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Open-File Report 2018–1080

U.S. Department of the Interior
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Conversion Factors

U.S. customary units to International System of Units

Multiply	By	To obtain
	Length	
inch (in.)	2.54	centimeter (cm)
inch (in.)	25.4	millimeter (mm)
foot (ft)	0.3048	meter (m)
mile (mi)	1.609	kilometer (km)
mile, nautical (nmi)	1.852	kilometer (km)
yard (yd)	0.9144	meter (m)
	Volume	
barrel (bbl; petroleum, 1 barrel=42 gal)	0.1590	cubic meter (m^3)
ounce, fluid (fl. oz)	0.02957	liter (L)
pint (pt)	0.4732	liter (L)
quart (qt)	0.9464	liter (L)
gallon (gal)	3.785	liter (L)
gallon (gal)	0.003785	cubic meter (m^3)
gallon (gal)	3.785	cubic decimeter (dm^3)
million gallons (Mgal)	3,785	cubic meter (m^3)
cubic inch (in^3)	16.39	cubic centimeter (cm^3)
cubic inch (in^3)	0.01639	cubic decimeter (dm^3)
cubic inch (in^3)	0.01639	liter (L)
cubic foot (ft^3)	28.32	cubic decimeter (dm^3)
cubic foot (ft^3)	0.02832	cubic meter (m^3)
cubic yard (yd^3)	0.7646	cubic meter (m^3)
cubic mile (mi^3)	4.168	cubic kilometer (km^3)
acre-foot (acre-ft)	1,233	cubic meter (m^3)
acre-foot (acre-ft)	0.001233	cubic hectometer (hm^3)
	Mass	
ounce, avoirdupois (oz)	28.35	gram (g)
pound, avoirdupois (lb)	0.4536	kilogram (kg)
ton, short (2,000 lb)	0.9072	metric ton (t)
ton, long (2,240 lb)	1.016	metric ton (t)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F = (1.8 \times °C) + 32.$$

Temperature in degrees Fahrenheit (°F) may be converted to degrees Celsius (°C) as follows:

$$°C = (°F - 32) / 1.8.$$

Abbreviations

ANOVA	analysis of variance
ATP	adenosine triphosphate
Cl^-	chloride ion
°C	temperature in degrees Celsius
d	day(s)
DOD	U.S. Department of Defense
EPA	U.S. Environmental Protection Agency
g	gram
g/dL	grams per deciliter
h	hour(s)
Hb	hemoglobin
HCO_3^-	bicarbonate anion
Hct	hematocrit
IACUC	Institutional Animal Care and Use Committee
iCa	ionized calcium
K^+	ionized potassium
KCl	potassium chloride
L	liter
LC_{50}	lethal concentration (50 percent)
LSC	Leetown Science Center
M	molar
mg	milligram
mg/dL	milligrams per deciliter
mg/L	milligrams per liter
mL	milliliter
mm	millimeter
mmol/L	millimoles per liter
mOsm/L	milliosmoles per liter
n =	number of
Na^+	ionized sodium
ng/mL	nanograms per milliliter
NOEC	no observed effect concentration
NYDEC	New York Department of Environmental Conservation
PCV	packed (blood) cell volume

ppm	parts per million
PVC	polyvinyl chloride (synthetic polymer piping)
sec	second(s)
TCO_2	total carbon dioxide
µL	microliter
µS/cm	microsiemens per centimeter (electrical conductivity measurement)
USDA-NCCCWA	U.S. Department of Agriculture, National Center for Cool and Cold Water Aquaculture
USFWS	U.S. Fish and Wildlife Service
USGS	U.S. Geological Survey

An Evaluation of the Toxicity of Potassium Chloride, Active Compound in the Molluscicide Potash, on Salmonid Fishes and Their Forage Base

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Abstract

Potash, with the active ingredient potassium chloride (KCl) is a chemical that is currently being evaluated for potential use as a molluscicide to combat invasive zebra mussels and quagga mussels in Western United States waters. Although data available for other freshwater fishes indicate that recommended treatment levels of potash as a molluscicide are sublethal, this has not been demonstrated for all salmonid species. The objectives of this study were to perform toxicity testing to determine the lethality of potassium chloride against selected species of salmonid fish (brook trout and Chinook salmon) and selected invertebrate forage, and to identify any potential adverse physiological impacts of KCl to these salmonids in water at treatment levels used for mollusk eradication. Minimal mortality (n=1 fish) was observed during 96-hour toxicity testing at KCl concentrations of 0 to 800 milligrams per liter (mg/L), indicating that the lethal concentration (LC_{50}) values in these salmonid species were considerably higher than realistic molluscicide treatment concentrations. Sublethal effects were examined through evaluation of behavioral and morphological (histological) observation as well as specific blood chemistry parameters (electrolytes, osmolality, glucose, and cortisol). There was no strong evidence of significant physiological impairment among the two salmonid species due to KCl exposure. Whereas statistically significant differences in some parameters were observed in association with KCl treatments, it is unlikely that these differences indicate adverse biological impacts. Acute toxicity tests were conducted with invertebrate species at KCl exposure concentrations of 0–3,200 mg/L. Daphniid exposure trials resulted in differences in mortality among the test groups with higher mortality evident among the higher KCl exposure concentrations with a calculated LC_{50} value of 196 mg/L KCl for a 48-hour exposure. Crayfish exposed to higher concentrations of KCl at or above 800 mg/L as specimens exhibited death or reversible paralysis. Chironomid larvae exposures were largely inconclusive because of cannibalistic behavior among the various test groups.

Introduction

Background

As a leading authority in the interagency Aquatic Nuisance Species Task Force, the U.S. Fish and Wildlife Service (USFWS) is responsible for the successful development and implementation of control measures for invasive aquatic mussels in United States waters. The U.S. Geological Survey (USGS), in keeping with its mission, provides science in support of the USFWS management goals. Invasive species of mussels that currently represent widespread threats in native aquatic habitats throughout the Nation include two species of *Dreissena* mussels, the zebra mussel (*D. polymorph*a) and the quagga mussel (*D. rostriformis bugensis*). Development of new methods and technologies that successfully kill or neutralize invasive mussels while doing no harm to native wildlife or ecosystems are an important part of a management strategy for these nuisance species.

Potash, with the active ingredient potassium chloride (KCl), is a chemical that is currently being evaluated for potential use as a molluscicide to combat invasive zebra mussels and quagga mussels in Western United States waters. Potassium-based compounds have been evaluated experimentally for their efficacy in killing zebra mussels, particularly targeting the veliger larval stage (Fisher and others, 1991). Research results indicated that the potassium salts used, including KCl, demonstrated killing capability against this invasive mussel species. During the winter of 2006, KCl was used at a concentration of approximately 100 milligrams per liter (mg/L) (or parts per million, ppm) to successfully eradicate zebra mussels from a 12-acre abandoned rock quarry in Virginia without harming other aquatic fauna (Virginia Department of Game and Inland Fisheries, 2005). Whereas KCl is not an U.S. Environmental Protection Agency (EPA) approved pesticide and requires special permitting for use, it is generally considered a reasonably safe alternative to other molluscicides because of its low potential to impact

non-target species (Virginia Department of Game and Inland Fisheries, 2005). The potential mechanism of action selectively impacting *Dreissena* mussels is thought to target the branchial epithelium interfering with Na^+/K^+/ATPase activity and impacting functional gas exchange (Fisher and others, 1991). Although no impacts on aquatic wildlife or human health at a target concentration for zebra mussel eradication of 100 mg/L, have been reported there is not an extensive body of literature available to describe the potential chronic impacts of KCl on aquatic wildlife, nor have the impacts on all potential non-target aquatic wildlife species of interest been described. Before using such a new control measure, an understanding of the full potential scope of its effects on native wildlife must be developed. This body of knowledge would be especially valuable when considering application in habitats that support species of concern. Listed species of Pacific salmonid fish represent an important example.

Research into the efficacy of potash as a control method for dreissenid mussels under different water chemistry conditions and habitat have demonstrated that water chemistry plays an important role relative to the impacts of KCl as a molluscicide (Moffitt and others, 2016). Specifically, low conductivity water with low levels of sodium supports the efficacy of KCl treatment. High conductivity and total dissolved solids (TDS) in water reduce the effectiveness of KCl as a biocide for dreissenid mussels (C. Moffitt, USGS, oral commun., 2016). It is likely that the same mechanisms and principles of action of KCl (based on dysfunction of the branchial Na^+/K^+/ATPase activity) may be anticipated for other aquatic invertebrates and fish. Therefore, water chemistry conditions that provide low conductivity and low dissolved solids, particularly sodium, are probably best suited to demonstrate the potential toxicity-related impacts on these species of interest.

Although the use of liquid KCl-based potash may be a viable control mechanism against invasive *Dreissena* mussels in the Pacific Northwest region, consideration must first be given to the potential scope of effects on the endangered populations of salmonid fish that may cohabitate these waters. Acute toxicity testing for potash and other chemical mixtures with KCl as the active ingredient have been reported for some species (Vijayavel and Balasubramanian, 2007), but very little data specific to salmonid fish are available. Whereas extrapolation of data available for other freshwater fish indicates that these treatment levels of potash as a molluscicide are sublethal, this has not been demonstrated for all salmonid species. In addition, data that describe the potential subclinical physiological effects of this chemical on these species are not widely available.

Information Needs Addressed

Control of aquatic invasive species is an important component of the mission of the USFWS. Likewise, protection of listed species such as endangered northwest salmonid populations is central to the mission of the USFWS. Both of these issues are addressed by this study. The acute and chronic toxicity testing of KCl provides valuable information related to the potential scope and side effects of KCL-based potash as a molluscicide to control invasive *Dreissena* mussels in sensitive habitat. The effects were assessed using relevant salmonid fish species and invertebrate forage species as surrogates for the endangered salmonid populations and their prey items that would likely cohabitate waters of the Pacific Northwest where molluscicide treatment may be used. Thus, the acute (lethal) toxic effects of this compound on species of concern and their forage base were analyzed. In addition, the study determined whether potentially chronic, sublethal effects may exist with additional consequences for overall health status and survival of these salmonid fish populations. The scope of work has broad applicability for use of potassium-based compounds as molluscicides in waters inhabited by salmonid fish.

