 and 2019

Under subcontract to EEI and funded by PTAC, Nautilus (2016; 2019) examined the ameliorating effects of hardness on sodium chloride toxicity for the following species:

1. *C. (Neocloeon) triangulifer* (mayfly)
2. *L. pipiens* (northern leopard frog)

The long-term static-renewal test for *C. triangulifer* was conducted on the organisms <48 hr old, and lasted for 28 days, comparing the soft (42 mg/L), moderately hard (84 mg/L), hard (85 mg/L) and very hard (300 mg/L) water levels. Biological endpoints, such as mortality, emergence, and biomass, were measured. Since emergence test is highly susceptible to variability (Elphick; *in pers. comm*), the final data pool was based on mortality and biomass endpoint values. The consequent ameliorating effect was observed at the moderately hard to very hard water level, where LC_{20} (mortality) was increased from 243 to 502 mg Cl/L. At the same time, LC_{25} of 361 mg/L was observed at the soft water, which is 118 mg/L higher than the value recorded for the moderately hard water.

The long-term static-renewal test for *L. pipiens* (tadpole stage) lasted for 2,040-2,136 hours, and included three water hardness levels, such as very soft, moderately hard, and very hard water (8, 90, and 300 mg/L, respectively). Mortality, biomass, and length biological endpoints were evaluated. The most sensitive biomass IC_{20} substantially increased from 801 mg Cl/L in the very soft water to 2,723 mg/L at the moderately hard water; however, no substantial effect was observed between moderately hard and very hard water treatments.

5.4 SPECIES WITH NO, CONFLICTING, OR EQUIVOCAL HARDNESS EFFECT ON CHLORIDE TOXICITY

After reviewing the literature, four instances were found, where no ameliorating effect (or conflicting or equivocal results) were observed for hardness influence on chloride toxicity:

1) *S. tenue and S. simile*;
2) *C. dubia;*
3) *P. promelas;* and,
4) *G. parvus* and *P. heterostropha.*

Three out of four above mentioned occurrences were presented in studies / data tables with data quality (or lack of detail) issues, and the results were limited for use in guideline derivation.

S. tenue and S. simile (Fingernail clams)

Wurtz and Bridges (1961) examined the toxicity of Cl, in soft and hard waters, towards *S. tenue*; however, no control survival was reported. This study did not find any hardness amelioration of Cl toxicity (EC_{50} of 698 and 667 mg Cl^-/L for the soft and hard waters of 20 and 100 mg $CaCO_3$/L, respectively). In contrast, tests for another species (*S. simile*) from the same clam genus by Soucek *et al.* (2011), where supporting information was provided, have demonstrated an ameliorative effect (EC_{50} of 740 and 1,100 mg Cl^-/L for the hardness of 51 and 192 mg $CaCO_3$/L, respectively). The study by Wurtz and Bridges (1961) is limited by the lack of reporting of control survival data and a smaller range in tested hardness level (*i.e.*, 20 and 100 mg $CaCO_3$/L). The more recent data from Soucek *et al.* (2011) was considered more indicative of a potential hardness effect on chloride toxicity for clams.

C. dubia (Water flea)

In WSLH (2007), the original data was not available. In contrast to the case of *C. dubia* results reported by WSLH, where hardness amelioration was not observed over a rather narrow range in hardness (acute toxicity values of 1,677 and 1,499 mg/L at hardness values of 84.8 and 169.5 mg/L, respectively). Four other studies demonstrated ameliorating effects of hardness for this species over a wider hardness range. Elphick *et al*. (2011a), Lasier *et al*. (2006), Lasier and Hardin (2010), and PTAC-funded research by WSLH (2016) observed a positive correlation between chloride tolerance and hardness, suggesting by weight of evidence that hardness is ameliorating for chloride toxicity in *C. dubia.* Elphick *et al.* (2011a) and WSLH (2016) used a relatively wide hardness range of 10 – 320 mg/L and 10 – 350 mg/L, respectively.

P. promelas (Fathead minnow)

WSLH (2007; cited in: US EPA, 2011) tested the effect of hardness on chloride toxicity and did not identify an ameliorative effect in soft and hard waters (81.4 and 169.5 mg $CaCO_3$/L). However, the original report was not available for review, and these data were not used (CCME, 2011). A hardness amelioration effect has been observed on SO_4^{2-} toxicity for *P. promelas* (Elphick *et al.,* 2011b) suggesting a relationship with chloride is possible.

The more recent PTAC-funded research (UGARF 2016a) tested the effect of hardness on chloride toxicity with five waters, ranging from the very soft/soft to extremely hard (11-42, 85, 169, 338, and 677 mg $CaCO_3$/L). The two highest target hardness concentrations were not reached, and results were not included in the final dataset.

Although the data on fish does not appear to indicate amelioration, it was difficult to produce toxic effect on fish from elevated Cl, possibly due to the challenges for freshwater fish to keep ions in their tissue in a hypoosmotic environment. Since fish are less sensitive to chloride, the fish toxicity endpoints will have a lesser relevance to hardness-adjusted SSDs protective of more sensitive species that drive the water quality guideline.

G. parvus and P. heterostropha (Planorbid snails)

An amelioration of chloride toxicity was observed for the planorbid snail *P. heterostropha* (Wurtz and Bridges, 1961) in soft and hard waters (20 and 100 mg $CaCO_3$/L). In contrast, no such relationship was found by Soucek *et al.* (2011) using the planorbid *G. parvus*. The authors suggest that the unique respiration mechanism of planorbid snails (they lack gills) may explain the results. Wurtz and Bridges (1961) did not report control survival, and the relationship was relatively week for hardness amelioration of chloride toxicity. In addition, the number of testing animals was small, with considerable variability in the results.

Soucek *et al.* (2011) tested *G. parvus* of variable ages (3-5 mm diameter) and evaluated different Ca^{2+}: Mg^{2+} ratios between the soft and hard water treatments (56 and 212 mg $CaCO_3$/L respectively). During the softer water treatment, the ratio of Ca^{2+} to Mg^{2+} ions was 4.44 (13.5 mg Ca^{2+}/L for 3.04 mg Mg^{2+}/L), whereas the harder 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 Ca^{2+} to Mg^{2+} (Welsh *et al.,* 2000). As a result, the two-fold difference in Ca^{2+} to Mg^{2+} ratios between the soft and hard water treatments for *G. parvus* may explain the lack of an ameliorating effect of water hardness in the planorbid snail *G. parvus*. Furthermore, if Ca^{2+} is of greater importance in ameliorating toxicity compared to Mg^{2+}, as has been suggested in some literature (Leblanc and Surpenant, 1984; Jackson *et al.,* 2000; Welsh *et al.,* 2000), then the absence of an ameliorating effect may be speculated to be the result of a lower Ca^{2+} to Mg^{2+} ratio in the harder versus softer water treatments. These snails were found to be relatively less sensitive to chloride toxicity, and any hardness relationship would be less relevant for adjusting the SSD and associated guideline based on differences in hardness levels.

5.5 SUMMARY

The peer-reviewed literature demonstrates a weight of evidence support for the hypothesis that an ameliorating relationship exists between increasing water hardness and chloride toxicity, and it is possible that Ca^{2+} may have a stronger ameliorating effect than Mg^{2+}. The key priority was to establish hardness-toxicity relationships for the species making up the lower portions of the acute and chronic SSDs, to make sure that any future hardness-adjusted guidelines are protective of the most sensitive aquatic organisms.

PTAC-sponsored research was completed on three unionid species (*L. siliquoidea*, *L. fasciola*, and *A. subangulata). L. fasciola* was particularly sensitive to chloride toxicity on the short-term and long-term basis, and *L. siliquoidea* experienced the high short-term sensitivity, as reflected by their position on the SSD curves. *A. subangulata* (western ridged mussel) was not previously assessed by CCME (2011). It was determined that the chloride sensitivity of *L. siliquoidea* may be in part a function of the brooding time and source waters from which the brooding mothers were obtained. Unionid tests frequently involve the use of wild populations, which differ from many traditional toxicity test organisms that are more genetically identical and are reared in the lab. Regardless, for *L. siliquoidea*, *L. fasciola*, and *A. subangulata*, chloride toxicity was found to be ameliorated by increasing hardness based on research conducted at the University of Georgia by Dr. Bringoff, an expert in unionid glochidia toxicity testing (Appendix A).

PTAC-sponsored research was conducted on the mayfly species *C. (Neocloeon) triangulifer,* the sensitive *Ephemeroptera*. Nautilus (2019) conducted the series of Cl toxicity testing, and observed a trend of ameliorating hardness effect. The hardness of 84, 168, 292 mg CaO_3/L had a corresponding IC_{20} chloride of 243, 373, and 502 mg/L; however, the soft water (42 mg CaO_3/L) had an IC_{20} of 361 mg/L chloride. The control mortalities were noticed higher in soft and hard water, which may partially affect the soft water results.

More distinct hardness-chloride toxicity relationship was found by Nautilus (2016) in another PTAC-sponsored research, completed on *L. pipiens* (northern leopard frog). The 3-fold increase in IC_{20} chloride concentration was observed with hardness change from 8 to 90 mg CaO_3/L.

PTAC-sponsored work was completed on the algae species (*P. subcapitata*), identified as potentially sensitive from the work of Simmons (2012), where an effect concentration of 96 mg Cl/L was determined. Results indicate that *P. subcapitata* is not necessarily sensitive based on research conducted under funding from PTAC (WSLH, 2016), which involved multiple replicates and controlled experimental conditions. Apparent, statistically significant ameliorating effect of hardness was observed.

An extensive PTAC-sponsored study enhanced the *C. dubia* (water flea) dataset. The research conducted by WSLH (2016) supported the distinct hardness-toxicity relationship, previously observed by Elphick (2011).

6 HARDNESS-ADJUSTED LONG-TERM GUIDELINES

The following section outlines the results from toxicological analysis of the long-term dataset adjusted for water hardness. The derivation of the long-term hardness adjusted WQG for chloride is outlined using chronic data with associated water hardness values available. The long-term hardness-toxicity relationships with an overall best-fit hardness-toxicity slope is evaluated, the SSD adjusted to 50 mg $CaCO_3$/L is created. Any value could be selected (other than 50 mg $CaCO_3$/L) – the value of 50 was considered a reasonable estimate for typical softer waters in Canada as a starting point. Ultimately, this value selection does not alter the final relationship of chloride toxicity at different levels of water hardness – in other words, it does not affect the hardness adjustment equation to derive long-term chloride WQGs.

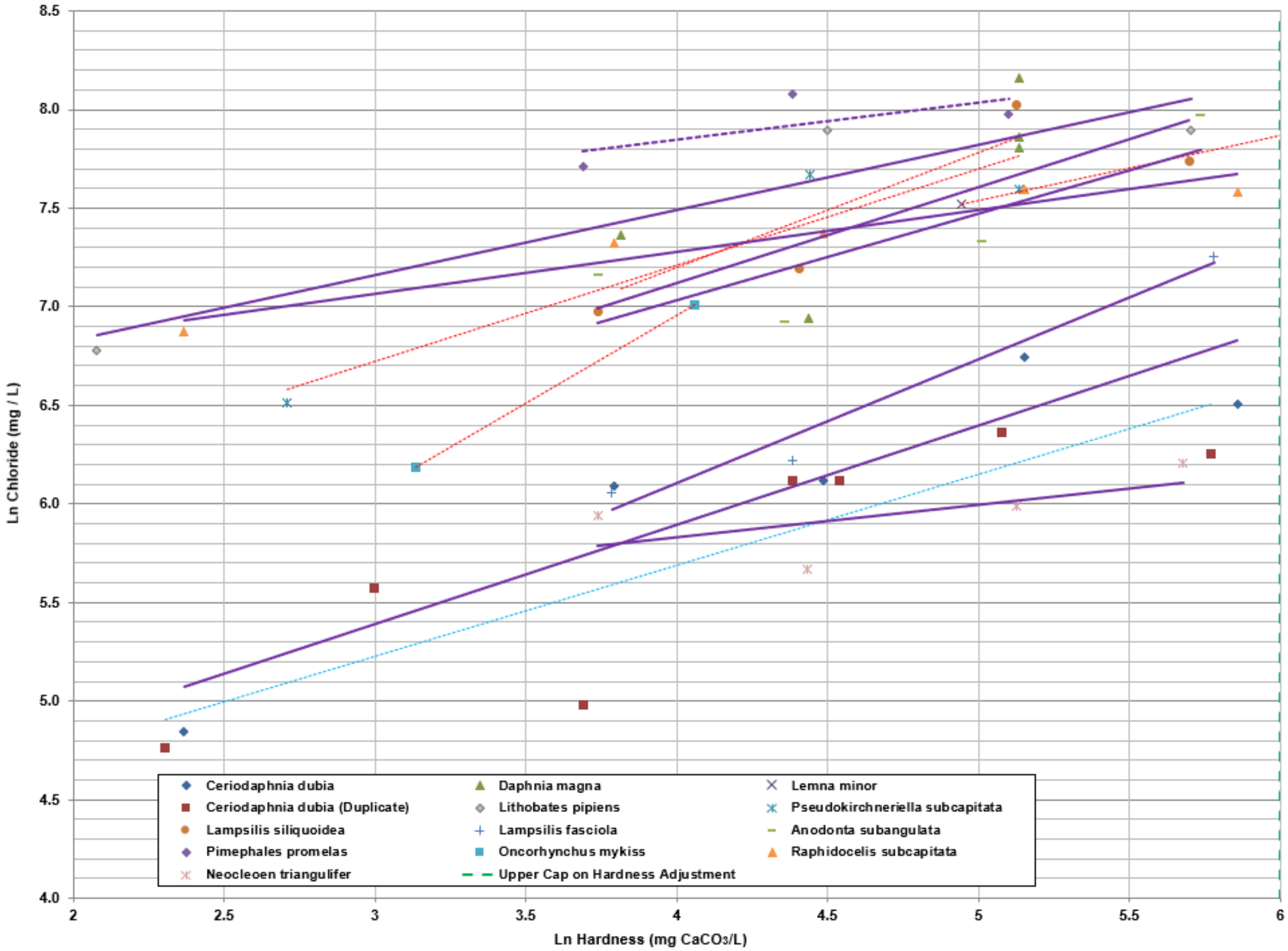
6.1 HARDNESS ADJUSTMENT REGRESSION SLOPES

For the long-term endpoint, eight datasets for seven species had comparable effect concentration across the minimum required hardness range: *C. dubia* (water flea), *C. triangulifer* (mayfly), *A. subangulata* (freshwater mussel), *L. fasciola* (freshwater mussel), *L. siliquiodea* (freshwater mussel), *L. pipiens* (frog), 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. For comparing natural log chloride versus natural log hardness, a linear regression was used to calculate a best-fit hardness-toxicity slope for each of the seven species.

