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Hazard/Risk Assessment

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

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

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INTRODUCTION

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

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

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

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

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

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

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

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

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

METHODS

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

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

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

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

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

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

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

[a] Formerly *Chironomus tentans*.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

RESULTS AND DISCUSSION

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

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

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

Table 3. Results of sublethal toxicity tests[a]

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

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

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

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

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

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

[a] LC50 = median lethal concentration; IC25 = 25% inhibition concentration; NC = not calculated by the U.S. EPA [9].
[b] Mean (±2 SD) for 20 tests conducted as reference toxicant tests.
[c] The chronic test for *Hyalella* was less sensitive than the acute test and, consequently, for calculation of the acute-to-chronic ratio, the chronic test value was assumed to be the LC25 (25% lethal concentration) from the acute test. The actual IC25 for biomass of *Hyalella* was 1,705 mg/L.

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

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

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

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

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

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

[a] IC = inhibition concentration values (IC*x*) associated with 10 and 25%; LC10 = 10% lethal concentration; MATC = maximum acceptable toxicant concentration; EC50 = median effect concentration.
[b] Point estimates were calculated using linear interpolation based on original data provided in Birge et al. [26].
[c] Point estimates were calculated using multiple linear estimation (Probit) based on original data provided in Birge et al. [26].

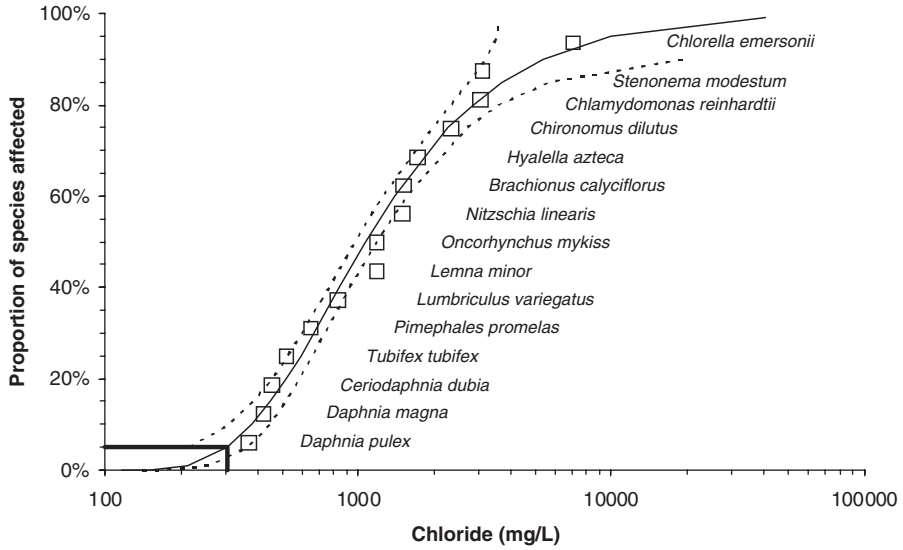

Fig. 1. Species sensitivity distribution for chronic toxicity test data using chloride. The squares represent the empirical data, the solid line represents the modeled distribution, and the dashed lines represent the 95% confidence limits.

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

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

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

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

[a] IC = inhibition concentration values (IC*x*) associated with 25 and 50%; CL = confidence limits; LC50 = median lethal concentration.
[b] Mean and 2 SD range of 20 data points for chronic toxicity tests using chloride.

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

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

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

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

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

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

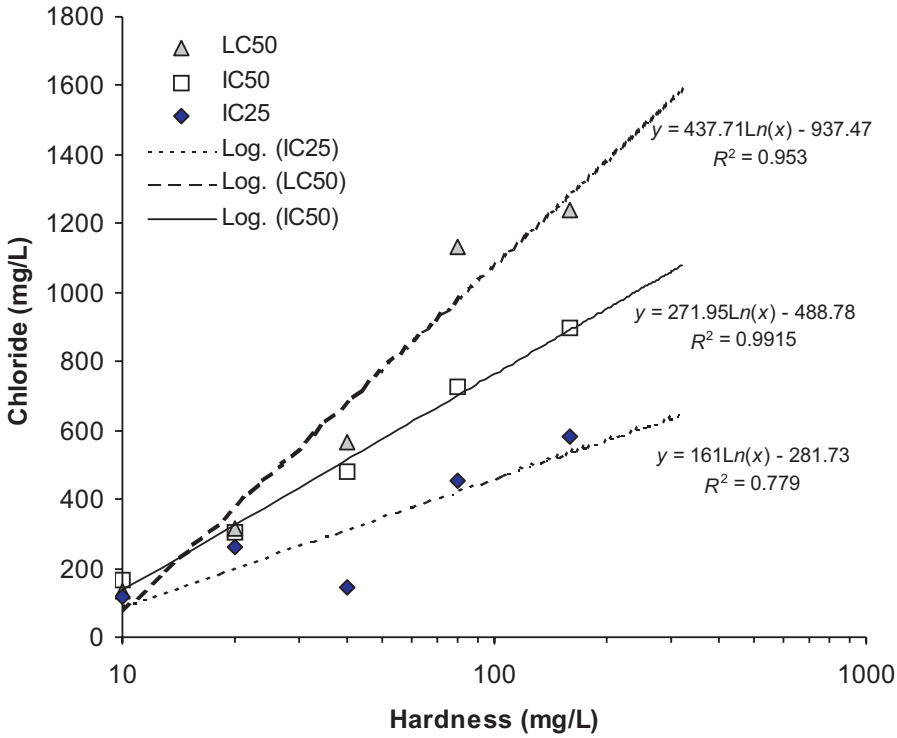

Fig. 2. Relationship between hardness and sensitivity to chloride for reproduction (IC25 and IC50 inhibition concentrations) and survival median lethal concentration (LC50) endpoints.

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

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

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

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

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

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

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

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

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

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