Preventing the establishment of aquatic invasive species in general, and dreissenid mussels in particular, is a high conservation priority for USFWS Region 1, the Pacific Region including Hawaii, Idaho, Oregon, Washington State, and the Pacific Islands . The establishment of dreissenid mussel populations in the Columbia River Basin in the Pacific Northwest could have real and significant consequences on efforts to protect listed species, but the ecological effects of invasive mussel establishment go beyond listed species to the overall health of aquatic communities as well. For instance, ssenid mussel infestations have been implicated in shifts in invertebrate and fish communities, an increased frequency of toxic algal blooms, and the spread of avian botulism (Getchell and Bowser, 2006; Nalepa, 2010). Alternatively, any rapid response to a mussel introduction must be predicated on minimizing harm to our trust resources. The investigation into the potential negative effects of potash on salmonids provides managers of Pacific Northwest waters and elsewhere better information on the most appropriate and effective tools for eradicating dreissenid mussels before they become established.

Although it is not possible to accurately predict when and where an invasion will take place, it is known that the range of dreissenid mussels is encroaching upon the Pacific Northwest. As a result, there is an increased risk of mussel introduction and establishment. The more information available to support the development of best management practices before a mussel introduction occurs, the greater the likelihood that rapid response efforts will be successful. This initial investigation into the toxicity of potash to salmonids and their prey items addresses critical matters of potential ecological impact to inform responsible application of KCl-based molluscicides in the Columbia River Basin.

Objectives

1. To perform acute toxicity tests to evaluate the lethality of potash (KCl, proposed for use as a molluscicide in Pacific Northwest salmonid habitat) against representative species of salmonid fish (brook trout and Chinook salmon) and their forage base.

2. To perform toxicity tests to identify any potential physiological impacts of KCl in water at relevant treatment levels on selected species of salmonid fish.

Applied Methodology and Procedures

Acute Toxicity of Potassium Chloride to Juvenile Salmonid Fish

Toxicity testing was performed with two species of salmonid fish, one each from the genera *Oncorhynchus* and *Salvelinus*, which were representative of salmonid fish populations of concern in the Pacific Northwest region. Juvenile Chinook salmon (*Oncorhynchus tshawytscha*) and juvenile brook trout (*Salvelinus fontinalis*) were utilized for this study (Densmore and others, 2018). Brook trout were used as a proxy species for the Endangered Species Act listed bull trout (*Salvelinus confluentus*; U.S. Fish and Wildlife Service 1999), as these fish are difficult to obtain in quantities sufficient for toxicity testing, and physiological reactivity to KCl is expected to be similar between the two species. The use of these fish in this study was performed in accordance with an Institutional Animal Care and Use Committee (IACUC) approved protocol (Leetown Science Center IACUC Protocol 2016-001). Approximately 1,500 Chinook salmon fry (about 2.5 grams [g]/fish) were obtained from New York Department of Environmental Conservation, Salmon River Fish Hatchery (Altmar, N.Y.). Approximately 1,000 brook trout fry (about 3 g/fish) were obtained from the U.S. Department of Agriculture, National Center for Cool and Cold Water Aquaculture (Kearneysville, W.Va.). After delivery, fish were maintained in 1,000-liter (L) holding stock tanks with flow-through spring water (at a temperature of 12.5 degrees Celsius, or °C) supplied to Leetown Science Center (LSC) wet laboratories. Approximately 1 week prior to the onset of the experiments, the fish were transferred to closed recirculating holding tank systems for acclimation to experimental water maintained at 12–13 °C. All fish were maintained using standard laboratory protocols for transport, care, and handling in accordance with both the LSC Fish Health Laboratory Standard Operating Procedures for the Acquisition, Care, and Handling of Laboratory Animals (Leetown Science Center, USGS, written commun., 2016) as well as the associated IACUC-approved protocol.

Toxicity experiments were performed with replicate treatment groups and unexposed control groups. Reagent grade KCl was utilized for testing, as KCl is the active ingredient in industrial potash at over 99.9 percent standard content, and the mechanism of action of potash on dreissenid mussels is ascribed to KCl toxicity (Fisher and others, 1991). Furthermore, there are variations in the formulation of industrial grade potash so that reagent grade KCl provides for better uniformity and consistency across experiments (United Nations Industrial Development Organization–International Fertilizer Development Center, 1998). Experimental concentrations of KCl dilutions were monitored throughout experimental exposures with a portable meter measuring potassium anions in water (Horiba LAQUAtwin potassium K^+ compact ion meter, Horiba Scientific Ltd., Kyoto, Japan). Other water-quality parameters were measured as needed with standard portable submersible meters (Yellow Springs Instrument Co., Yellow Springs, Ohio) or water-quality test kits (Hach Chemical Company, Loveland, Ohio). Previous investigations to evaluate the impacts of KCl in water on dreissenid mussels have demonstrated that conductivity and TDS significantly impacted the efficacy of KCl as a molluscicide (C. Moffitt, USGS, oral commun., 2016). Because of this finding, two formulations of experimental water were used for each species of fish and acute toxicity tests were performed at both high and low water conductivity levels. Undiluted spring water that supplies the LSC wet laboratories was used for the high conductivity tests. Undiluted (flowing) spring water had a conductivity of approximately 680 microsiemens per centimeter (µS/cm), alkalinity (HCO_3^-) of 120 mg/L, Na^+ of 3.5 mg/L, and K^+ of 1.8 mg/L. A second test formulation was developed using spring water diluted with deionized laboratory water to low conductivity of approximately 150 µS/cm; this low conductivity water was also used for both acute toxicity tests and acute sublethal effects testing with brook trout. Water samples including both high and low conductivity formulations, with and without the addition of KCl reagent at a calculated 800 mg/L, were analyzed by a commercial water-quality laboratory (Reliance Laboratories Inc., Martinsburg, W.Va.) for the presence of total calcium (Ca), total magnesium (Mg), total potassium (K), total sodium (Na), and total chloride (Cl).

Acute toxicity tests were conducted as static replacement 96-hour toxicity test*s* (U.S. Environmental Protection Agency, 1996b) to determine the lethality of KCl and ascertain any sublethal histological changes associated with KCl exposures. Experimental exposures were conducted in triplicate at concentrations of 0, 25, 50, 100, 200, 400, and 800 mg/L KCl. Circular tanks containing 18 L of water with supplemental aeration were used as the static testing vessels. These tanks were partially submerged in troughs with flowing spring water to maintain a consistent suitable temperature of approximately 13 °C in the testing tanks. Seven fish were present in each replicate tank (n=21 fish in total per treatment level; n=147 fish in total per experimental trial). The toxicity testing was performed four times: high and low background water conductivity using either brook trout or Chinook salmon. For each test, a 100-percent water change with KCl replacement was performed 48 hours into the experimental period. Fish were monitored four times daily throughout the experiment for presence of moribund or dead fish. Daily observations related to both gross appearance and behavior were quantitatively recorded. At the conclusion of the 96-hour test period, all remaining fish were rapidly euthanized with a lethal dose of tricaine methanesulfonate (500 mg/L, loss of righting reflex in less than 30 seconds) applied to test water, followed by transection of the proximal spinal cord. Gross necropsy was

performed for each specimen and any abnormalities were recorded. All fish from one replicate tank for each exposure group (n=7 fish) were preserved in Z-fix (buffered formalin based) tissue fixative for potential histological analysis. The seven specimens from the 0, 100, and 800 mg/L exposure groups were processed for histopathological analysis by light microscopy using standard methodology (Luna, 1992; Reimschuessel and others, 1992). For each species, seven additional fish were taken directly from the holding tank and euthanized/handled as previously described for histological sampling to provide "non-experimental" control groups for histological reference. In addition to evaluation for the presence of lesions and abnormalities, chloride cell counts were performed for gill tissues. Chloride cells were counted in interlamellar spaces (five consecutive lamellae) for three randomly selected gill segments from each fish.

Sublethal Physiological Impacts of Potassium Chloride to Juvenile Brook Trout—Acute Exposure

Modified toxicity testing was similarly performed using brook trout to evaluate the potential sublethal physiological effects of KCl on fish health. Brook trout were held in triplicate (3 fish/tank) in 14 replicate tanks (aquaria with 36 L of water and supplemental aeration) for both control (no KCl added) and treatment (200 mg/L KCl added) groups. Static exposure tests were performed for 24 hours, and aquaria were maintained at 12-13 °C, as previously described. Fish were observed for activity and behavior throughout the exposure period, also as previously described. At the end of 24 hours, fish were rapidly euthanized with a lethal dose of tricaine methanesufonate (500 mg/L, loss of righting reflex in less than30 seconds) applied to test water. Morphometric parameters (total length, total weight) were measured and blood was immediately collected by use of caudal venipuncture using 1-milliliter (mL) syringes with 26-gauge needles and held on ice in 2-mL heparanized microcentrifuge tubes. Gross necropsy was performed for all specimens, and gill tissues were preserved in Z-Fix tissue fixative (Anatech Ltd., Battle Creek, Mich.) and archived for processing and evaluation. For one specimen from each replicate, whole blood was used for blood chemistry analysis with the iStat clinical chemistry analyzer and the CHEM8+cartridge (Abbott Point of Care, Inc., Princeton, N.J.). Plasma chemistry parameters evaluated included sodium, potassium, chloride, ionized calcium, total CO_2, glucose, hematocrit, and hemoglobin. Plasma was collected from the remainder of the blood samples using centrifugation and frozen at -80 °C pending further analysis. Plasma samples were used to evaluate plasma osmolality with an Osmette™ osmometer (Precision Systems, Inc., Natick, Mass.) using 50-microliter (µL) plasma samples in duplicate. Plasma cortisol was determined using a competitive enzyme-linked immunoassay as described by Carey and McCormick (1998), with modifications. Adaptations of this assay included the sourcing of the cortisol-HRP-conjugate and rabbit anti-cortisol antibody from Fitzgerald Industries (Acton, Mass,). In addition, SureBlue Select (KPL Inc.; Seracare, Milford, Mass.) was used as the enzyme substrate for color development. Optical density was read using a SpectraMax M4 (Molecular Devices, Sunnyvale, Calif.) and plasma cortisol concentrations were determined by use of interpolation to a standard curve using SoftMaxPro v. 6.2.2 (Molecular Devices, Sunnyvale, Calif.). A four-parameter curve was used for sample interpolation. The range of the assay defined by the standard curve was 1–400 nanograms per milliliter (ng/mL).