Two distinct datasets (Elphick, 2011; WSLH, 2016) were available for *C. dubia*, and each slope was evaluated by degradation model (stability test), with a p-value of 0.0285 and 0.0032 for Elphick (2011) and WSLH (2016) data, respectively. The WSLH (2016) dataset with a smaller p-value was included in the final regression, to represent *C. dubia*. Datasets included in the final regression are shown in Figure 6.1 in black and purple dashed lines.

Datasets for four species, *D. magna* (water flea), *L. minor* (duckweed), *P. promelas* (fathead minnow), and *O. mykiss* (rainbow trout) were found to demonstrate hardness-toxicity relationships. In order for the datasets for three of these species (*D. magna*, *L. minor*, and *O. mykiss*) to be used, data from different studies and laboratories (and thus potentially different experimental conditions) would need to be pooled to achieve the minimum required hardness range – as a result, these datasets were excluded from the slope calculations. The dataset for *P. promelas* (discussed in Section 5.4) was excluded due to conflicting results and concerns regarding the experimental setup. Datasets excluded from the final regression are listed in Table 5.3 and shown in Figure 6.1 in red dashed lines.

Figure 6.1. Long-Term Hardness-Toxicity Relationship



***Notes**:*
Purple dashed lines represent PTAC-sponsored work included in the final dataset (Table 5.2); purple dashed line indicates the data for P. promelas, not included in the final slope evaluation due to conflicting results and concerns regarding the experimental setup (Section 5.4).
Blue dashed line represents the second available dataset for C. dubia (Elphick 2011), not included in the final slope evaluation.
Red dashed lines indicate data points from different studies, different laboratories, and different experimental conditions - considered unreliable and often not meeting US EPA requirements; not included in the final dataset; Table 5.3

The slope evaluation and statistical analysis was performed with the degradation model (stability test). T-test confirmed that the pooled slope of 0.38 for the long-term data was statistically significant ($P < 0.0001$), and intercepts were significant with relation to the pooled slope (Table 6.1). Individual dataset slopes ranged from 0.17 to 0.75, with statistically significant relationship in two slopes, namely *R. subcapitata,* and *C. dubia*. T-test for *A. subangulata* is nearly significant at the 0.05 level. Test for *C. triangulifer* was not statistically significant on the hardness range from 42 mg/L to 292 mg/L; however, the slope became statistically significant on the 84 mg/L – 292 mg/L hardness range, when the soft water value was excluded. Three individual dataset slopes (*L. fasciola, L. siliquoidea, and L. pipiens*) showed no statistical significance. Mean standard errors were acceptable. Statistical analyses results are present in Table 6.1. Individual hardness-chloride relationships for each species are shown in Figure 6.2.

An additional pooled slope evaluation was completed on *R. subcapitata* and *C. dubia* showing statistically significant individual slopes, borderline significant *A. subangul*ata, and *C. triangulifer* without soft water value. The pooled slope was equal to 0.35, indicating generally similar results to the pooled slope of 0.38 including dataset that did not have statistically significant individual slopes. Statistical analyses results are present in Table 6.2.

Thus, the pooled slope of 0.38 from the main dataset did not substantially differ from the slope derived from statistically significant regressions. This slope was chosen, to incorporate more variability within species and chloride-hardness relationship.

The calculated pooled slope of 0.38 for the long-term data was then used to normalize toxicity values in the long-term SSD (Section 4) to a hardness of 50 mg/L using equation 5.3.

$$\mathbf{LT}\boldsymbol{EC_{x(50\ \mathrm{mg\ CaCO3/L})}} = \mathbf{exp}^{\{\,(\,[\,\ln(50) - \ln(\mathrm{hardness})\,]\ \times\ \mathrm{LTPS}) + \ln(\mathrm{LTECx})\,\}} \qquad \textit{(Eq. 5.3)}$$

where

LT*EC*$_{x(50\ \mathrm{mg\ CaCO_3/L})}$:	a given long-term effect concentration normalized to 50 mg $CaCO_3$/L
LT*ECx:*	a given long-term effect concentration
LTPS:	the pooled slope of 0.38 derived from long-term data

Table 6.1. Long-Term Hardness-Toxicity Regression Slopes

Parameter	Species	Estimate	Std. Error	t Ratio	Prob> I t I
Intercept Slope	*Anodonta subangulata*	5.44 0.38	0.62 0.12	8.51 3.05	0.0034 0.0555
Intercept Slope	*Centroptilum (Neocloeon) triangulifer*	4.89 0.21	0.95 0.20	5.04 1.03	0.0371 0.4098
Intercept Slope	*Ceriodaphnia dubia*	3.17 0.56	0.35 0.08	10.9 8.66	0.0016 0.0032
Intercept Slope	*Lampsilis fasciola*	2.41 0.75	1.24 0.26	1.66 2.45	0.3454 0.2469
Intercept Slope	*Lampsilis siliquoidea*	4.22 0.61	1.81 0.38	2.34 1.63	0.1444 0.2452
Intercept Slope	*Lithobathes pipiens*	6.00 0.37	0.52 0.12	11.55 3.09	0.0550 0.1991
Intercept Slope	*Raphidocelis subcapitata*	6.29 0.17	0.22 0.05	41.6 5.43	<.0001 0.0056
Pooled slope		0.38	0.06	6.53	<.0001

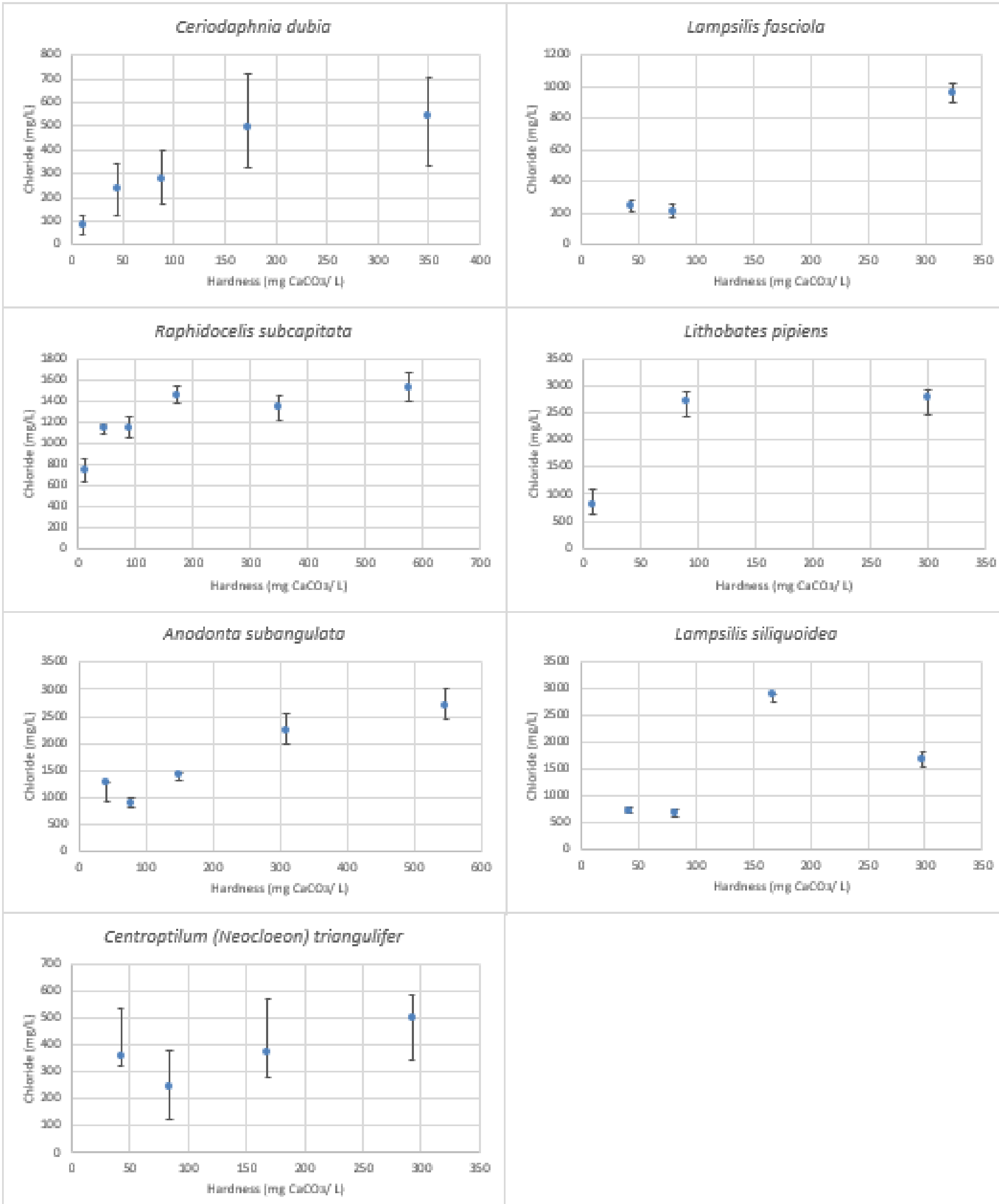
Notes:
Red font indicates statistically significant relationship with P<0.05; orange font indicates the borderline significant result
Test for C. triangulifer was not statistically significant on the hardness range from 42 mg/L to 292 mg/L

Table 6.2. Significant Long-Term Hardness-Toxicity Regression Slopes

Parameter	Species	Estimate	Std. Error	t Ratio	Prob> I t I
Intercept Slope	*Ceriodaphnia dubia*	3.17 0.56	0.29 0.06	10.91 8.66	0.0016 0.0032
Intercept Slope	*Raphidocelis subcapitata*	6.29 0.17	0.15 0.03	41.56 5.43	<.0001 0.0056
Intercept Slope	*Anodonta subangulata**	5.44 0.38	0.64 0.13	8.51 3.05	0.0034 0.0555
Intercept Slope	Centroptilum triangulifer	2.91 0.58	0.12 0.02	24.87 25.43	0.0256 0.0250
Pooled slope		0.34	0.06	6.07	0.0001

Notes:
Red font indicates statistically significant relationship with P<0.05
*Orange font indicates the borderline significant relationship with P=0.0555, added to statistical pool
The slope for C. triangulifer became statistically significant on the 84 mg/L – 292 mg/L hardness range, when the soft water value was excluded.

Figure 6.2. Individual Species Hardness-Chloride Relationship



Individual species charts show calculated toxicity endpoints (Table 5.2) with 95% confidence intervals; species with observed statistically significant pattern (charts on the left) were additionally evaluated for the pooled slope (Table 6.2).

6.2 HARDNESS ADJUSTMENT SSD AND BENCHMARK CONCENTRATION

For the hardness-adjusted long-term SSD, eight of the 35 species from the unadjusted SSD curve (Table 4.1) were excluded, since no hardness data were available for these data points. The species excluded due to a lack of reported study hardness data were: *M. securis* (fingernail clam), *S. natans* (floating fern), a species of fresh water snail (*Physa sp.)*, *G. pseudolimnaeus* (amphipod), and four species of algae (*C. humicola, C. vulgaris*, *C. minutissimo*, and *Synechocystis sp.*). With the exception of *M. securis* (HPP = 0.04), 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 27 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 0.40 derived in the previous section. The normalized data set is shown in Table 6.2. SSD derivation followed the same methods as described in Section 2.3, and normalized effect concentrations converted to the base 10 logs, with corresponding HPP ranks were assessed by EasyFit 5.6 Professional software (Mathwave Technologies, 2015).

Among six models recommended by CCME (2007), including Burr Type III, Gumbell, Logistic, Lognormal, Normal, and Weibull cumulative distribution functions, the Logistic distribution had the highest rank. The logistic model was selected based on goodness-of-fit, model feasibility, and minimizing the scaled residuals. Anderson-Darling analyses chosen for the better sensitivity towards the ends, put Logistic distribution on the seventh place out of 65 models compared.

The general Logistic function equation was the same as utilized for the unadjusted long-term data in the current report and in CCME (2007):

$$y = 1/(1 + \exp(-z)) \quad \textbf{(Eq. 4.1)}$$

$$\textit{where } z = (x - \mu)/\delta$$

where y is the proportion of species affected, and x is the base 10 log of the chloride concentration. The continuous scale parameter δ and continuous location parameter μ were calculated by EasyFit as 0.23 and 2.71, respectively. Note that parameters δ and μ are calculated individually for each data set.

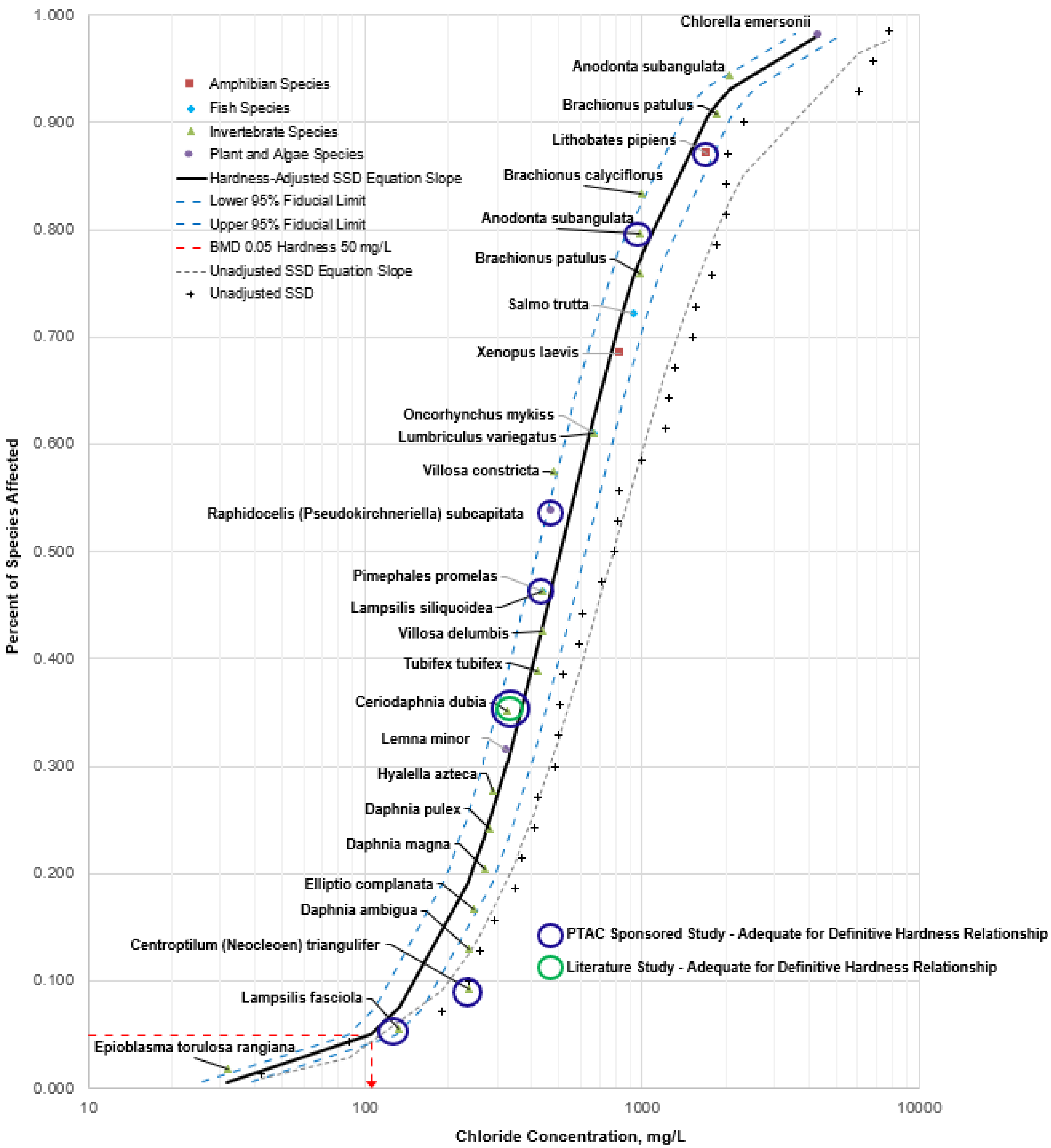
Figure 6.3 shows the long-term hardness-adjusted SSD made up of no- and low-effect concentrations normalized to 50 mg $CaCO_3$/L and corresponding Hazen plotting position values (Table 6.3), along with the fitted Logistic function (Eq. 4.1). The long-term benchmark concentration, defined as the 5th percentile along the derived Logistic function (Eq. 4.1) was **106** mg/L chloride with a lower fiducial limit of 85 mg/L chloride, at a hardness level of 50 mg/L.

Table 6.3. Long-Term Hardness-Adjusted Effect Concentrations

Species	Common name	Toxicological Endpoint	Biological Endpoint	Normalized No/Low EC (mg Cl/L)	HPP	Source
Epioblasma torulosa rangiana	Freshwater mussel	24 h EC_{10}	Glochidia functional survival	32.4	0.019	Gillis, 2011
Lampsilis fasciola	Wavy-rayed Lampmussel	24 h EC_{10}	Glochidia functional survival	**134.7**	0.056	**see Table 4.2***
Centroptilum (Neocloeon) triangulifer	Mayfly	336 h IC_{25}	Survival	**236.3**	0.093	**see Table 4.2***
Daphnia ambigua	Water flea	240 h EC_{10}	Mortality and reproduction	237.2	0.130	Harmon *et al.,* 2003
Elliptio complanata	Fatmucket clam	24 h EC_{10}	Survival	255.5	0.167	Bringolf *et al.,* 2007
Daphnia magna	Water flea	504 h EC16-IC25	Reproduction	**271.0**	0.204	**see Table 4.2***
Daphnia pulex	Water flea	504 h IC_{10}	Reproduction	286.2	0.241	Birge *et al.,* 1985
Hyalella azteca	Amphipod	672 h EC_{25}	Growth, dry weight/ amphipod	296.4	0.278	Bartlett *et al.,* 2012
Ceriodaphnia dubia	Water flea	168 h IC_{25}	Reproduction	**322.5**	0.315	**see Table 4.2***
Lemna minor	Common duckweed	168 h EC_{10}	Growth	335.4	0.352	Simmons, 2012
Tubifex tubifex	Tubificid worm	672 h EC_{10}	Reproduction	425.0	0.389	Elphick *et al.,* 2011a
Lampsilis siliquoidea	Freshwater mussel	96 h EC_{10}	Glochidia functional survival	**447.5**	0.426	**see Table 4.2***
Villosa delumbis	Freshwater mussel	24 h EC_{10}	Survival	450.6	0.463	Bringolf *et al*., 2007
Pimephales promelas	Fathead minnow	816 h LC_{10}	Mortality	461.1	0.500	Birge *et al.,* 1985
Raphidocelis (Pseudokirchneriella) subcapitata	Microalgae	96 h EC_{10}	Fluorescence and growth	**488.6**	0.537	**see Table 4.2***
Villosa constricta	Freshwater mussel	24 h EC_{10}	Survival	496.5	0.574	Bringolf *et al.,* 2007
Lumbriculus variegatus	California blackworm	672 h IC_{25}	Biomass	675.6	0.611	Elphick *et al.,* 2011a
Oncorhynchus mykiss	Rainbow trout	168 h EC_{25}	Embryo viability	709.1	0.648	Beak, 1999
Salmo trutta	Brown trout	192 h NOEC	Mortality	815.3	0.685	Camargo and Tarazona, 1991
Xenopus laevis	African clawed frog	168 h LC_{10}	Mortality	952.4	0.722	Beak, 1999
Brachionus patulus	Rotifer	336 h NOEC	Population increase	993.3	0.759	Peredo-Alvarez et al., 2003
Brachionus calyciflorus	Rotifer	48 h IC_{10}	Reproduction	1,016.2	0.833	Elphick *et al.,* 2011a
Anodonta subangulata	Freshwater mussel	24 h EC_{10}	Glochidia functional survival	**1,027.9**	0.796	**see Table 4.2***
Lithobates pipiens	Northern leopard frog	2,040-2,136 h EC_{20}	Biomass	**1,705.5**	0.870	Nautilus, 2016
Chironomus dilutus / tentans	Midge	480 h EC_{10}	Biomass	1,896.6	0.907	Elphick *et al.,* 2011a
Stenonema modestum	Mayfly	336 h MATC	Development	2,070.8	0.944	Diamond *et al*., 1992
Chlorella emersonii	Green alga	192-336 h MATC	Growth	4,497.9	0.981	Setter *et al*., 1982

Normalized No/Low EC *– effect concentrations from Table 4.1 normalized to a hardness of 50 mg $CaCO_3$/L utilizing Eq. 2.3*
HPP -*Hazen plotting position (Section 2.3)*
**Data shown is the geomean value taken from multiple studies. Intra-study comparisons use the most sensitive endpoint, whereas inter-study comparisons use geometric mean. Multiple study data are summarized in Table 4.2.*

Figure 6.3. Long-Term SSD Adjusted to 50 mg/L Hardness



Notes:*1) SSD derived by plotting no- and low-effect concentrations (preferred endpoints: 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$_{50}$) adjusted to a hardness of 50 mg CaCO$_3$ /L for 27 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 106 mg Cl/L; 3) Grey hashed curve and black crosses represent the unadjusted SSD (Figure 4.1).*

Note that PTAC-sponsored studies for L. siliquoidea were included in the final hardness slope calculation, whereas the values for P. promelas were considered unacceptable (Section 5.4).

6.3 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 Stephen *et al.* (1985). The function was derived using the long-term hardness-toxicity slope (m) of 0.38 calculated in Section 5.3.1 and the derived long-term WQG of 106 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 3.18. Using this value, the equation of the line to determine the long-term hardness dependant water quality guideline concentration can be expressed as:

$$\textit{Long-term WQG} = \exp^{[0.38\,(\ln(hardness)) + 3.18]} \qquad \textit{(Eq. 6.1)}$$

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

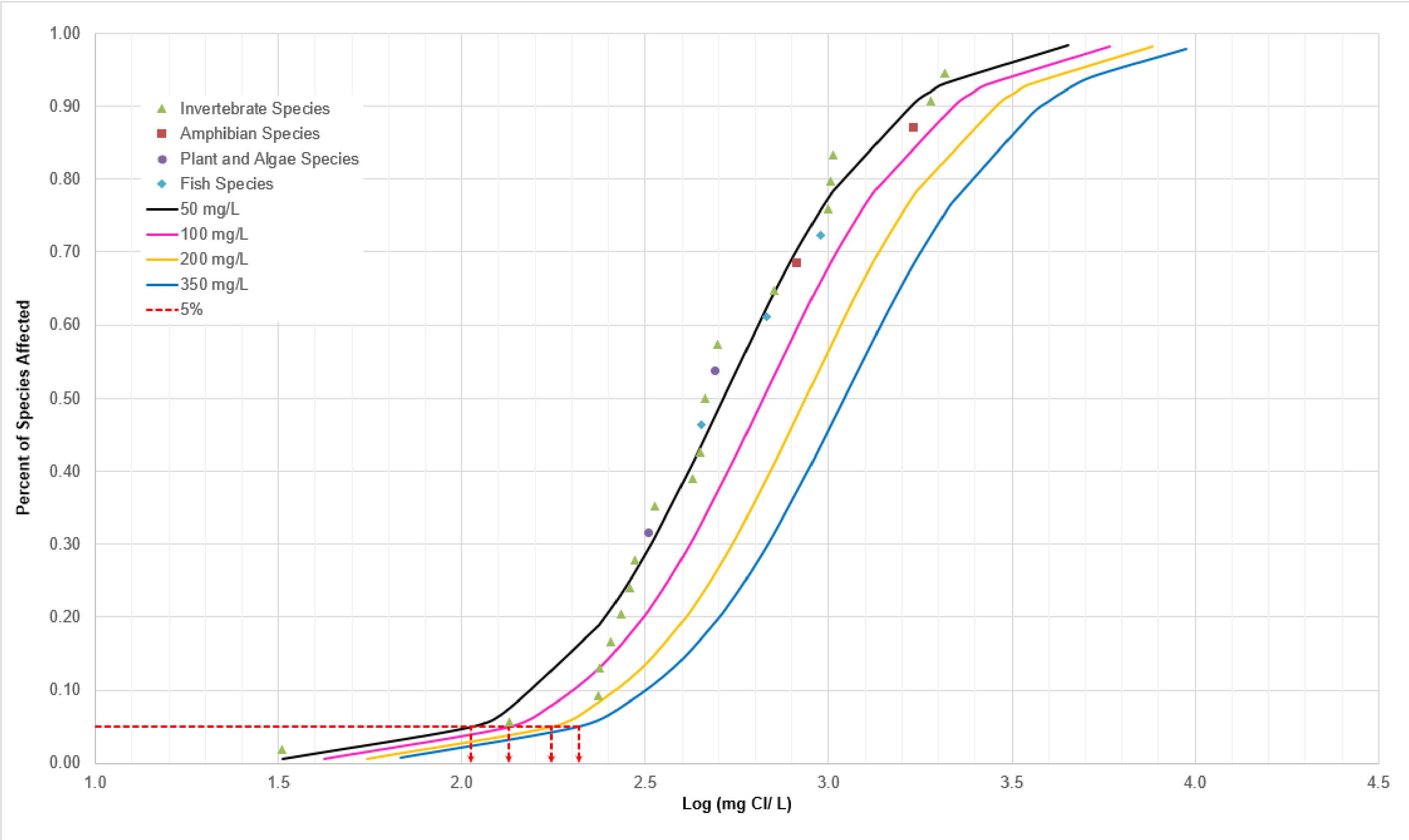
Figure 6.3 provides a visual representation of the 50 mg $CaCO_3$/L hardness adjusted SSD along with modeled curves at hardness concentration of 100, 200, and 350 $CaCO_3$/L. These values represent the upper bound thresholds for water classified as soft, moderately soft, moderately hard, and hard, respectively. The long-term WQG chloride concentrations corresponding to these hardness levels are indicated in Figure 6.3 and are summarized in Table 6.3.