Sublethal Physiological Impacts of Potassium Chloride to Juvenile Chinook Salmon—Subchronic Exposure

A subchronic toxicity test using Chinook salmon was performed in a 190-gallon recirculating holding tank with chilled (12 °C) water formulated to conductivity of approximately 300 µS/cm. Two test trials were run consecutively for 10 days, with added KCl levels of 0 mg/L (control) and 200 mg/L (treatment). For each test trial, 60 fish were used. Twelve fish per group were removed from the study on days 1 and 6, with the remainder on day 10. Fish were rapidly euthanized with a lethal dose of tricaine methanesulfonate (500 mg/L, loss of righting reflex in less than 30 seconds). Subsequent tissue sampling and analyses were performed as described for sublethal impacts analysis with brook trout (gross and behavioral observations, plasma chemistry parameters, osmolality, and cortisol evaluations).

Acute Toxicity of Potassium Chloride to Invertebrate Forage of Salmonid Fish

Daphnia magna and chironomid midge larvae (bloodworms) were purchased from a commercial supplier (Sachs Systems Aquaculture, St. Augustine, Fla.). Acute toxicity tests (U.S. Environmental Protection Agency, 1996a) were performed with these specimens using two-fold serial dilutions of KCl (0–3,200 mg/L) in LSC laboratory spring water diluted with deionized water to a baseline conductivity of 270 µS/cm. Baseline water conductivity levels were derived based on a combination of acclimation trials with these species and previous observations of the effects of conductivity on KCl efficacy as a molluscicide (C. Moffit, USGS, oral commun., 2016). A 3-molar (M) solution of aqueous KCl (Sigma Aldrich Co., St. Louis, Mo.) was used to prepare the KCl test solutions. Upon receipt, invertebrates of each species were acclimated to the 270 µS/cm baseline test water at 15 °C in the holding vessels over an approximate 24-hour period with 10 individual specimens per container. Acid-washed glass 150-mL beakers containing 50 mL of test solution were used as test vessels in a 15 °C incubator to maintain the temperature. Following the acclimation period, specimen viability was

checked by motility and condition, and the proper amounts of aqueous KCl solution were added to each test container and mixed to achieve the target concentrations. Serial dilutions of KCl and a negative control exposure were run in triplicate at the following KCl concentrations: 0 mg/L (control), 25 mg/L, 50 mg/L, 100 mg/L, 200 mg/L, 400 mg/L, 800 mg/L, 1,600 mg/L, and 3,200 mg/L. The acute toxicity test was conducted for 48 hours, and organism viability (motility and condition) were checked at 2, 4, and 24 hours. Mortality was assessed and recorded at each observation interval and at the 48-hour conclusion.

Additional 48-hour trials were conducted with crayfish (*Procambarus* sp., approximately 32 millimeters [mm] average length) purchased from the same commercial supplier. Crayfish were held at five KCl test concentrations (0, 200, 400, 800, and 1,600 mg/L) in four replicate tanks (1 L total water volume) with small sections of 0.75-inch-diameter polyvinyl chloride (PVC) tubing along the tank bottoms to offer cover. Baseline water conductivity of 270 µS/cm was used, similar to the water used in the other invertebrate toxicity trials, and water temperature was maintained at approximately 21 °C, and an airstone provided aeration for each tank. Each replicate tank contained three crayfish. Crayfish were monitored for apparent mortality at 0, 9, 24, 32, and 48 hours. At each observation interval, presumptively dead crayfish were identified based on lack of righting reflex and lack of response to tactile stimuli. These presumptively dead specimens were removed from the tanks and placed in individual freshwater containers (0 mg/L KCl with all other parameters as previously described) for 24 hours to verify mortality based on persistence of lack of response/righting reflex. Observations of cannibalism (loss of all or part of an individual specimen) within a replicate tank resulted in the removal of the replicate from the remainder of the study.

Results

Water Quality and Water Chemistry

Water chemistry analysis for select inorganic components (Reliance Laboratories Inc., Martinsburg, W.Va.) from the representative holding tanks (high and low conductivity; 0 mg/L and 800 mg/L KCl) is summarized in appendix 1. Ionized potassium values for each exposure level as determined by use of the portable meter at the onset of each experimental trial are provided in appendix 2. Additional water-quality data collected daily from all experimental tanks throughout these trials are summarized in appendixes 3*A*–3*G* for each of the seven independent toxicity trials. Water-quality and water chemistry parameters were consistent throughout the experiments. Water temperatures were stable and comparable across experimental trials, ranging from approximately 12.7 to 13.8 °C in the smaller replicate tanks and 11.7 to 12.8 °C in the large recirculating system. Dissolved oxygen levels were near saturation for the duration of the experiments due to the constant aeration, and the pH of experimental water was mildly alkaline (approximately 7.8–8.5). Water conductivity varied among the test groups/tanks in direct proportion to the KCl levels added for each toxicity trial. Ionized potassium (mg/L K^+) was generally found to be in the range of 40–50 percent of the total KCl solution concentration (mg/L or ppm) throughout the experimental trials.

Acute Toxicity of Potassium Chloride to Juvenile Salmonid Fish

Minimal mortality (one fish) occurred across all KCl exposures. There was no mortality observed in the negative control groups (0 mg/L KCl) for any of the trials. The single mortality was a Chinook salmon tested at 200 mg/L KCl at low baseline water conductivity. The mortality occurred at the end of the trial, and was noted at the termination of the 96-hour exposure. At necropsy, this fish was generally pale, yet there were no gross lesions noted and no obvious proximate cause of death. Because this was the only mortality observed for the toxicity trials, no LC_{50} value could be determined for KCl exposure for either species at either baseline conductivity. Statistical analysis using one-way analysis of variance (ANOVA) and the Tukey-Kramer post-hoc test for paired means with $p<0.05$ revealed no significant differences in mortality among any of the groups tested over the 96-hour exposures.

Abnormalities observed among the fish for each exposure trial are described in appendix 4. Sporadic noteworthy behavior was seen among the test groups. Most commonly, hyper- excitable swimming behavior was noted in one to a few fish in the various test chambers, more commonly among the Chinook salmon. Other morphological or behavioral anomalies were infrequently noted, including mild generalized hyperpigmentation or hypopigmentation, deformed opercles, increased ventilation, coughing, lethargic swimming behavior, and unusual tail flexure or undulation. Upon post-mortem examination of all test fish, no other gross abnormalities were noted. Statistical analysis of the frequency of abnormalities among treatment groups (hyper-excitability, lethargy, ventilatory changes, pigmentation status, locomotor changes, and opercular defects) was performed for each 24-hour observation interval for each of these trials. Analyses using one-way ANOVA and the Tukey-Kramer post-hoc test for paired means with $p<0.05$ revealed no significant differences among any of these parameters.

Histologically, few lesions were observed among the fish, and there were no obvious associations with KCl exposure levels (appendix 5). Mild epithelial lifting was noted among both species and all test groups; this change is believed to be a post-mortem artifact related to the tissue collection and processing. Histological lesions among the Chinook salmon held at low or high water conductivity included renal edema (n=1), altered renal foci (n=1), and hepatocellular vacuolation (n=2); all were deemed mild in severity. Histological changes among

the brook trout included gill epithelial hyperplasia (n=3), renal mineralization/nephrocalcinosis (n=1), and protozoal parasites on the surface of skin or gill tissue (n=3); all of these changes were also mild in severity. Epithelial lifting was noted as moderate in severity in a few individual fish among both Chinook salmon (n=3) and brook trout (n=1), somewhat above the baseline (probable artifactual) level noted throughout all other specimens. Analysis of these changes was evaluated for each test group using Fisher's exact test, and no statistical differences related to KCl exposure level were evident ($p<0.05$).

Chloride cell abundance was compared among the groups using one-way ANOVA with the Tukey-Kramer post-hoc test for pairs of means and $p<0.05$ (table 1; appendix 5). There were no significant differences in chloride cell abundance across the experimental treatments for either species, however, differences in abundance were noted between the fish maintained in the holding tank and a few experimental groups. For the Chinook salmon at low conductivity, chloride cell abundance was significantly greater only at 100 mg/L exposure compared with the Chinook salmon from the holding tank. For brook trout at low conductivity, chloride cell abundance was greater only for the 0 mg/L exposure group as compared to the holding tank. For brook trout at high conductivity, the same was observed for the 800 mg/L exposure group compared to the holding tank.

Table 1. Chloride cell abundance among test groups.

[Chloride cell counts determined per five lamellae and the four adjoining inter-lamellar spaces. Mean numbers (standard error values displayed parenthetically) were determined for each species at high and low water conductivity. Baseline values are presented for additional groups of fish collected from the holding tanks to determine baseline readings from fish in the holding tank outside the scope of the experiment. Statistical differences as measured with analysis-of-variance (ANOVA) at $p<0.05$ with Tukey-Kramer post-hoc testing were only apparent between the baseline group for each species and those experimental groups marked with an *. µS/cm, microsiemens per centimeter; mg/L, milligrams per liter]

Species	Water conductivity (value)	Potassium chloride concentration, in mg/L	Mean number of chloride cells (standard error)
Chinook salmon	Baseline		5.1 (0.28)
	High (680 µS/cm)	0	6.1 (0.54)
		100	7.1 (0.84)
		800	7.2 (0.57)
	Low (150 µS/cm)	0	7.7 (0.63)
		100	9.2 (1.12)*
		800	7.5 (0.6)
Brook trout	Baseline		5.9 (0.51)
	High (680 µS/cm)	0	7.2 (0.46)
		100	7.9 (0.75)
		800	10.0 (1.02)*
	Low (150 µS/cm)	0	8.7 (0.96)*
		100	7.2 (0.39)
		800	7.6 (0.53)

Sublethal Physiological Impacts of Potassium Chloride to Juvenile Brook Trout —Acute Exposure

No mortality was noted among brook trout in control or treatment groups in either high or low water conductivity. No differences in mean length or weight for fish between the control and treatment groups for either conductivity level tested were observed, based on Kruskal-Wallis statistics ($p<0.05$). During post-mortem examination, sporadic external abnormalities were noted, including frayed fins, opercular deformity, ocular hemorrhage, frayed gill, and kyphosis (spinal curvature). There were no significant differences in lesion occurrence among control and KCl-exposed fish at either water conductivity trial, based on Fisher's exact test ($p<0.05$).