Table 6.3. Long-Term Hardness-Adjusted WQG Concentrations

Hardness (mg $CaCO_3$/L)	Long-term WQG (mg Cl^-/L)
Current derivation	***($\exp^{[0.38(\ln(hardness)) + 3.18]}$)***
5 (very soft)	**44**
50 (soft)	**106**
100 (moderately soft)	**138**
200 (moderately hard)	**180**
350 (hard)	**222**

Note, *that a maximum hardness influence cap was set at 350 mg/L hardness, due to potential toxicological interference in extremely hard waters. For the very hard water (>350 mg/L), the maximum computable guideline of 222 mg/L is recommended.*

Figure 6.3. Long-Term SSD Adjusted to the Various Hardness Levels



Notes:
X-axis shows chloride concentrations, converted to the base 10 log for better visibility;
Black curve represents the 50 mg $CaCO_3$/L hardness adjusted SSD with associated species (Table 6.2);
Red dashed line indicates the 5th percentile (y-axis) and the resulting long-term WQG concentrations (x-axis) of 106, 138, 180, and 222, mg Cl/L, corresponding to hardness values of 50, 100, 200, and 350 mg $CaCO_3$/L, respectively.

7 ASSESSMENT OF GUIDELINE PROTECTIVENESS

To evaluate the extent to which the long-term hardness adjusted guidelines are protective of other conditions such as $CaCl_2$ and $MgCl_2$ salt exposures, an assessment of protectiveness was conducted. The approach involved plotting the long-term concentrations from acceptable data sources by corresponding hardness values. The associated long-term guidelines were then plotted, and data points occurring below these respective guidelines examined in detail.

7.1 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=7) and studies (n=8). The appropriateness of this guideline for extrapolation to other species, including sensitive species in the chronic SSD that drive 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 non-adjusted for hardness were plotted against the hardness-adjusted guideline (Figure 7.1). Some of the data points plotted in Figure 7.1 have been incorporated into the SSD (a well as in CCME's, 2011 SSD), but in other cases the data were not included in the SSD for various reasons.

Of the 501 acceptable long-term values plotted in Figure 7.1, two fell below the long-term WQG for chloride (Table 7.1). The lowest point comes from the 24 h EC_{10} value of 24 mg Cl/L (Bringolf *et al*., 2007); however, the most recent data obtained for *L. fasciola* by the same researcher in the same laboratory (UGARF, 2016a), suggest higher 24 h EC_{10} values (indicated by arrows in Figure 7.1). This discrepancy may be related to the brood origin and to the difference between ecosystems where the mussels were collected prior to experiment.

The second lowest point corresponds to 42 mg Cl/L (Gillis, 2009) for *E. torulosa rangiana*. Both mussels are considered species at risk by COSEWIC, and *L. fasciola* is classified as special concern, while *E. torulosa rangiana* is classified as endangered. In the CCME (2011) derivation 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. The same protection clause can be applied for the current guidelines, based on the lowest values observed:

> "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".

Due to the different brood sensitivity, observed by Bringolf (2007) and UGARF (2016a) between the *L. fasciola* collected in various ecosystems, it is recommended that in some waters the guidelines might be set up at 24 mg/L, to ensure protection of the most sensitive population. In other waters in the different geographical regions, the guidelines for *L. fasciola* may be constrained by 188 mg/L, a geometric mean from Bringolf (2007) and UGARF (2016a) studies.

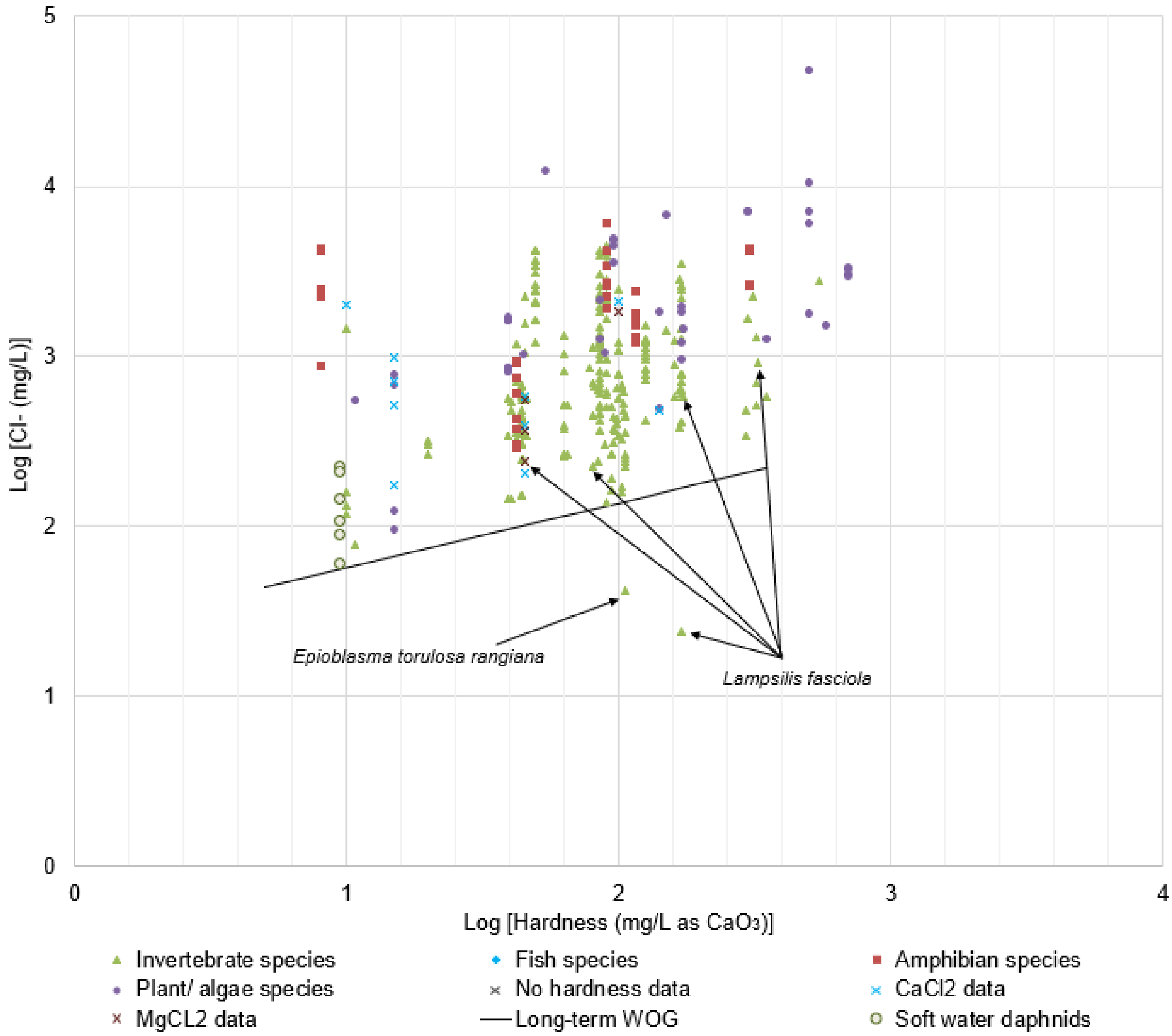
The two lowest sensitive endpoints for freshwater mussels and clams are coming from studies examining the glochidia life stage. Evidence suggests that free-living glochidia survive in the water column for durations varying between 24 h to 10 days (ASTM, 2013; Cope *et al.,* 2008). In the current dataset, glochidia endpoints at durations greater than 24 h were available from four studies (Valenti *et al.,* 2007; Pandolfo *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 more toxic results (*e.g.* Bringolf *et al.* (2007): *V.constricta* EC_{50} of 1,674 (24 h) and 1,571 mg Cl^-/ L (48h); *E. complanata* EC_{50} of 1,620 (24 h) and 1,353 mg Cl^-/ L (48h); and *L. fasciola* EC_{50} of 1,116 (24 h) and 1,056 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 2,008 (24 h) and 2,202 mg Cl^-/ L (48 h)). 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 historical glochidia data at exposure durations of ≤ 24 h, and includes more recent data obtained during PTAC-funded projects for *L. fasciola*, *L. siliquiodea*, and *A. subangulata.* The more recent 24 h EC_{10} (glochidia functional survival) chloride concentrations observed for these species are ranging from 877 to 2,701 mg/L for *A. subangulata*, from 212 to 964 mg/L for *L. fasciola*, and from 730 to 2,877 for *L. siliquoidea* (UGARF, 2016a; discussed in detail in Section 5.3).

The next sensitive species appearing on the hardness-adjusted SSD curve, is a mayfly *C. triangulifer.* The available endpoints for *C. triangulifer* included mortality, emergence, and biomass. Since emergence test is highly susceptible to variability (Elphick; *in pers. comm*), the biomass was considered to be more suitable and reliable indicator of the chronic toxicity. The final long-term endpoints included the dry weight of emerged organisms and mortality rate, ranging from 139 mg Cl/L (Struewing *et al.,* 2015) to >642 mg Cl/L (Nautilus, 2019). None of these points falls below guidelines (Figure 7.1).

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). The dataset used in the process of the guidelines development, includes 126 toxicological endpoints for four daphnia species (*C. dubia*, *D. ambigua*, *D. magna*, and *D. pulex*), ranging from 80 mg Cl/L (IC_{10}, *C. dubia* reproduction; WSLH, 2016) to 4,070 mg Cl/L (LOEC, *D. magna*, mortality; Rescan, 2007). None of these points falls below guidelines (Figure 7.1).

Figure 7.1. Long-Term Data Points Compared to the Hardness-Adjusted WQG



***Notes**:*
All toxicity values from acceptable studies, including those not utilized in the SSD derivation, are plotted.
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.).

Effect concentrations with no reported hardness data are plotted on a y-axis for context.

7.2 DISCUSSION OF $CACL_2$ TOXICITY DATA POINTS

CCME (2011) developed the chloride guidelines, using tests with NaCl and $CaCl_2$. Following the CCME (2011) approach, NaCl and $CaCl_2$ toxicity data were evaluated and incorporated in the current guidelines, and among with NaCl, $CaCl_2$ toxicity data are included in assessment of WQG protectiveness.

The short-term SSDs curves (Section 3.1), literature reviews and the most recent multi-ion toxicity data (discussed in Section 9.1), suggests the relative toxicity of NaCl ≈ $CaCl_2$. It should be noted, that on the molar basis, $CaCl_2$ salts have a higher (1.6-fold) chloride concentration compared to NaCl, and therefore, the $CaCl_2$ toxicity is considered equal to NaCl, if a $CaCl_2$ EC_{10} or EC_{25} is a 1.6-fold for the same species and endpoint. If a NaCl to $CaCl_2$ ratio of the same endpoint is less than 1.6, the NaCl toxicity is considered to be relatively higher. If this ratio is greater than 1.6, the $CaCl_2$ toxicity is considered to be relatively higher. These considerations were taken into account when selecting the representative points for the final SSD data pool.

For photosynthetic organisms (green algae and plants), available NaCl toxicological endpoints were less or similar to the corresponding endpoints for the same species tested with $CaCl_2$. Diatoms *Navicula seminulum* have been tested by Academy of Natural Sciences (1960) with a single toxicological endpoint of LC_{50}, reported as 1,474 mg/L for NaCl, and 2,000 mg/L for $CaCl_2$ (<1.6-fold). These values were omitted from the final SSD data set, since the endpoint was greater then EC_{25}. Green algae *P. subcapitata*, tested by Simmons (2012) had an EC_{10} of 124 mg/L for NaCl vs 518 mg/L for $CaCl_2$ (population growth), and 96 mg/L for NaCl vs 177 mg/L for $CaCl_2$ (fluorescence), which implies relatively higher NaCl toxicity (<1.6-fold). Plants (*L. minor*) were assessed by Simmons (2012) with EC_{10} of 496 mg/L for NaCl and 482 mg/L for $CaCl_2$ (growth), which also implies relatively higher NaCl toxicity (<1.6-fold). Thus, the lowest values, selected for the SSD data pool, were based on the NaCl endpoints of 96 mg/L (*P. subcapitata*) and 496 mg/kg (*L. minor*).

For *D. magna*, research showed an opposite effect, with a relatively higher $CaCl_2$ toxicity. Biesinger and Christensen (1972) reported an IC_{16} of 1,049 mg/L for NaCl vs 205 mg/L for $CaCL_2$ (reproduction), with a ratio greater than 1.6. To address potentially higher $CaCl_2$ toxicity for *D. magna*, the lowest number, 205 mg/L of $CaCl_2$, was included in the SSD data pool (Table 4.2).

7.3 RECENTLY PUBLISHED CANADIAN STUDIES

7.3.1 Very Soft Water Zooplankton

Several recently published studies (Arnott et al., 2020; Greco *et al.*, 2021; Isanta-Navarro *et al.*, 2021; Valleau *et al.*, 2020), pointed out an importance of an adequate protection of zooplankton in very soft waters. Arnott *et al.* (2020) and Isanta-Navarro *et al.* (2021) have completed toxicity testing in the laboratory medium. Greco et al. (2021) performed an experiment using the microcosm at Long Lake, Ontario. Valleau *et al.* (2021) conducted a paleolimnology studies of five of the saltiest lakes (38.2-90.9 mg Cl/L) in the Muskoka River Watershed, Ontario.

Arnott *et al.* (2020) investigated multiple daphnid varieties from water bodies with underlying Precambrian Shield. The chronic (long-term) studies were conducted in laboratory FLAMES medium, which reflects the chemistry of soft water Canadian shield lakes (Arnott *et al*., 2020). The purpose of the study was to examine the effects of road salts on natural water environments with very low hardness levels. It was noticed, that the chloride impacts daphnids at concentrations below current water quality guidelines. The interpreted toxicity results from Arnott *et al.* 2020 are discussed in detail in Section 7.3.2.

Greco *et al.* (2021) exposed the freshwater zooplankton communities to thirty chloride concentration increments for six weeks, crossed with either ambient or high nutrient treatments. Additionally, researchers investigated the effects of the CWQG concentrations on soft water zooplankton. Greco et. al (2021) have detected a 69% decrease in total zooplankton biomass and a 62% decrease in cladoceran abundance at 120 mg Cl/L. The microcosm experiment was performed at Long Lake, Ontario, in 2018 (Greco *et al*., 2021).