Plasma chemistry evaluation (Kruskal-Wallis test statistic, $p<0.05$) determined that there were no significant differences in ionized calcium, hematocrit, or hemoglobin values between the control (0 mg/L KCl) and 200 mg/L KCl exposed groups in trials at both water conductivity levels. Additionally, there was no significant difference in plasma glucose among control and KCl-exposed groups at the high water conductivity. Plasma potassium levels for all groups could not be determined as they all measured below the detection limit for this test (2 millimoles per liter, or mmol/L). Plasma sodium and chloride were both significantly lower in fish exposed to the 200 mg/L KCl compared to unexposed controls for both the high and low water conductivity experiments. Total plasma carbon dioxide (CO_2) was higher in the KCl-exposed fish for both baseline conductivity levels, and plasma glucose was higher in the KCl-exposed fish for the low water conductivity trial. Plasma osmolality was not significantly different between the unexposed and KCl-exposed fish for either test trial. Plasma chemistry data for brook trout are summarized in table 2.

Plasma cortisol was compared between control and treatment groups following acute KCl exposure in either low or high conductivity water (fig. 1). In order to determine if the initial plasma cortisol concentrations between these experiments were similar, plasma cortisol concentrations were compared between control groups. No significant differences were observed between control groups. Median concentrations ranged from 34.1–35.8 nanograms per milliliter (ng/mL). Plasma cortisol was not significantly different between control and treatment groups following acute exposure to 200 mg/L KCl in low conductivity water ($p=0.11$). Median plasma cortisol was significantly increased following the same 24-hour exposure in high conductivity water (Mann-Whitney U test; $p=0.042$), and there was a significant change in the probability distribution as determined by the Kolmogorov-Smirnov test ($p=0.021$). A treatment effect was evident across all groups (Kruskal-Wallis H test ($p=0.009$).

Table 2. Plasma chemistry data for brook trout following 24-hour exposure to potassium chloride at 200 milligrams per liter.

[KCl, potassium chloride; Na^+, sodium; Cl^-, chloride; iCa, ionized calcium; TCO_2, total carbon dioxide; Hct, hematocrit; Hb, hemoglobin; µS/cm, microsiemens per centimeter; mg/L, milligrams per liter; mmol/L, millimoles per liter; mg/dL, milligrams per deciliter; %, percent; g/dL, grams per deciliter; mOsm/L, milliosmoles per liter; Values shown are means with standard errors in parentheses, and statistically significant differences between the 0 mg/L and 200 mg/L exposure groups are indicated with an * (Kruskal-Wallis test statistic with $p<0.05$)]

Baseline water conductivity (value)	KCl, in mg/L	Na^+, in mmol/L	Cl^-, in mmol/L	iCa, in mmol/L	TCO_2, in mmol/L	Glucose, in mg/dL	Hct, in %	Hb, in g/dL	Osmolality, in mOsm/L
Low (150 µS/cm)	0	143.6 (0.9)	127.8 (0.7)	1.84 (0.03)	10.9 (0.2)	109.3 (5.6)	42.2 (1.5)	14.4 (0.5)	309.8 (2.0)
	200	141.1 (0.8)*	124.5 (0.9)*	1.86 (0.02)	11.6 (0.3)*	134.3 (6.8)*	46.0 (1.4)	15.6 (0.5)	306.6 (1.9)
High (680 µS/cm)	0	141.8 (0.8)	124.0 (0.7)	1.72 (0.04)	11.1 (0.3)	153.2 (8.7)	43.8 (1.3)	14.9 (0.5)	298.9 (1.5)
	200	138.2 (1.1)*	117.6 (1.2)*	1.74 (0.07)	13.9 (0.5)*	176.0 (12.4)	48.0 (1.9)	16.3 (0.6)	291.7 (2.8)

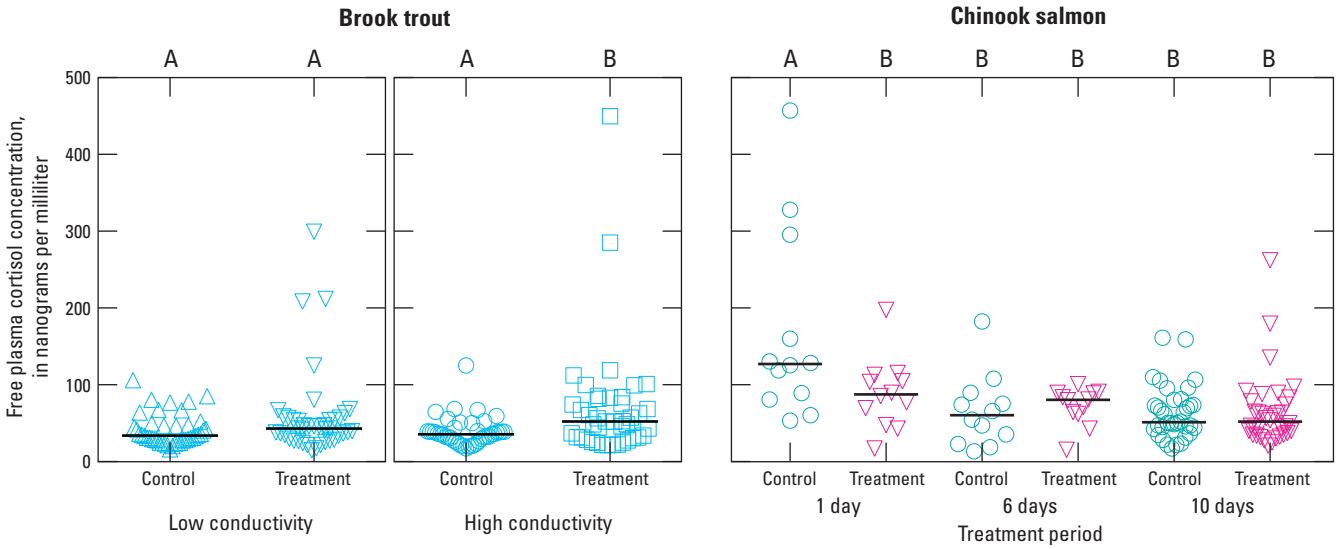

Figure 1. Plasma cortisol values for brook trout and Chinook salmon exposed to 0 and 200 milligrams per liter (mg/L) of potassium chloride. Plasma cortisol was not significantly different among brook trout between control and treatment groups following acute exposure to 200 mg/L of potassium chloride (KCl) in low conductivity water, but median plasma cortisol was significantly increased following the same 24-hour exposure in high conductivity water. Among the Chinook salmon, plasma cortisol decreased across groups over time during the course of this experiment, and median plasma cortisol was significantly higher in control fish compared to KCL-exposed fish at the 24-hour time point. Horizontal bars indicate the median. Treatment denoted with the same capital letter are not significantly different ($p \leq 0.05$).

Sublethal Physiological Impacts of Potassium Chloride to Juvenile Chinook Salmon—Subchronic Exposure

No mortality was noted among Chinook salmon control or treatment groups during the exposure trial. There were no differences in mean fish length or weight between the control and treatment groups for either conductivity level based on Kruskal-Wallis statistics ($p<0.05$). During post-mortem examination, sporadic abnormalities were noted, which included mild ocular hemorrhage and renal mineralization. There were no significant differences in the occurrence of lesions among control and KCl-exposed fish based on Fisher's exact test at $p<0.05$.

Plasma chemistry parameters were evaluated for changes over the course of the exposure for each test group sampled at 1 day and 10 days (table 3*A*). These parameters were also evaluated for differences between the 0 mg/L and 200 mg/L exposures at each distinct time interval (table 3*B*). Analyses were performed with the Kruskal-Wallis test statistic at $p<0.05$. Again, plasma potassium levels for all groups could not be determined as they all were below the detection limit for this test (2 mmol/L). Four of the plasma chemistry parameters as well as hematocrit and hemoglobin changed significantly over the course of the experiment for the unexposed control group (0 mg/L): plasma sodium, chloride, and ionized calcium decreased whereas plasma glucose, hematocrit, and hemoglobin all increased from day 1 to day 10. Only total carbon dioxide (TCO_2) levels were significantly increased from day 1 to day 10 for the test group exposed to 200 mg/L KCl. Significant differences between the 0 and the 200 mg/L exposure groups also were noted among three of the plasma chemistry parameters, each at two distinct time intervals: plasma sodium was higher in the KCl exposure group at days 6 and 10; plasma chloride was lower in the exposure group at days 1 and 6; and plasma glucose was higher in the exposure group at days 1 and 6. In addition, both hematocrit and hemoglobin were lower for the 200 mg/L exposure group compared to the 0 mg/L control group on day 10.

Table 3*A*. Plasma chemistry data for Chinook salmon over time for 0 milligrams per liter and 200 milligrams per liter potassium chloride exposure groups.