Isanta-Navarro *et al.* (2021) tested *Daphnia pulicaria*, originally isolated from Red Chalk Lake, Ontario, for acute (short-term) chloride toxicity. Daphnia was reared in laboratory FLAMES medium, to address the major and trace element chemistry of soft-water Canadian Shield lakes (Isanta-Navarro *et al.*, 2021). Researchers studied the impact of essential dietary lipids on NaCl tolerance with six different concentrations, similar to the range of concentrations found in the Muskoka area, where the study was conducted. Authors found a strong positive linear relationship between food quantity and 14-day chloride LC_{50}, implying that daphnids in oligotrophic lakes might be more vulnerable to the salts. The 14-day chloride values were ranging from 49.2 to 138.1 mg Cl/L, with most individuals surviving the current CWQG threshold of 120 mg Cl/L on a supplementary cholesterol diet.

Valleau *et al.* (2021) examined Cladocera subfossils in dated sediment cores from six lakes within the Muskoka River watershed, Ontario. This paleolimnogical study investigated long-term effect of Cl runoff on Cladocera in soft waters, and found distinct community-level changes concurrent with road-salt application (Valleau *et al*., 2021). The zooplankton community changes were observed in the lakes with Cl concentrations from 32.8 mg/L to 90.9 mg/L. In two of these lakes, the changes were distinct and associated with road salt (Valleau *et al*., 2021).

The studies mentioned above demonstrated the need for a better evaluation of the Cl toxicity in soft waters, and importance of implementing the hardness-tailored guidelines, for an adequate protection of the soft-water communities. Such importance was also admitted by daphnid toxicity studies in Minnesota, US. Wersebe *et al.* (2021) investigated the long-term salinization effect on the hard water daphnids, with consistent increase in *Daphnia pulicaria* body size, which is opposite to the trend observed in Canadian soft waters.

7.3.2 Long-Term Toxicity Values Estimation (Arnott *et al*., 2020)

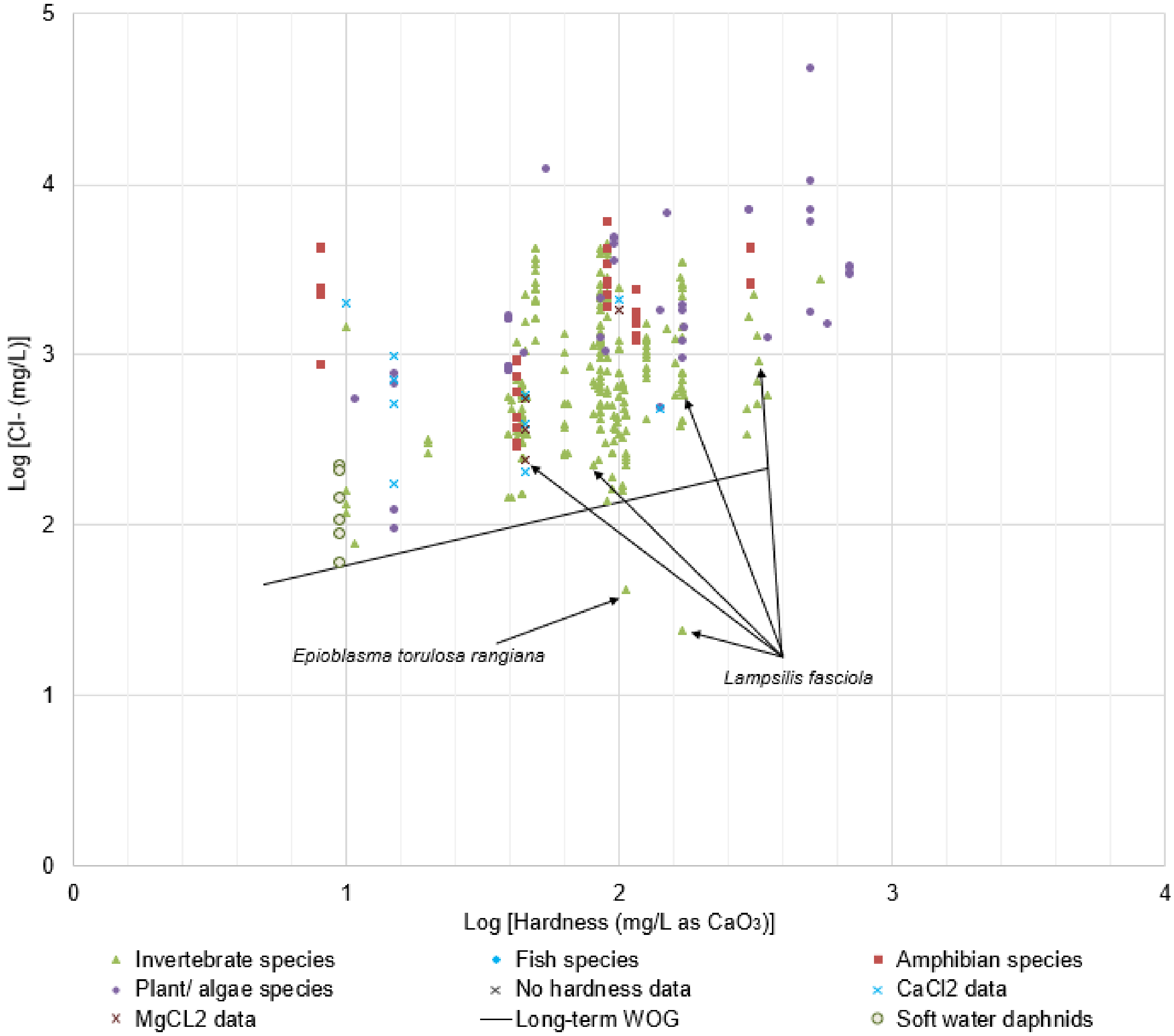
Arnott et al. (2020) conducted the 21-day laboratory life-history experiment on six species commonly found in soft water Canadian Shield Lakes. Five out of six species, *Daphnia catawba*, *Daphnia* *mendotae*, *Daphnia minnehaha*, *Daphnia pulicaria*, and the hybrid *D. pulicaria*D. pulex* were originally collected from soft water lakes in Muskoka, Ontario, and cultivated in laboratory for two years prior to experiments (Arnott *et al.* 2020). The sixth species, *D. pulex*, was originally collected from a pond in Oregon, USA, and cultivated in laboratory for more than a decade (Arnott *et al.,* 2020).

A soft water medium, mimicking the chemical characteristics of two lakes in Muskoka, was used for the long-term cultivation and experiment. The measured hardness was equal to 9.41 mg $CaCO_3$/L, reflecting the low Ca concentrations in Shield lakes, and control group survival was reported as greater than 95% (Arnott *et al.,* 2020). Seven chloride concentrations (NaCl), ranging from 0.39 mg/L (control group) to 145.5 mg/L, were used. Biological endpoints included the number of neonates, days before reproduction begins, clutch size, and an average mortality rate (Arnott *et al.,* 2020).

To compare results from Arnott *et al.* (2020) studies to the hardness-adjusted guidelines, EEI examined the supporting information. Since experimental results shown a high variability in control group for all reproduction endpoints, and an absence of testing of significance between different dose levels, an average mortality rate was chosen to estimate the EC_{10}-EC_{20} range. Dose-response relationship was estimated from supplementary charts, and corresponding toxicological endpoints were calculated with the newest BMDS version (V3.2) software, currently used by US EPA for the dose-response modeling.

The calculated EC_{10} value was the lowest for *D. minnehaha* (60 mg/L), followed by *D. catawba* (89 mg/L), and *D. mendotae* (107 mg/L). *D. pulicaria*, hybrid *D. pulicaria*D. pulex*, and *D. pulex* appeared to be less sensitive to chloride with an EC_{10} value of 210 mg/L, 226 mg/L, and greater than 145 mg/L, respectively. Calculated EC_{10} values were plotted against hardness-adjusted chloride guidelines, extrapolated to the lower bound of the hardness range, or very soft water (Figure 7.2). The hardness-adjusted chloride guideline, corresponding to a very soft water hardness level of 9.41 mg $CaCO_3$/L, was equal to 56 mg Cl/L, which is lower than the estimated EC_{10} toxicity endpoint for the most sensitive of the soft water daphnid species tested by Arnott *et al.* (2020) (*D. minnehaha*). In other words, the methods presented herein for hardness adjustment according to the equations and pooled slope for amelioration of chloride toxicity, supports the empirical work by Arnott *et al.* (2020) and is protective of those species in a soft water environment from an unacceptable risk of adverse effect. The Arnott *et al.* (2020) dataset provided the ability to test the methods herein for sensitive species in very soft waters.

Figure 7.2. Soft Water Daphnids Data Compared to the Hardness-Adjusted WQG



Notes:
Soft water daphnids data are estimated from Arnott et al. (2020) supporting information using BMDS version 3.2.

7.3.3 Hardness Slope and Toxicity Data Extrapolation

Although reproduction EC_{10} endpoints from Arnott *et al.* (2020) were not calculated due to the limited raw data and higher dose-response variability, an EC_{10} based on mortality rate may be compared with available published data. One out of six soft water daphnids, *D. pulex,* assessed by Arnott *et al.* (2020) with a hardness of 9.41 mg/L, was also studied by Birge *et al.* (1985) with a hardness of 96.9 mg/L.

To compare results from both studies, an estimated EC_{10} from Arnott *et al*. (2020) was extrapolated to a hardness reported by Birge *et al*. (1985), using a modified Eq. 2.2 for the hardness adjustment:

$$\mathbf{LT}\boldsymbol{EC_{x(96.9\ mg\ CaCO_3/L)}} = \mathbf{exp}^{\{ ([\ln(96.9) - \ln(9.41)] \times 0.38) + \ln(145) \}} \qquad \textbf{\textit{(Eq. 5.2)}}$$

where:

LT$EC_{x(96.9\ mg\ CaCO3/L)}$: a given long-term effect concentration extrapolated to 96.9 mg $CaCO_3$/L

96.9 $_{(mg\ CaCO3/L)}$:	a hardness reported by Birge *et al*. (1985)
9.41 $_{(mg\ CaCO3/L)}$:	a hardness reported by Arnott *et al*. (2020)
0.38	the pooled slope derived from long-term data (Section 6.1.1)
145 $_{(Cl\ mg/L)}$	an estimated EC_{10} of > 145 mg/L from Arnott *et al.* (2020) supporting data charts

Using the values, shown above, an EC_{10} of >351.7 mg/L (an average mortality rate) was calculated. Birge *et al.* (1985) reported an EC_{10} of 368 mg/L (reproduction endpoint), which is close to an extrapolated value of >351.7 mg/L. Similar extrapolation may be used for potential data evaluation working with intra-species variability due to the species origin (i.e., daphnids from soft waters in Ontario vs daphnids from hard waters in Alberta).

8 DISCUSSION

NaCl was one out of the four major chloride salts (NaCl, $CaCl_2$, KCl, $MgCl_2$), which data satisfied the long-term toxicological and statistical requirements for Type A guideline derivation (SSD method; CCME (2007). Following the rational of the CCME (2007), the current guidelines development was based on NaCl and $CaCl_2$ toxicity data, without addressing the KCL and $MgCl_2$ salts. Comparison of the short-term SSDs (Section 3.1) was consistent with the literature reviews and the most recent data (discussed in Section 9.1), suggesting the relative toxicity order of KCl > $MgCl_2$ > NaCl ≈ $CaCl_2$.

The long-term (NaCl) data met the requirements for Type A guideline derivation with 35 species-specific data points. The logistic model provided the best fit to the data, and the derived long-term WQG was 125 mg/L, which is 4% higher than the value established by the CCME (2011). Based on toxicity-hardness relationships from seven species, a pooled slope of 0.38 was calculated and used to adjust the long-term SSD to a hardness of 50 mg $CaCO_3$/L, producing a long-term WQG of 106 mg/L applicable to a hardness of 50 mg/L. The pooled slope statistical value was used to derive an equation to calculate a long-term water quality guideline:

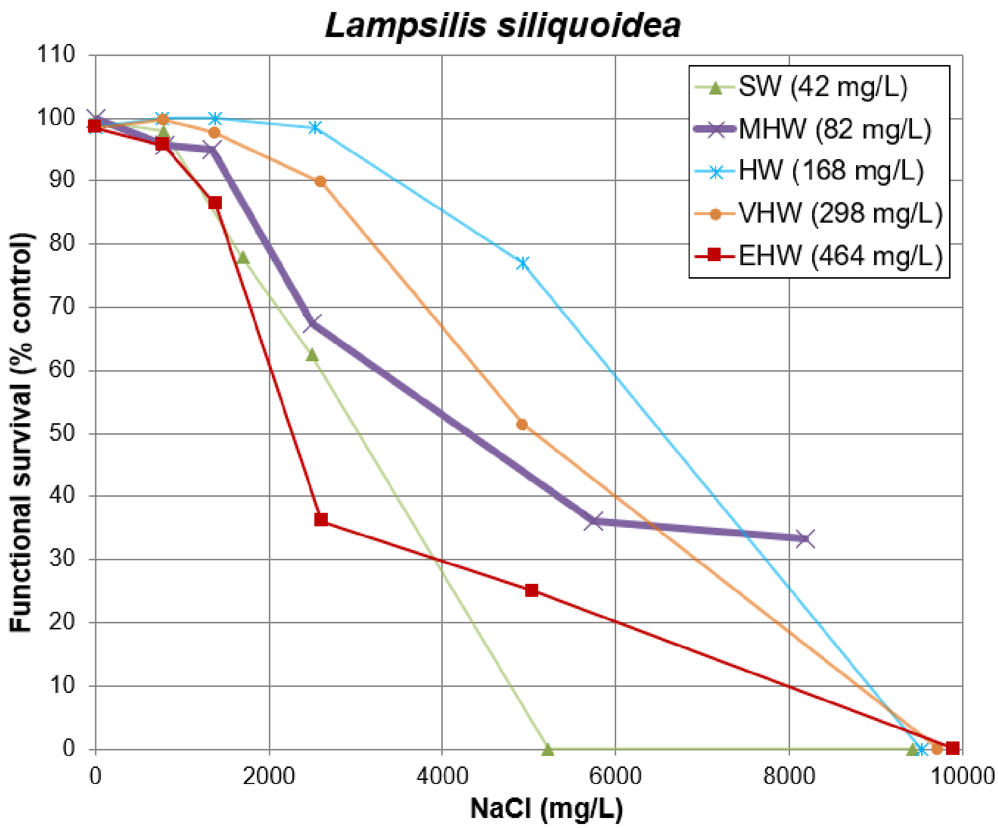
$$\textit{Long-term WQG} = \exp^{[0.38\,(\ln(\text{hardness})) + 3.18]} \qquad \textit{(Eq. 6.1)}$$

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

Note that the long-term hardness equation is used to calculate guidelines between 5 and 350 mg $CaCO_3$/L. For hardness levels < 5 mg $CaCO_3$/L, the lower limit of 44 mg/L is applied. For hardness >350 mg $CaCO_3$/L, the upper limit of 222 mg/L is applied.