[KCl, potassium chloride; Na^+, sodium; Cl^-, chloride; iCa, ionized calcium; TCO_2, total carbon dioxide; Hct, hematocrit; Hb, hemoglobin; mg/L, milligrams per liter; mmol/L, millimoles per liter; mg/dL, milligrams per deciliter; %, percent; g/dL, grams per deciliter; mOsm/L, milliosmoles per liter; Values shown are means with standard errors in parentheses, and statistically significant differences between the 1-day and 10-day measured parameters for the 0 mg/L and 200 mg/L exposure groups are indicated with an * next to the mean for the 10-day measured value in each test group (Kruskal-Wallis test statistic with $p<0.05$)]

Test period	Na^+, in mmol/L	Cl^-, in mmol/L	iCa, in mmol/L	TCO_2, in mmol/L	Glucose, in mg/dL	Hct, in %	Hb, in g/dL	Osmolality, in mOsm/L
				0 mg/L KCl				
Day 1	144.1 (1.0)	127.3 (0.7)	1.79 (0.02)	11.9 (0.3)	63.4 (3.9)	40.3 (1.0)	13.7 (0.3)	295.3 (1.5)
Day 10	140.6 (0.6)*	124.2 (1.2)*	1.94 (0.04)*	12.5 (0.3)	73.1 (3.2)*	48.5 (1.6)*	16.5 (0.5)*	299.6 (2.0)
				200 mg/L KCl				
Day 1	142.6 (1.0)	123.1 (1.1)	1.78 (0.02)	11.6 (0.3)	72.3 (3.5)	42.4 (1.4)	14.4 (0.5)	295.3 (3.3)
Day 10	143.8 (0.7)	123.0 (0.6)	1.87 (0.03)	12.3 (0.3)*	79.1 (4.3)	41.1 (1.3)	14.0 (0.5)	302.0 (1.4)

Table 3*B*. Plasma chemistry for Chinook salmon throughout the 10-day potassium chloride exposure trial.

[KCl, potassium chloride; Na^+, sodium; Cl^-, chloride; iCa, ionized calcium; TCO_2, total carbon dioxide; Hct, hematocrit; Hb, hemoglobin; mg/L, milligrams per liter; mmol/L, millimoles per liter; mg/dL, milligrams per deciliter; %, percent; g/dL, grams per deciliter; mOsm/L, milliosmoles per liter; Values shown are means with standard errors in parentheses, and statistically significant differences between the 0 mg/L and 200 mg/L exposure groups are indicated with an * next to the mean for the 200 mg/L cell for each individual time period (1, 6, or 10 days; Kruskal-Wallis test statistic with $p<0.05$)]

Test period	KCl, in mg/L	Na^+, in mmol/L	Cl^-, in mmol/L	iCa, in mmol/L	TCO_2, in mmol/L	Glucose, in mg/dL	Hct, in %	Hb, in g/dL	Osmolality, in mOsm/L
Day 1	0	144.1 (1.0)	127.3 (0.7)	1.79 (0.02)	11.9 (0.3)	63.4 (3.9)	40.3 (1.0)	13.7 (0.3)	295.3 (1.5)
	200	142.6 (1.0)	123.1 (1.1)*	1.78 (0.02)	11.6 (0.3)	72.3 (3.5)*	42.4 (1.4)	14.4 (0.5)	295.3 (3.3)
Day 6	0	143.1 (0.4)	127.1 (0.6)	1.74 (0.02)	11.2 (0.3)	55.5 (2.4)	41.1 (1.4)	14.2 (0.4)	301.4 (3.0)
	200	144.9 (0.4)*	125.1 (0.6)*	1.82 (0.03)	11.0 (0.3)	67.2 (3.9)*	40.2 (1.3)	13.7 (0.4)	300.0 (2.4)
Day 10	0	140.6 (0.6)	124.2 (1.2)	1.94 (0.04)	12.5 (0.3)	73.1 (3.2)	48.5 (1.6)	16.5 (0.5)	299.6 (2.0)
	200	143.8 (0.7)*	123.0 (0.6)	1.87 (0.03)	12.3 (0.3)	79.1 (4.3)	41.1 (1.3)*	14.0 (0.5)*	302.0 (1.4)

Plasma osmolality was not significantly different between the unexposed and KCl-exposed fish at the 1-, 6-, or 10-day time interval, nor did plasma osmolality differ significantly between day 1 and day 10 of the exposures for either the 0 mg/L or 200 mg/L exposure groups.

Plasma cortisol concentrations were compared across treatments and time for the KCl exposures for Chinook salmon (fig. 1). Significant differences were identified across all groups (Kruskal-Wallis H test; p=0.009). Median plasma cortisol was significantly higher in control fish (170.3 ng/mL) compared to KCl-exposed fish (89.0 ng/mL) at the 24-hour time point (Kolmogorov-Smirnov test; p=0.034). In general, plasma cortisol decreased across groups over time during the course of this experiment indicating that the increased plasma cortisol at the 24-hour time point reflected handling stress initiated at time zero. By day 10, median plasma cortisol was 52.7 and 53.4 ng/mL between the control and treated groups, respectively. The analysis of resting plasma cortisol in Chinook salmon indicated great variability (14.4–80.4 ng/mL; median, 57.3).

Acute Toxicity of Potassium Chloride to Invertebrate Forage of Salmonid Fish

Whereas mortality was observed among the chironomids including both control and KCl treatment groups, no significant trends in mortality related to the KCl exposure levels were noted. Assessment of mortality was confounded by the cannibalistic or scavenging behavior of the chironomid larvae, as observed by the disappearance of whole specimens or portions of specimens from the test chambers. ANOVA (one-way, $p<0.05$) did not show any significant differences in mortality among the test concentrations (fig. 2).

Mortality among the daphniids showed significant differences among test groups for each observation interval (2 hours, 4 hours, 24 hours, 48 hours) as evaluated with the Kruskal-Wallis test statistic at $p<0.05$ (fig. 3). Calculation of KCl LC_{50} concentrations was performed using log probit analysis (Miller and Tainter, 1944) for the daphniids. The LC_{50} decreased in concentration value with increasing time, so that the 2-hour exposure LC_{50} was approximately 1,375 mg/L

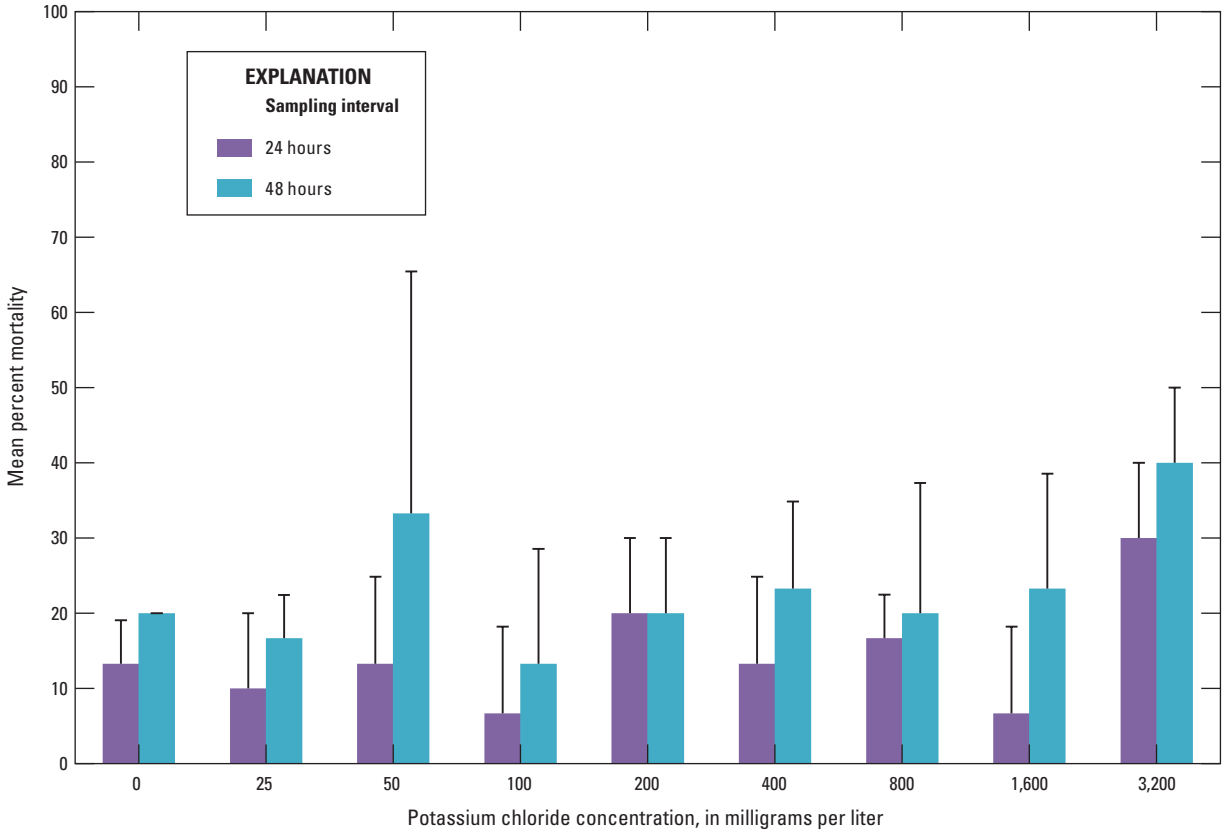

Figure 2. Mortality of chironomid larvae (bloodworms) during toxicity testing with potassium chloride exposures of 0–3,200 milligrams per liter. Mean percent mortality of chironomids (with standard deviation bars) for all three replicates at each test concentration for 24-hour (h) and 48-h intervals. No mortality was noted at 2 h and 4 h.

whereas the 48-hour exposure LC_{50} was calculated as 196 mg/L (fig. 4; appendix 6).

Mortality was also observed among the crayfish (fig. 5). Cannibalism occurred at all test concentrations except the 0 mg/L exposure group at or after 24 hours; therefore, half or more of the replicates from each test concentration were removed from the experiment beyond this point. As a result, differences among each test concentration were only evaluated through the 9-hour and 24-hour exposures. No mortality was evident at the 9-hour test interval among any test groups, but a significant difference ($p<0.05$) among groups was noted at 24 hours by use of one way-ANOVA ($p=0.026$). The Tukey-Kramer post-hoc test identified a significant difference only between the 0 mg/L and the 800 mg/L exposure groups, with decreased survival among the 800 mg/L exposure group. A total of seven crayfish from the 800 mg/L concentration and one crayfish from the 1,600 mg/L concentration were presumed dead and removed from the experimental tanks to freshwater holding tanks without KCl. Within 24 hours, four of the seven crayfish from the 800 mg/L exposure group recovered their righting reflex and response to tactile stimuli. The crayfish from the 1,600 mg/L exposure group did not recover and was presumed dead.