The lowest value comes from the reasonable extremes of water hardness values for Canadian surface waters (CCME, 2011). The upper limit of 350 mg/L addresses potential toxicological interference in extremely hard waters. Such interference was observed in UGARF (2016a) studies for *L. siliquoidea,* with an EC_{10} of 730, 681, 2,877, 1,162, and 565 mg/L at 42, 82, 168, 298, and 464 mg/L hardness, suggesting that ameliorating effect of hardness may drop with increasing salt concentrations (Figure 8.1).

Figure 8.1. Dose-Response Curve at Various Hardness Level



***Notes:** hardness measured as mg/L as $CaCO_3$; SW -soft water, MHW -medium hard water; HW-hard water, VHW – very hard water, EHW – extremely hard water*

In the CCME (2011) guideline derivation process for chloride, reasonable limits of water hardness values in Canada are presented as 5 to 240 mg $CaCO_3$/L. These values were based on a national survey of Canadian surface waters, with 41 locations chosen as representative of Canadian waters. The survey was conducted over the period 1975 to 1977, with an average hardness level from 7 to 180 mg $CaCO_3$/L in British Columbia; 5 to 179 mg $CaCO_3$/L in Northwest Territories; 98 to 329 mg $CaCO_3$/L in Alberta; 12 to 132 mg $CaCO_3$/L in Saskatchewan; and 15 to 716 mg $CaCO_3$/L in Manitoba.

Table 8.2 is adapted from the data presented in CCME (2011). An average hardness in Alberta surface waters was reported as 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 are consistent 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 8.1) suggests that chloride guidelines could be expected to range within approximately 131 – 188 mg Cl/L for major surface waters in Alberta.

For contaminated site work in Alberta and some other provinces of Canada, water quality guidelines are applied at the hyporheic zone where groundwater interfaces with surface water. Often in these situations, water hardness will be greater than measurements in surface water. Table 8.3 (a,b) summarizes the surface water hardness by major Canadian cities, reported by tertiary sources, Aquatell (2021) and CWQA (2013). These sources included drinking water, and therefore, reflect both surface water and groundwater hardness, which may be higher than those reported by CCME (2011). The values in Table 8.2 show a similar hardness pattern as in Table 8.1, with relatively softer waters in Atlantic provinces and in British Columbia, and relatively harder waters in Alberta, Manitoba, and Saskatchewan.

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 (Mitchell 1990). In Maritime provinces, 90th percentile falls below 5 mg $CaCO_3$/L. For extremely soft waters (<5 mg/L), the lowest limit of 45 mg/L of the hardness-adjusted guideline should be used.

Alternatively, water hardness in sloughs and other small water bodies can reach concentration much higher than the 90th percentile shown in Table 8.2. The upper 10th percentile in ten out of twelve provinces and territories had a hardness level exceeding the 350 mg/L threshold of extremely hard water, where current guidelines may be extrapolated. For these values, the maximum of 216 mg/L chloride guidelines, calculated for 350 mg/L of hardness, should be used, assuming the plateau effect rather than ameliorating hardness effect in extremely hard waters.

Table 8.1. 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]	1,920[1] 1,920[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:
Adapted from CCME (2011); NA = data were not available.
Referred in CCME (2011):
[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.

Table 8.2. Water Hardness Level by Canadian City
a) Reported by Aquatell

Province	Hardness (mg $CaCO_3$/L)					
	Min	10th percentile	50th percentile	90th percentile	Max	n
Newfoundland and Labrador	23	23.7	26.5	29.65	30	3
Nova Scotia	1.10	10.5	28	178.9	250	20
New Brunswick[4]	10	35.4	51	137.8	172	15
P.E.I.	134.4	145.8	191.6	243	248.7	3
Quebec[4]	6	19.5	61	215.75	365	67
Ontario	10	78.4	166.8	479.1	975	220
Manitoba	81	136.2	229	558	650	10
Saskatchewan	7	37.3	262.5	657.9	800	29
Alberta	8	165	181	259.9	325	49
British Columbia	4.37	7.46	60.15	251.6	340	36
Yukon	NA	NA	NA	NA	NA	NA
Northwest Territories	NA	NA	NA	NA	NA	NA
Nunavut	NA	NA	NA	NA	NA	NA

Notes:
Adapted from Aquatell (2021); NA = data were not available
n – number of Cities (datapoints), included in statistics
Multiple values per City were pre-averaged to the single datapoint, when applicable
Note that Newfoundland and Labrador, and P.E.I data might be not representative due to relatively low n.

Table 8.2. Water Hardness Level by Canadian City
b) Reported by Canadian Water Quality Association

Province	Hardness (mg $CaCO_3$/L)					
	Min	10th percentile	50th percentile	90th percentile	Max	n
Newfoundland and Labrador	6.8	6.8	6.8	6.8	6.8	1
Nova Scotia	13.7	22.2	30.8	118.7	133.4	19
New Brunswick[4]	34.2	42.8	56.4	108.8	171	13
P.E.I.	116.3	118.8	129.1	139.4	141.9	2
Quebec[4]	17.1	24.8	62.4	261.6	513	66
Ontario	17.1	51.3	171	422.4	2,052	136
Manitoba	256.5	276.2	350.6	487.4	547.2	6
Saskatchewan	150.5	237.7	434.3	829.7	1,239.8	25
Alberta	17.1	97.5	200.1	292.4	530.1	25
British Columbia	4.37	7.46	60.15	251.6	340	36
Yukon	NA	NA	NA	NA	NA	NA
Northwest Territories	NA	NA	NA	NA	NA	NA
Nunavut	NA	NA	NA	NA	NA	NA

Notes:
Adapted from Danamark Water Care Ltd (2013); NA = data were not available
n – number of Cities (datapoints), included in statistics
Multiple values per City were pre-averaged to the single datapoint, when applicable
Note that Newfoundland and Labrador, and P.E.I data might be not representative due to relatively low n.

The unadjusted long-term SSD was compared to the CCME (2011). The resulting WQG of 125 mg Cl/L is essentially similar to the CCME derived guideline of 120 mg/L (4% difference). In both derivations (CCME and herein), the Logistic model was found to best fit the data.

Three important deviations from the CCME (2011) dataset should be noted. The first is data for a freshwater mussel *L. fasciola* where the 24 h EC_{10} endpoint shifted from 24 mg/L (Bringolf *et al.,* 2007) to 188 mg Cl/L based on a geometric mean that incorporated a second datapoint produced for this species and toxicological endpoint from the same research lab ranging from 247 to 964 mg Cl/L (Bringolf *et al.,* 2007; UGARF, 2016). The second important deviation is the endpoint for a fingernail clam *M. secures* where the LOEC was shifted from 121 mg/L (natality) as used in CCME (2011) to an EC_{25} (natality) of 88 mg/L, calculated by EEI from the raw data presented in Mackie (1978). Preferably, toxicity endpoints used in SSD datasets are EC_{10} or EC_{25} values as opposed to LOECs. The third deviation is the addition of mayfly *C. triangulifer* data, a sensitive species with an EC_{20} for survival of 236 mg Cl/L, which previously was not included in the CCME (2011) dataset.

These data point represents a departure from the CCME derivation at the most sensitive lower end of the SSD curve. The four lowest values of 24, 42, 121, and 259 mg/L for *L. fasciola, E. torulosa rangiana, M. securis*, and *D. ambigua* were replaced by 42, 88, 187, and 236 mg/L from *E. torulosa rangiana, M. securis, L. fasciola,* and *C. triangulifer*, respectively, and *D. ambigua* was shifted to the 5th HPP of the SSD.

Two SSD data points for *C. dubia* and *L. minor* plotted lower in the curve herein compared to CCME (2011). The endpoint concentrations decreased from 454 to 340 mg Cl/L, and from 1,171 to 496 mg Cl/L, respectively, making the lower part of the SSD curve smoother with the addition of data from Lasier and Hardin (2010), Elphick *et al.* (2011a), Simmons (2012), Struewing *et al*. (2015), and WSLH (2016). This shifting affected the lower end of the SSD curve shape, making it more ‘rounded’ at the 5th percentile threshold point (Figure 4.1).

9 MULTI-ION TOXICITY EFFECT

The major Cl ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, and HCO_3^-/CO_3^{2-}) are present in all fresh waters, and their toxicity can vary depending on the concentrations of other ions (Mount *et al.,* 2016). One of the most comprehensive studies was conducted by Mount et al. (2016) for *C. dubia.*

9.1.1 Invertebrates

Na salts were significantly less toxic than salts of other cations with the same anion, and NaCl was less toxic than the other Na salts on a molarity rather than mass basis. K salts were found more toxic than the corresponding salts with other cations by approximately an order of magnitude, indicating that K is the principal source of toxicity for these salts (Mount *et al*., 2016). Mg salts were less toxic than K salts, but more toxic than corresponding Na salts, suggesting Mg salts toxicity was primarily driven by cations (Mount *et al.,* 2016). $CaCl_2$ was more toxic than NaCl on a total molarity basis, but it showed no clear evidence of relative ion toxicities, since $CaCl_2$ as twice as many Cl ions compared to NaCl (Mount *et al*., 2016). In general, the relative toxicity of the four major chloride salts is consistent with the same order discussed in Section 3:

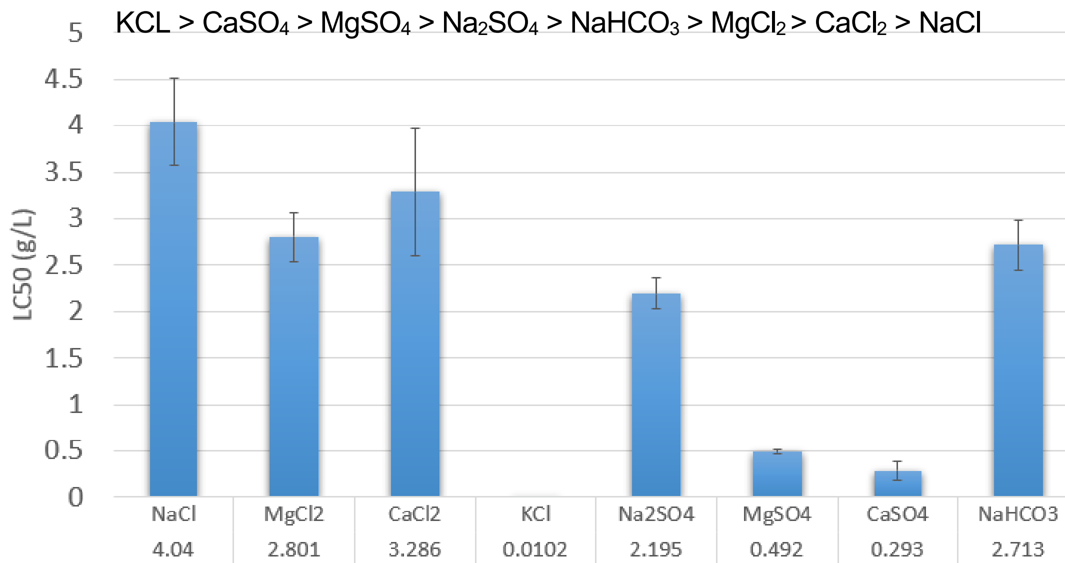
KCl >> $MgCl_2$ > $CaCl_2$ <=> NaCl

with toxicity values of 221, 546, 1,216, and 1,273 mg Cl^-/L, respectively for these ion pairs, in moderately hard reconstituted water (water hardness of 84.4 mg $CaCO_3$/L). The same order was observed for the amended Lake Superior water (water hardness of 53 mg $CaCO_3$/L), where LC_{50} of KCl, $MgCl_2$, NaCl, and $CaCl_2$ were equal to 167, 641, 1,158, and 1,216 mg Cl^-/L, respectively. These Cl concentrations suggest substantial difference between KCl, $MgCl_2$, and NaCl/$CaCl_2$ toxicities, but not between NaCl and $CaCl_2$ (Mount *et al*., 2016).

A similar order of salt ion pair toxicity was observed from the PTAC-funded research (UGARF, 2016b) with the freshwater mussel *L. siliquoidea*, involving 24 h glochidia functional survival/ viability test. Since glochidia are considered the most sensitive life stage of freshwater mussels, the study is an important addition to the comparative toxicity database. Mussels were exposed to seven salts (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, and HCO_3^-) to examine major ion toxicity effects in moderately hard water (water hardness of 100 mg $CaCO_3$ mg/L). The greatest effect was observed for KCl, whereas the lowest toxicity was shown for NaCl. Toxicity values on a whole salt basis are compared in Figure 9.1, and toxicity values on an anion basis are illustrated in Figure 9.1(b).