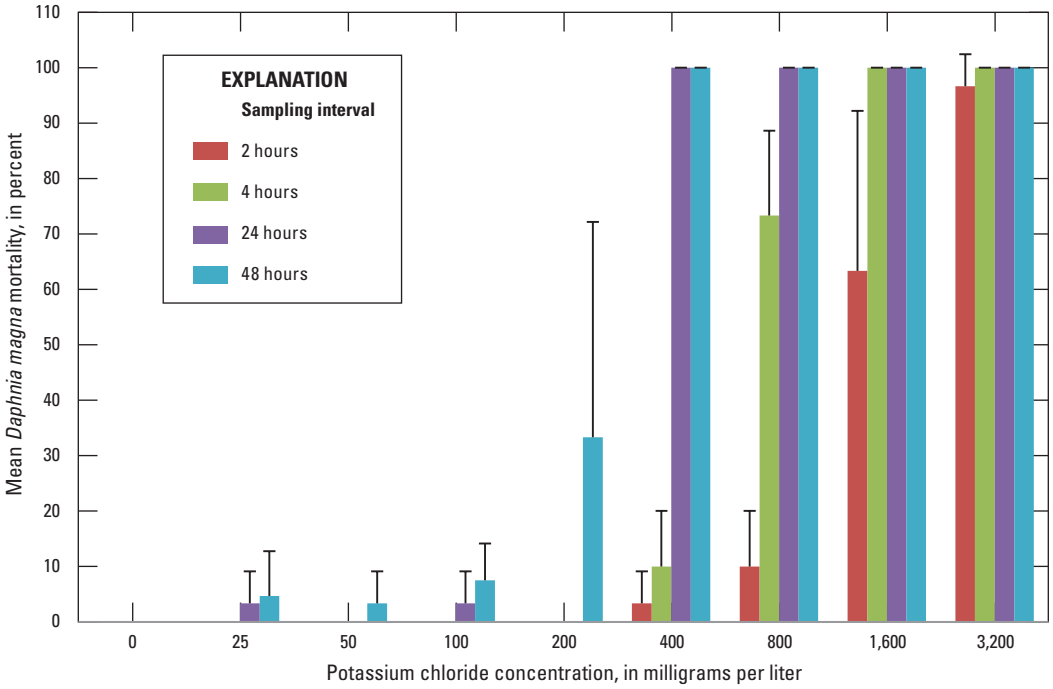

Figure 3. Mortality of *Daphnia magna* during toxicity testing with potassium chloride exposures of 0–3,200 milligrams per liter. Mean percent mortality of *Daphnia* (with standard deviation bars) for all three replicates are given for each test concentration and test interval.

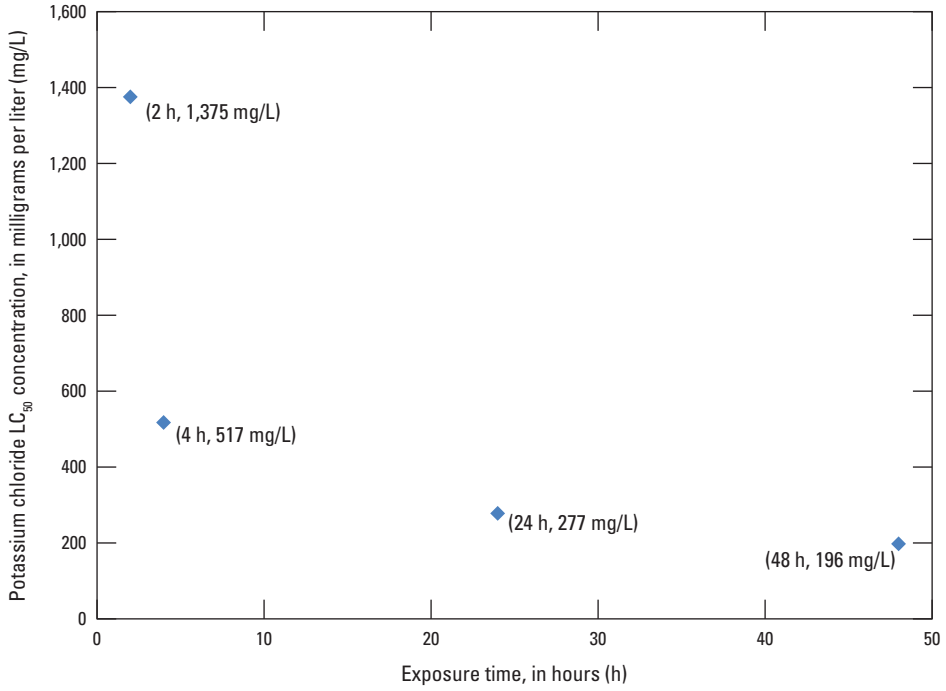

Figure 4. Lethal concentration (LC_{50}) values for daphniid exposure to potassium chloride. Values were derived by use of log probit analysis, and demonstrate that the LC_{50} decreases with increasing time of exposure. LC_{50} values are shown in milligrams per liter for 2, 4, 24, and 48 hours.

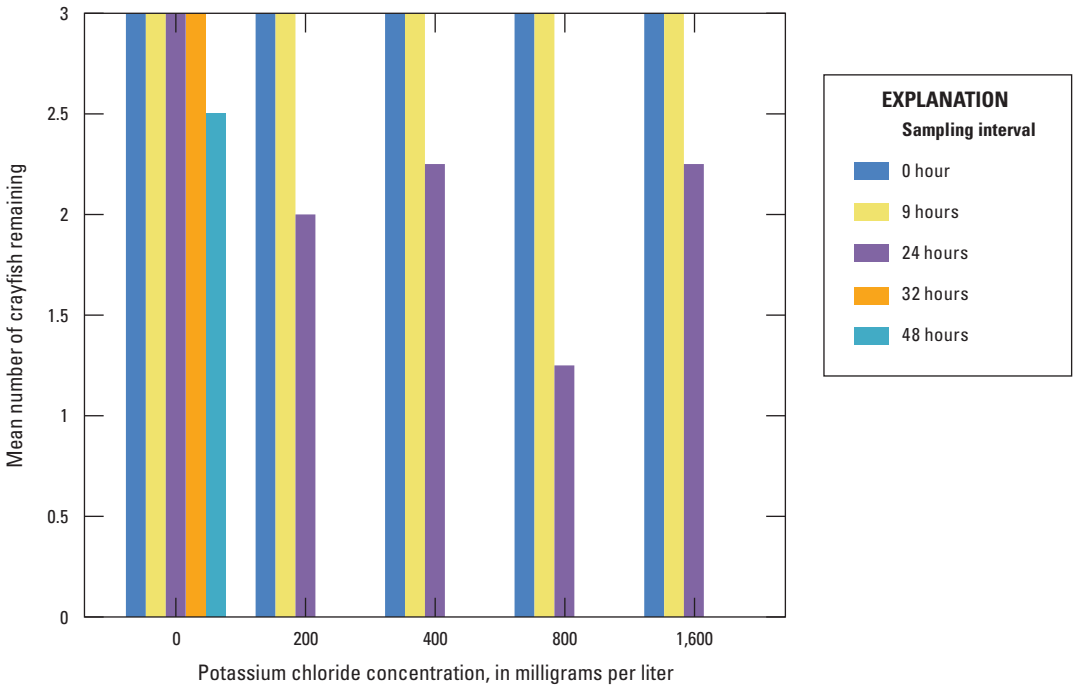

Figure 5. Acute exposure to potassium chloride in crayfish. Mean survival (three crayfish per replicate) at each interval (h, hours) for each potassium chloride concentration. No data are provided for replicates removed from the study due to cannibalism at 32 and 48 h.

Interpretations and Conclusions

Based upon the acute toxicity testing of KCl using both juvenile brook trout and juvenile Chinook salmon, acute lethal effects of potash on these salmonids at these life stages are not expected at concentrations commonly utilized to control invasive dreissenid mussels (100 mg/L). Exposure concentrations of as much as 800 mg/L KCl, eight times greater than the dose of KCl used as a molluscicide, were applied to these fish in static systems for 96 hours; there was no evidence of mortality attributable to KCl exposure among either species. On the basis of this observation, it is clear that the lethal concentration (LC_{50}) is well above the KCl concentrations used in this study. The no observed effect concentration (NOEC) for KCl under these test conditions is also beyond the scope of KCl concentrations used in this study.

Behavioral or gross morphological effects on these fish from KCl-based molluscicide applications at levels up to 800 mg/L were also not indicated within the scope of this study. There were no sublethal behavioral effects noted for either species in association with the KCl exposures through the 800 mg/L concentrations tested. Behavioral changes related to respiration and locomotion and noted infrequently among the fingerlings of both species were not associated with KCl concentration, nor were they statistically different among the test groups. Similarly, mild gross morphological changes including pigmentation differences and opercular defects were noted, however, were neither consistent within certain test groups nor statistically different among groups. In addition, necropsy of the brook trout assessed for sublethal physiological impacts (24-hour exposure at 200 mg/L KCl) revealed no morphological changes associated with KCl exposure.

Histological examination of tissues was utilized to determine the potential for overt or subtle microscopic morphological changes to provide indications of physiological impairment related to KCl exposure. In particular, changes to osmoregulatory and ionoregulatory organs and tissues (skin, gill, excretory kidney) could be anticipated with exposure to high concentrations of inorganic salts. Histologically, few changes were evident among these fish. There was no statistical significance attributed to any of the changes, and no apparent correlation to changes at either 100 or 800 mg/L KCl exposure compared to the 0 mg/L KCl exposure control fish. Because of the lack of histological findings among specimens held at these KCl concentrations among both species, fish tissue samples collected from the other specimens were not processed for evaluation.

Quantitative histological evaluation of chloride cell presence along the base of the lamellae in the gill epithelium was included in the histological analysis, as the mitochondrion-rich chloride cells play an important role in ionoregulation, and chloride cell proliferation is a common response to environmental challenges to fish involving ionoregulatory responses (Perry, 1998). For both marine and freshwater teleosts, chloride cells are sites of ion transport processes through various mechanisms such as Na^+/K^+/ATPase, electrogenic proton extrusion, and anionic exchange (Evans and others, 2005), and chloride cell numbers reflect metabolic activity in gill tissue (Perry and Walsh, 1989). Physiological stress posed by elevated concentrations of environmental salts such as KCl may therefore be expected to result in increased numbers of chloride cells in the branchial epithelium; however, this was generally not observed in this study. Whereas significant differences in chloride cell numbers were observed for both species, these differences were seen between fish maintained in the holding tanks and fish in three of the different experimental groups (table 1). Differences were inconsistent in that they were noted in three of four experimental trials at both baseline conductivity levels for the test water and among three KCl exposure levels (0, 100, and 800 mg/L). These differences, if physiologically relevant, represented increased chloride cell numbers in response to other water chemistry parameters in flowing holding water compared to static test water rather than changes attributable to KCl exposures.

Plasma chemistry parameters provide further information relative to the overall health of fish, and have longstanding use as clinical indicators of disease among salmonid fish (Hille, 1982). However, there may still be considerable variability among normal or reference range for blood parameters related to environmental conditions and life history variables for a given species of salmonid fish (Hille, 1982; Holmes and Donaldson, 1969). Statistical differences were noted among some of these clinical chemistry parameters (such as plasma sodium and chloride) when compared between KCl-exposed and unexposed fish. However, these differences do not necessarily indicate associated pathological impacts of KCl. The plasma chemistry values, though statistically distinct between the groups, still appear to fall within established ranges of "normal" values as reported in the literature (Holmes and Donaldson, 1969). For instance, plasma sodium values in this study were lower in the brook trout exposed to 200 mg/L KCl compared to the unexposed control fish at both high and low water conductivity levels. Although statistically different, these mean plasma sodium values were still close (within a few millimoles per liter) and were comparable to previously reported normal values or reference ranges for trout in freshwater *(*Holmes and Donaldson, 1969). Thus, although the differences in some plasma electrolyte values between groups were statistically significant, they were not sufficiently divergent to indicate impactful physiological differences. There is an inherent challenge related to the interpretation of such results, and differences in one such parameter must be considered in light of the remainder of the clinical data collected to determine their meaning.

Similar to the blood chemistry results, although statistically significant differences in plasma cortisol associated with KCl treatments were observed, the biological ramifications of these differences are not clear. The KCl treatment of brook trout led to increased plasma cortisol in high conductivity water. Differences in plasma cortisol among Chinook salmon, however, did not follow this same trend as cortisol was

initially higher among the unexposed control fish and their cortisol levels decreased over time during the 10-day trial.

Plasma glucose, another non-specific indicator of physiological stress among vertebrates, was also higher among the KCl-exposed fish compared to control fish for brook trout tested at low water conductivity for 24 hours and for Chinook salmon during 24 hours and 6 days of exposure. Consistent with the other blood chemistry changes noted, the differences in glucose levels between unexposed and KCl-exposed fish for each species were not extreme and are well within the scope of normal physiological response to an environmental stressor (Wagner and Congleton, 2004; Densmore and Panek, 2013). By comparison, a greater disparity in blood glucose levels were noted between the unexposed control Brook trout held at low (150 microsiemens per centimeter, or µS/cm) water conductivity (109.3 g/dL) and unexposed control brook trout held at high (680 µS/cm) water conductivity (153.2 g/dL).

Significant differences in physiological stress responses between brook trout and Chinook salmon may be confounded by the genetic histories and life histories of the two tested species. The brook trout used in this study were obtained from an aquaculture facility that breeds strains of fish for commercial fish production. Previous research has shown that aquaculture strains of fish species are more resilient and recover more quickly from handling and tank confinement than wild fish (Huntingford, 2004; Overli and others, 2005). The Chinook salmon utilized in this study came from a New York Department of Environmental Conservation hatchery, which spawns wild broodstock that swim upriver into holding areas during the natural spawning season; they have not been "domesticated" or bred in captivity for several generations. Presumably, they are less adapted to confinement in captivity (Stickney, 1994). In addition, most brook trout populations are stenohaline salmonids that are adapted to cold, very clean freshwater (Benke, 2002), whereas Chinook salmon are an anadromous species that spawn in freshwater, travel downriver and mature in saltwater, and return to natal freshwater rivers to spawn (National Oceanic and Atmospheric Administration Fisheries, 2016). Anadromous species are euryhaline, and are adaptable to a wide range of salinity or ion concentrations. Changes in ion concentrations are less likely to affect Chinook salmon, as indicated by the increase in cortisol immediately following KCl addition, which decreases over exposure time.

Although few to no impacts were noted among the salmonid fish that may indicate mortality or significant physiological impairment resulting from KCl exposure, the data derived from the invertebrate exposure trials indicates otherwise. The chironomid larvae exposures were largely inconclusive because of cannibalistic behavior among the various test groups; mortality could not be definitively attributed to KCl exposure. Furthermore, no statistical differences in mortality among the groups were present to indicate a potential impact of acute KCl exposure on survival. Daphniid exposure trials, however, resulted in statistically significant differences in mortality among the test groups with higher mortality evident among the higher exposure concentrations (figs. 3 and 4) and a LC_{50} calculated as 196 mg/L for 48 hours of exposure. Previous application of potash as a KCl-based molluscicide was at concentrations of 100 mg/L, less than half of this calculated LC_{50} value (Virginia Department of Game and Inland Fisheries, 2005). In light of the target application of potash as a molluscicide directed against the veliger stage of dreissenid mussels, significant mortality among sensitive aquatic invertebrates, such as daphniids, is not unexpected.

Crayfish, the other crustacean species examined in this study, also appeared to show some degree of sensitivity to high concentrations of KCl. Crayfish mortality occurred in test groups exposed to KCl, particularly among the higher concentrations, at 24 hours and beyond, although the mortality rate was not specifically dose-dependent. Cannibalism confounded the interpretation of results, particularly beyond 24-hour exposure. Immobilization of many specimens held at higher concentrations of KCl (800 and 1,600 mg/L), mimicking the appearance of death through both loss of righting reflex and failure to respond to tactile stimuli, was also of interest. Half of the specimens presumed to be dead from these test groups were able to completely recover in freshwater within 24 hours. Even complete recovery from this level of immobilization, however, presents a cause for concern for the survival of individual animals, as crayfish would be potentially vulnerable to predation and other environmental pressures if they were unable to respond accordingly for a substantial period of time. Causality related to this finding in this experiment was not determined, but it is likely that the immobility was related to disruption of neuromuscular function through altered ionic potential across cell membranes. Crayfish possess a stretch receptor organ, which functions analogously to the muscle spindle in vertebrates (Rydqvist and others, 2007), and disruption of this organ's function and resultant paralysis may possibly be attributed to high environmental concentrations of ions (K^+ in particular). Further investigation related to this phenomenon could help discern the causality of the immobility, and whether prolonged exposure to lower doses of KCl may also represent a threat to crayfish or other invertebrate forage species.

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Appendixes

Appendix 1. Water Chemistry Analysis

[µS/cm, microsiemens per centimeters; mg/L, milligrams per liter; inorganic analytes (calcium, magnesium, potassium, sodium, and chloride) were measured in experimental water at high (680 µS/cm) and low (150 µS/cm) baseline water conductivity, either supplemented (800 mg/L) or not supplemented (0 mg/L) with potassium chloride (KCl); Sample analysis was performed by a commercial water-quality laboratory (Reliance Laboratories Inc., Martinsburg, WV); ND, analyte not detected and presumably below detection limits]

Approximate baseline water conductivity, in µS/cm	KCl, in mg/L	Analyte concentration, in mg/L				
		Calcium	Magnesium	Potassium	Sodium	Chloride
680	0	84.1	10.8	1.65	4.43	8.4
150	0	30.1	1.89	ND	0.64	1.85
680	800	80.2	9.18	552	3.85	393
150	800	28.7	1.64	450	1.09	379

Appendix 2. Ionized potassium measurements—96-hour acute toxicity tests.

[KCl, potassium chloride; mg/L, milligrams per liter; K^+, ionized potassium; DI, deionized; Ionized potassium values from test water used in the four acute toxicity tests (high and low baseline water conductivity with brook trout and Chinook salmon) are provided in this table. Potassium anion concentration in test water was measured at the onset of each trial with a portable meter (Horiba LAQUAtwin potassium K^+ compaction meter, Horiba Scientific Ltd., Kyoto, Japan)]

Species	Baseline water conductivity level	KCl, in mg/L	Measured K^+, in mg/L
Brook trout	High	DI water	0
		0	1
		25	11
		50	24
		100	44
		200	100
		400	190
		800	360
	Low	DI water	0
		0	1
		25	11
		50	23
		100	45
		200	96
		400	190
		800	380
Chinook salmon	High	DI water	0
		0	1
		25	12
		50	23
		100	44
		200	91
		400	190
		800	390
	Low	DI water	0
		0	1
		25	11
		50	22
		100	47
		200	96
		400	200
		800	370

Appendix 3*A*. Water-quality measurements (temperature, pH, conductivity, and dissolved oxygen) collected daily from all experimental tanks for the 96-hour potassium chloride (KCl) toxicity test, with brook trout at high baseline water conductivity.