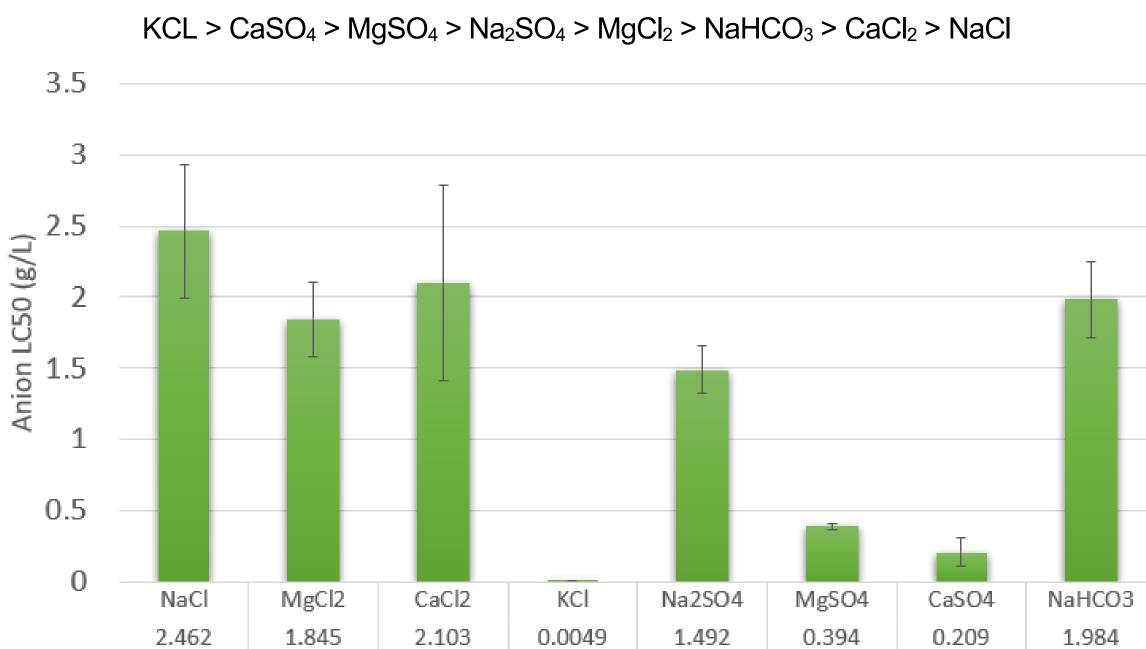
Figure 9.1. Comparative Toxicity for *L. siliquoidea* (UGARF, 2016b)

a) Whole Salt Basis



Notes: LC_{50} values (g/L) are shown on the x-axis under corresponding salt bar

b) Anion Basis



Notes: LC_{50} values (g/L) are shown on the x-axis under corresponding salt bar

Generally, the salt-based toxicity values and the anion-based toxicity values had a similar order, increasing from KCl to NaCl (KCl more toxic, NaCl less toxic), as shown in Figure 9.1(a) and (b). SO_4 salts were all less toxic than KCl. Among three sulphate salts, $CaSO_4$ was the most toxic, followed by $MgSO_4$ and Na_2SO_4. Next two places were taken by $MgCl_2$ and $NaHCO_3$, where $MgCl_2$ was slightly less toxic (LC_{50} of 2.801 g/L for $MgCl_2$ *versus* 2.713 g/L for $NaHCO_3$), and vice-versa on an anion-basis (LC_{50} of 1.845 g/L for $MgCl_2$ *versus* 1.984 g/L for $NaHCO_3$). The next salt ion pair with a lower toxic potency (higher LC_{50} value) was $CaCl_2$, with the least toxic salt ion pair being NaCl. Differences in toxic potency between $MgCl_2$, $CaCl_2$, and NaCl in Figures 9.1a and 9.1b were not statistically significant at a p value of 0.05.

A similar pattern of relative salt ion pair toxicity was observed by Bringolf et al. (2017) where three freshwater mussel species (*L. siliquoidea, L. fasciola*, and *Anodonta suborbiculata*) were tested for the major common ion pairs using NaCl, Na2SO4, $NaHCO_3$, $MgCl_2$, $MgSO_4$, $CaCl_2$, $CaSO_4$, and KCl, with moderately hard reconstituted water (MHRW). The 24 hr EC_{50} toxic potency was as follows:

KCL > $CaSO_4$ > $MgSO_4$ > Na_2SO_4 > $NaHCO_3$ > $MgCl_2$ > NaCl> $CaCl_2$

9.1.2 Algae

Algae are major primary producers in the aquatic food chain and setting a chloride WQG that is protective of these species is imperative for overall ecosystem protection. Available toxicological data suggests *P. subcapitata* was more sensitive to Cl compared to other species, which was a key rationale for the inclusion of this species in PTAC-funded research conducted by WSLH (2017).

WSLH (2017) completed a multi-salt test based on a multi-ion toxicity evaluation scope established by Equilibrium Environmental, in order to investigate relative Cl toxicity between several salt ion pairs including NaCl, Na_2SO_4, $NaHCO_3$, $MgCl_2$, $MgSO_4$, $CaCl_2$, $CaSO_4$, and KCl, completed in MHRW. Initially, algae were tested in a medium with a standard nutrient volume (0.1mL/100mL), and subsequently, in a medium composed of 10X phosphorus (P) and 10X selenium/molybdenum (Se/Mo) greater concentration.

For the initial standard nutrient test, salt toxicity (IC_{10}, fluorescence) ranged from 63 mg/L to 1,676 mg/L, in the following decreasing order of toxicity:

$CaSO_4$ > Na_2SO_4 > $MgCl_2$ > KCL > NaCl > $NaHCO_3$ > $MgSO_4$ > $CaCl_2$

The 10x nutrient had an effect on the lower end of the toxicity range where IC_{10} values ranged from 120 mg/L to 1, 406 mg/L, in addition to a change in order of relative toxicity between the salt ion pairs:

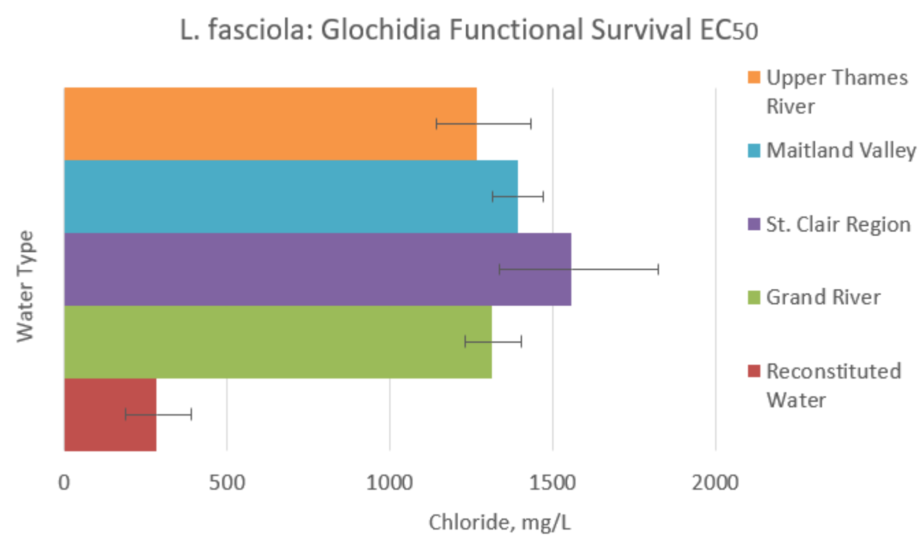
$CaSO_4$ > $CaCl_2$ > KCL > $MgSO_4$ > $NaHCO_3$ > $MgCl_2$ > Na_2SO_4 > NaCl

9.2 LABORATORY VERSUS FIELD DOSE-RESPONSE RELATIONSHIPS

Results from a number of studies suggest studies with reconstituted laboratory waters resulted in greater toxicological sensitivities compared to ‘wild’ in field water, for several species.

Gillis (2011) examined acute NaCl toxicity in freshwater mussel glochidia and compared laboratory chloride effect concentrations (EC_{50}) to those observed in the natural mussel’s water habitat. A series of tests were conducted with NaCl and moderately-hard reconstituted water (95-115 mg/L CaO_3/L), and with water collected from four significant mussel water body habitats in southern Ontario. Acute 24 h exposures in laboratory waters and NaCl-spiked natural waters with *L. fasciola* glochidia revealed a 4-fold difference in mussel sensitivity to chloride, with EC_{50} values of 1,265 to 1,559 mg/L for the natural waters, and 285 mg/L for the reconstituted water (Figure 9.2). These results may be explained in part by differences in water hardness as the natural waters were harder than reconstituted water (278 to 322 and 100 mg CaO_3/L, respectively). The 4-fold magnitude difference in effect could not be explained solely by hardness.

Figure 9.2. Chloride Toxicity Tests in Natural and Reconstituted Water (Gillis, 2011)



Values are adapted from Gillis (2011); whiskers represent the 95% confidence interval

Testing with another mussel species, *L. siliquoidea*, over variable hardness levels demonstrated a hardness effect within a range of 100-322 mg CaO_3/L, where EC_{50} values varied by less than 30% (ranging from 1,430 to 1,870 mg Cl^-/L; Gillis, 2011). A similar 30% difference in toxic response was observed for *L. fasciola* in natural waters. Work from the same lab completed by Prosser *et al.* (2017) involving natural water testing for *L. fasciola*, with 24 h LC_{50} of 903 mg Cl^-/L for the moderately hard water (100 mg CaO_3 mg/L) and 1,177 mg Cl-/L for very hard water (225 mg CaO_3 /L), determined an EC_{50} value in the natural waters with moderate hardness that was 3-fold greater than the EC_{50} for reconstituted lab water with the same hardness level, for the same species and life stage. Similarly, differences in toxic response to NaCl between reconstituted and natural waters could not be explained solely by hardness.

A similar effect has been observed with other cations. K^+ sub-lethal toxicity tests conducted by Nautilus (2015) for *C. dubia*, *D. magna*, and *P. promelas* (fathead minnow), using site water collected from the Ekati Diamond Mine in addition to laboratory water, demonstrated a 2-fold difference in toxic potency for *C. dubia* and *D. magna*, with the K^+ in site water demonstrating a lower toxic potency. For *C. dubia*, LC_{50} values were equal to 167 mg K/L for laboratory water, and 341 mg K/L for site water. For *D. magna*, LC_{50} values were 300 mg K/L and >628 mg K/L, respectively. The IC_{25} values demonstrated the same pattern. For *P. promelas*, toxicity values were similar for laboratory and site waters (or slightly lower for lab waters), with an LC_{50} of 401 and 369 mg K/L, and an IC_{25} of 250 and 226 mg K/L, respectively (Nautilus, 2015; ERM, 2015).

Johnson et al. (2015) investigated a brine salt (mixed NaCl and CaCl) effect on a growth and survivorship of mayfly (*C. triangulifer*) larvae. EEI examined the chemistry data table and dose-response chart, and calculated an EC_{20} value of 333 mg Cl-/L (growth endpoint) with BMDS version (V3.2) software. This endpoint is more than 2-fold higher than an EC_{25} biomass endpoint (139 mg Cl-/L) reported by Struewing (2015) for the same species in laboratory conditions.

Harless *et al*. (2011) investigated road salt toxicity towards amphibians with a series of 96-h acute toxicity tests using *R. sylvatica* tadpoles and filtered Portage Lake (Michigan, US) water, mixed with appropriate de-icers. The LC_{50} Cl concentrations of 4,586, 5,295, and 2,543 mg/L were observed for NaCl, $MgCl_2$, and $CaCl_2$, respectively. The LC_{50} Cl concentration of 4,586 mg/L is approximately 2-fold greater than those obtained by Collins and Russel (2008), and Sanzo and Hechar (2006) for the same species in laboratory waters. Collins and Russel (2008) observed a 96 h LC_{50} of 1,721 mg Cl/L, whereas Sanzo and Hechar (2006) estimated the value of 2,632 mg/L (an average between 1,608 and 3,117 mg Cl/L reported as calculated by Spearman-Karber and Probit methods, respectively).

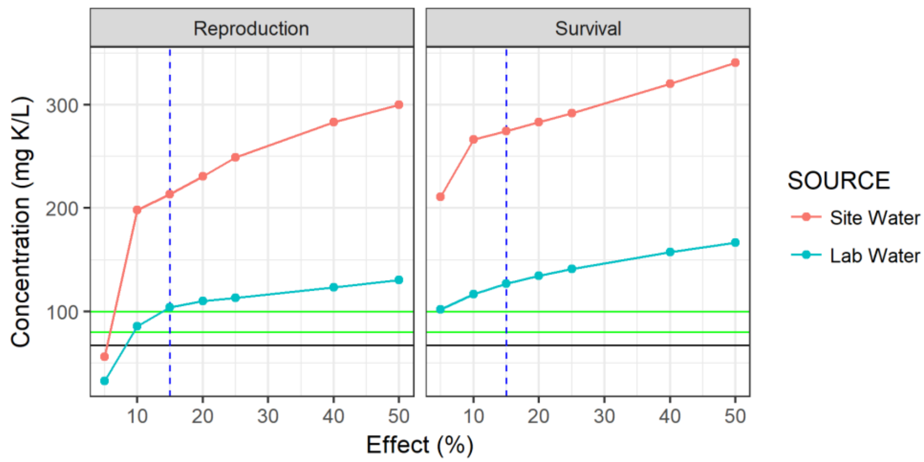
LGL (2018) tested water fleas (C. dubia and D. magna) and fish (P. promelas) using laboratory water and site water. Toxicity tests were completed for Ekati diamond mine (LGL, 2018). An approximately 2-fold higher K^+ toxicity for the water fleas was observed in the lab water. For the fish, K^+ toxicity in lab water was visible higher. Dose-response curves from LGL (2018) are shown in Figure 9.3.

Wang *et al.* (2007) used *L. siliquoidea* brooding mussels, collected at the same site, to test for the inter- and intra-laboratory variabilities. Variabilities were considered low, implying that difference in toxicity may be attributed to the particular batch/ strain rather than to the test methods and procedures.

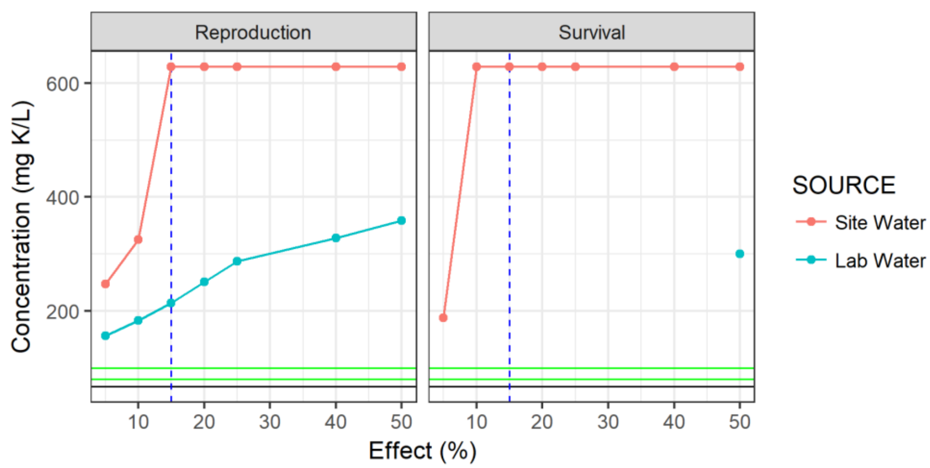
Although laboratory reconstituted water had an advantage of the consistency and comparison between the studies and species, it may not accurately predict the toxicity in natural environment (Gillis, 2011). Based on these studies, an approximately 2-fold greater sensitivity to laboratory waters may be expected for daphnids, mayflies, freshwater mussels, and frogs. Since current WQGs were derived based on laboratory data, the greater sensitivity may serve as an additional safety factor, and make the guidelines more conservative.

Figure 9.3. Potassium Toxicity Tests in Site and Lab Water (LGL, 2018)

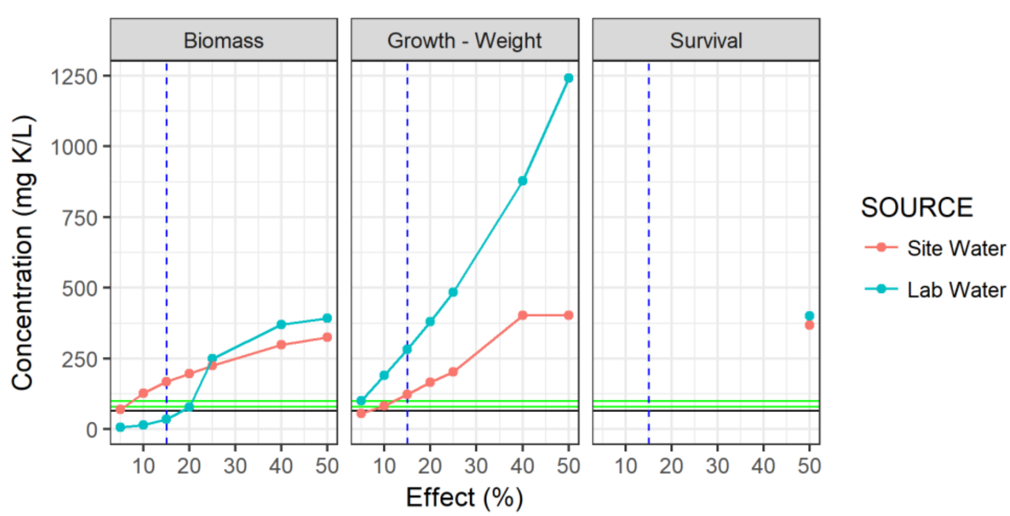
a) *C. dub*ia, 7-day Toxicity Test



b) *D. magna,* 21-day Toxicity Test



c) *P. promelas,* 7-day toxicity test



10 CONCLUSIONS

Data for NaCl and $CaCl_2$ was used to derive the WQGs herein and the available data satisfied the long-term toxicological and statistical requirements for a Type A guideline derivation (SSD) method as per CCME (2007). Comparison of the available long-term data with the literature reviews and CCME (2011) suggested that KCl is substantially more toxic than NaCl and $CaCl_2$, and $MgCl_2$ is more toxic when NaCl and $CaCl_2$, whereas NaCl and $CaCl_2$ toxicities are more similar. Thus, KCl and $MgCl_2$ salts were not assessed in the current work, as per the rationale and methods used by the CCME (2011) for deriving a Cl WQG for aquatic life.

The long-term (NaCl) data met the requirements Type A guideline derivation method with 35 species-specific data points. The logistic model provided the best fit to the data, and the derived long-term WQG (not normalized to a particular hardness) was **125 mg/L**, which is 5 mg/L higher (4% relative percent difference) than the 120 mg/L value established by the CCME (2011).

Based on Cl (as NaCl and $CaCl_2$) toxicity-hardness relationships, a pooled slope of **0.38** was calculated and used to adjust the long-term SSD to a hardness of 50 mg $CaCO_3$/L, producing a long-term WQG of **106 mg/L**. The pooled slope statistical value was used to derive an equation to calculate a long-term water quality guideline:

$$\textit{Long-term WQG} = \exp^{[0.38\,(\ln(\text{hardness})) + 3.18]} \qquad \textit{(Eq. 6.1)}$$

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

Guidelines for the Protection of Fresh Water Aquatic Life at Various Hardness Values

Water hardness (mg/L as $CaCO_3$)	Long-term exposure (mg Cl^-/L)
Lower limit (0-5)*	*44*
Soft (50)	*106*
Moderately hard (150)	*161*
Hard (300)	*209*
Upper limit (350 and greater)**	*222*

Notes: *the long-term hardness equation can be used for direct calculation from 5 to 350 mg $CaCO_3$ /L.*
** 44 mg Cl-/L is the lower limit long-term WQG value that applies to waters with 5 mg $CaCO_3$/L and less.*
*** 222 mg Cl-/L is the upper limit long-term WQG value that applies to waters of 350 mg $CaCO_3$/L and greater.*

To ensure the resulting chloride guideline provides an adequate protection for $MgCl_2$ and $CaCl_2$ toxicity, given the toxicological dataset was primarily NaCl based, an analysis of protectiveness was conducted. Acceptable toxicity values, non-adjusted for hardness, were plotted against the hardness-adjusted guideline. No $MgCl_2$ or $CaCl_2$ toxicity values fell below the guidelines, indicating the hardness-adjusted WQG derived from the NaCl toxicological dataset was also protective of aquatic life exposures to other chloride salts such as $MgCl_2$ and $CaCl_2$.

Comparative toxicity from the major salt ions, present in all fresh waters, such as Na+, K+, Ca^2, Mg^2, Cl-, SO_4^{2}-, and HCO_3^-, was evaluated additionally to the current guideline development. PTAC-funded studies by UGARF (2016b) and WSLH (2017) compared the long-term toxicity of the major salts to the freshwater mussel *L. siliquoidea*, and to the microalgae *R. subcapitata*. Generally, for the freshwater mussels the salt-based toxicity values and the anion-based toxicity values had the similar order as observed in the literature review and as mentioned in CCME (2011), decreasing from KCl (the most toxic salt) to NaCl/$CaCl_2$ (the least toxic salt). For the microalgae, $CaSO_4$ was observed as the most toxic salt, whereas the least toxic effect was observed in $CaCl_2$ or NaCl.

11 ADDITIONAL CONSIDERATIONS

The following section discusses the two additional considerations coming from the literature review and the most recent database. The first one refers to the intra-species variability for the strains collected from the different water bodies, with particular attention to the soft water and hard water background, and the second one comes from the limited data for endangered mussel species that drives SSD.

11.1 INTRA-SPECIES VARIABILITY

The freshwater mussels are known to be particularly sensitive to some waterborne contaminants (Gillis 2011). Not surprisingly, they are associated with the lowest EC values (Table 7.1-7.2) in the current database. Since the freshwater mussel studies involve the test organisms obtained from the brooding mussels collected in a wild environment, the potential interferences from the intra-specific variations should be considered.

For *L. fasciola* (glochidia stage), the 24 h EC_{10} was equal to 24 mg Cl/L (Bringolf *et al*., 2007), and around 10-fold greater (212-964 mg Cl/L) values in more recent studies completed by the same researcher in the same lab (UGARF, 2016). Personal communication between EEI and Dr. Bringolf indicated that the brooding time and the natural water system where the brooding mussels were collected, may have a significant influence on the sensitivity of this particular batch.

Another intra-specific variation in Cl sensitivity was observed by Gillis (2011) during glochidia acute toxicity test. The brooding mussels *L. fasciola* were collected from the same site (Grand River, ON) in different years, and their glochidia produced a 2-fold difference in EC_{50} values (113 mg/L from a 2008 brood and 285 mg Cl/L from a 2009 brood). For the same years, *L. siliquoidea* glochidia obtained from the mussels collected from two separate water bodies (Maitland River in 2009 and Cox Creek in 2008), produced EC_{50}s that varied by 8-fold (1,430 mg/L vs 168 mg/L). Gillis (2011) suggested that this kind of variability may be attributed to the health characteristics of the particular batch, or even to acquired tolerance to contaminants. It means that mussels from different water bodies may respond differently to Cl, and potential difference in contaminant sensitivity across watersheds should be taken into account when collecting brooding organisms (Gillis, 2011).

The intra-species differences appear to be a greater determinant of toxicological response variability when contrasted against differences between labs. Wang *et al*. (2007) investigated potential inter-laboratory and intra-laboratory variabilities, based on the *L. siliquoidea* brooding mussels, collected from the same site (Silver Fork of Perche Creek in Boone County, MO, USA). The brooding mussels were collected in March-April 2003, and testing organisms (glochidia) were obtained in laboratory conditions, eliminating possible effect of run-off or other timing-habitat interactions. Testing organisms were distributed to the different labs for the inter-laboratory comparison, and several tests were repeated in the same lab, for the intra-laboratory studies. In both cases, variations were considered low (Wang *et al*., 2007). This implies that toxicity endpoints variabilities, observed in the current dataset for the species, originated from the different natural waters, may be attributed to the particular batch/ strain rather than to the test methods and procedures.

Besides the sensitive freshwater mussels, the intra-species interference may come into play with more robust species, collected from the different waterbodies. Collins and Russell (2009) tested amphibians, and concluded that exposure to road salt may exclude salt-sensitive species from high Cl environments. Chloride analysis in the study ponds indicated that concentrations differ between individual ponds and between sampling periods (Collins and Russel, 2009). Therefore, organisms from the different ponds may exhibit different salt tolerance, potentially due to acclimatization.

The current SSD dataset includes the most sensitive endpoints and/or geometric means from the different studies for the same species and is based on a conservative approach. The intra-species variabilities were not assessed separately due to the lack of long-term studies coming from the different labs, which may lead to limitations. Future studies, comparing strain sensitivities (considering timing and the natural water habitat) may assist with estimating laboratory versus natural water safety factors and contribute to the better WQG precision, especially, when it comes to endangered/vulnerable species.

Potential difference in sensitivity may occur between species collected from the soft and hard waters. Toxicological sensitivity for organisms collected in environments with negligible or low salinity may be greater than for organisms originated from higher salinity environment. Guidelines based on toxicology endpoints obtained from the species, originated in the soft waters, might be overprotective for the same species inhabiting natural environments where background salinity is relatively high. At the same time, guidelines, based on toxicity values generated from species originating from natural waters with greater hardness will not be protective of soft water communities.

An example of this is the detailed work of Arnott *et al.* (2020) with daphnids involving very soft water. However, utilizing the hardness adjusting equation provided herein, these data are in alignment with other daphnid datasets generated by labs using higher hardness levels in the experimental water.

11.2 ENDANGERED SENSITIVE SPECIES RESEARCH RECOMMENDATIONS

Based on the updated toxicological database and the species sensitivity HPP, future research areas include endangered/ special concern species that may drive the SSD curve and subsequent WQG. Particularly, more data are needed for the COSEWIC endangered mussel *E. torulosa rangiana*, which occupies the lowest HPP rank on the current SSD, derived from a single study involving a single hardness level.

12 APPLICATIONS

The long-term hardness equation derived herein for adjusting chloride toxicity towards aquatic life species is applicable between hardness levels of 5 and 350 mg $CaCO_3$/L. The lowest hardness value comes from the minimum hardness specified in the CCME (2011) reasonable extremes of water hardness in Canada, whereas the upper limit is constrained by increased species sensitivity in extremely hard waters. Outside of the 5 to 350 $CaCO_3$/L range, the lower and upper calculated chloride guidelines of 44 mg/L and 222 mg/L should be applied.

Where appropriate, the Protection Clause (CCME, 2007) can be applied in areas where the COSEWIC special concern mussel (L. fasciola) or the COSEWIC endangered mussel (E. torulosa rangiana) have been identified. The increased sensitivity of these species in these waters may be due to a number of factors including sensitivity for broods evolved in waters of low background salinity and hardness.

13 CLOSURE

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EQUILIBRIUM ENVIRONMENTAL INC.

Equilibrium Environmental Inc.

Vicky Winter, M.Env.Tox., P.Biol.
Environmental Scientist/ Toxicologist
Data analyses, literature review, guideline calculations, writing

Anthony L. Knafla, M.Sc., DABT, P.Biol.
Founder/ Senior Scientist/ Toxicologist
Problem formulation, study design and scoping, guideline calculations, senior technical consultations

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