[mg/L, milligrams per liter; °C, degrees Celsius; µS/cm, microsiemens per centimeter; —, not applicable]

KCl added, in mg/L	Replicate	Water temperature, in °C	Water pH	Water conductivity, in µS/cm	Dissolved oxygen, in mg/L
			Day 1		
0	a	13.1	8.4	526	11.3
0	b	12.8	8.5	485	10.6
0	c	12.7	8.4	465	11
25	a	12.9	8.2	596	10.2
25	b	12.8	8.4	491	11.1
25	c	12.9	8.5	526	10.8
50	a	12.9	8.2	694	9.5
50	b	12.9	8.2	676	9.2
50	c	12.9	8.5	576	10.9
100	a	12.8	8.5	668	11
100	b	12.8	8.3	760	10.3
100	c	12.8	8.3	701	10.2
200	a	12.9	8.4	923	11.1
200	b	12.9	8.5	871	11
200	c	12.8	8.3	909	10.8
400	a	12.9	8.2	1,348	10
400	b	12.9	8.2	1,398	10.2
400	c	12.8	8.2	1,347	10.3
800	a	12.9	8.2	2,120	10
800	b	12.8	8.4	1,992	11
800	c	12.8	8.4	2,180	10.9
Spare	—	13	8.5	472	12.3
			Day 2		
0	a	13	8.5	476	10.6
0	b	12.9	8.3	433	11.2
0	c	12.8	8.4	423	10.7
25	a	12.9	8.3	565	10.6
25	b	12.8	8.5	456	10.8
25	c	12.9	8.5	490	11
50	a	12.9	8.4	639	10.4
50	b	12.8	8.4	615	10.4
50	c	12.8	8.5	536	11.2
100	a	12.8	8.5	633	11.2
100	b	12.8	8.3	731	10.1
100	c	12.8	8.3	687	10.6
200	a	12.9	8.5	888	11.3
200	b	12.9	8.5	840	11.2
200	c	12.8	8.3	877	10.9
400	a	12.9	8.3	1,322	10.3
400	b	12.9	8.3	1,370	10.1
400	c	12.8	8.3	1,377	10.5
800	a	12.9	8.3	1,998	10.4
800	b	12.8	8.4	1,974	10.9
800	c	12.8	8.4	2,150	10.8
Spare	—	13	8.6	398	12

KCl added, in mg/L	Replicate	Water temperature, in °C	Water pH	Water conductivity, in µS/cm	Dissolved oxygen, in mg/L
			Day 3		
0	a	12.9	8.4	590	11.1
0	b	12.8	8.5	553	11.5
0	c	12.8	8.5	562	11.3
25	a	12.8	8.3	673	10.6
25	b	12.8	8.5	593	11.5
25	c	12.8	8.4	606	11.3
50	a	12.8	8.4	707	10.9
50	b	12.8	8.4	701	10.9
50	c	12.8	8.5	664	11.3
100	a	12.8	8.5	756	11.3
100	b	12.8	8.3	833	10.6
100	c	12.8	8.3	833	10.6
200	a	12.9	8.5	985	11.7
200	b	12.9	8.5	967	11.4
200	c	12.8	8.3	1,015	11.1
400	a	12.8	8.3	1,458	10.9
400	b	12.8	8.3	1,433	10.6
400	c	12.8	8.4	1,414	11.1
800	a	12.9	8.3	2,250	10.5
800	b	12.8	8.5	2,180	11.2
800	c	12.8	8.4	2,120	11.3
Spare	—	13	8.5	366	12.2
			Day 4		
0	a	12.8	8.4	512	11.2
0	b	12.8	8.5	464	11.6
0	c	12.7	8.4	475	11.4
25	a	12.8	8.3	623	10.6
25	b	12.7	8.4	499	11.6
25	c	12.8	8.5	518	11.5
50	a	12.8	8.4	643	11.1
50	b	12.8	8.4	634	11.2
50	c	12.7	8.5	585	11.4
100	a	12.7	8.4	676	11.4
100	b	12.7	8.3	780	10.8
100	c	12.7	8.3	778	10.9
200	a	12.8	8.5	918	11.6
200	b	12.8	8.5	883	11.5
200	c	12.7	8.4	957	11.2
400	a	12.8	8.3	1,407	10.8
400	b	12.8	8.3	1,391	10.6
400	c	12.7	8.4	1,369	11.3
800	a	12.8	8.3	2,220	10.6
800	b	12.7	8.4	2,120	11.5
800	c	12.8	8.5	1,977	11.4
Spare	—	12.8	8.6	354	11.8

Appendix 3*B*. Water-quality measurements (temperature, pH, conductivity, and dissolved oxygen) collected daily from all experimental tanks for the 96-hour potassium chloride (KCl) toxicity test with brook trout at low baseline water conductivity.

[mg/L, milligrams per liter; °C, degrees Celsius; µS/cm, microsiemens per centimete; —, not applicable]

KCl added, in mg/L	Replicate	Water temperature, in °C	Water pH	Water conductivity, in µS/cm	Dissolved oxygen, in mg/L
			Day 1		
0	a	13.2	8	166	10.5
0	b	13	7.9	168	10.4
0	c	12.8	8	166	11.6
25	a	13	8	205	10.6
25	b	12.9	7.8	217	10.9
25	c	13	7.7	205	9.8
50	a	12.9	7.8	269	10.1
50	b	12.9	7.9	259	10.7
50	c	12.9	7.8	260	10.2
100	a	12.9	7.8	361	10.9
100	b	13	7.7	380	10.3
100	c	12.9	7.8	368	10.5
200	a	13.1	8	588	11.2
200	b	13.1	8	573	11
200	c	12.8	7.9	566	10.8
400	a	13	7.6	959	8.3
400	b	13	7.6	985	9.8
400	c	12.9	7.9	953	10.8
800	a	13	7.5	1,740	10.5
800	b	13	8	1,672	11.5
800	c	13	7.6	1,624	10.7
Spare	—	13.1	8.4	159	11.5
			Day 2		
0	a	13.1	8	177	10.7
0	b	12.8	7.8	176	10.7
0	c	12.7	8	171	11.4
25	a	12.9	8	216	10.9
25	b	12.8	7.9	215	10.9
25	c	12.8	7.6	217	10.2
50	a	12.9	8	276	10.6
50	b	12.9	7.7	271	10.9
50	c	12.8	7.7	278	10.4
100	a	12.9	7.8	373	10.9
100	b	12.8	7.6	394	10.4
100	c	12.8	7.7	381	10.7
200	a	12.9	8	597	11.2
200	b	12.9	7.7	587	11.1
200	c	12.9	7.8	581	10.9
400	a	12.9	7.8	976	10.6
400	b	12.9	7.7	1002	10.8
400	c	12.8	7.8	969	10.9
800	a	12.9	7.7	1,788	10.6
800	b	12.9	7.9	1,702	11.3
800	c	12.9	7.7	1,682	10.9
Spare	—	13	8.4	162	11.5

KCl added, in mg/L	Replicate	Water temperature, in °C	Water pH	Water conductivity, in µS/cm	Dissolved oxygen, in mg/L
			Day 3		
0	a	13	7.9	160	11.7
0	b	12.8	7.9	163	11.1
0	c	12.8	8.1	158	11.3
25	a	12.9	7.9	205	11.3
25	b	12.8	7.8	200	11.2
25	c	12.8	7.8	205	10.8
50	a	12.8	7.8	260	11.3
50	b	12.8	7.9	259	10.9
50	c	12.8	7.9	263	11.1
100	a	12.8	7.9	374	11
100	b	12.8	7.7	369	10.7
100	c	12.8	7.7	369	10.6
200	a	12.9	7.9	598	11.8
200	b	12.9	8	584	11.3
200	c	12.8	8	575	11.3
400	a	12.8	7.7	1,065	11.1
400	b	12.8	7.8	999	10.9
400	c	12.8	7.8	1,084	11
800	a	12.9	7.8	1,659	10.9
800	b	12.8	7.9	1801	11.4
800	c	12.9	7.7	1808	10.8
Spare	—	13	8.3	155	12.7
			Day 4		
0	a	13	8	169	11.3
0	b	12.8	7.9	171	10.9
0	c	12.8	8.1	166	11.3
25	a	12.9	7.9	208	11.2
25	b	12.8	8	209	11.2
25	c	12.8	7.8	212	10.8
50	a	12.8	7.8	268	11.1
50	b	12.7	7.9	270	10.9
50	c	12.8	8.0	270	11.1
100	a	12.8	7.9	382	11.1
100	b	12.8	7.8	381	10.7
100	c	12.8	7.8	378	10.6
200	a	12.8	7.9	605	11.5
200	b	12.9	8	592	11.2
200	c	12.8	8	586	11.3
400	a	12.8	7.8	1,076	11.1
400	b	12.8	7.7	1,005	11
400	c	12.8	7.8	1,094	11
800	a	12.8	7.8	1,675	11
800	b	12.8	8	1,812	11.4
800	c	12.9	7.8	1,818	10.7
Spare	—	13	8.3	157	12.2

Appendix 3*C*. Water-quality measurements (temperature, pH, conductivity, and dissolved oxygen) collected daily from all experimental tanks for the 96-hour potassium chloride (KCl) toxicity test with Chinook salmon at high baseline water conductivity.

[mg/L, milligrams per liter; °C, degrees Celsius; µS/cm, microsiemens per centimeter; —, not applicable]

KCl added, in mg/L	Replicate	Water temperature, in °C	Water pH	Water conductivity, in µS/cm	Dissolved oxygen, in mg/L
			Day 1		
0	a	12.9	8.3	577	11.3
0	b	12.9	8.3	588	10.9
0	c	12.8	8.4	529	11.2
25	a	12.9	8.4	614	11.3
25	b	12.8	8.4	591	11.3
25	c	12.7	8.3	631	10.6
50	a	12.8	8.4	695	11.1
50	b	12.9	8.3	688	10.8
50	c	12.9	8.5	607	11.2
100	a	12.8	8.4	752	11.2
100	b	12.8	8.4	718	11.3
100	c	12.8	8.4	741	11
200	a	12.9	8.4	930	11.5
200	b	12.9	8.4	882	11.4
200	c	12.8	8.4	930	11.2
400	a	12.9	8.2	1,389	10.6
400	b	12.9	8.3	1,384	10.9
400	c	12.8	8.3	1,312	11.2
800	a	12.9	8.3	2,240	11
800	b	12.9	8.4	2,180	11.4
800	c	12.9	8.4	2,150	11.1
Spare	—	13	8.4	544	12.2
			Day 2		
0	a	12.9	8.3	509	11.4
0	b	12.9	8.3	517	10.7
0	c	12.8	8.4	445	11.2
25	a	12.9	8.3	547	11.2
25	b	12.8	8.4	511	11.2
25	c	12.9	8.3	561	10.7
50	a	12.8	8.3	629	10.9
50	b	12.9	8.3	626	11
50	c	12.9	8.4	526	11.2
100	a	12.8	8.3	681	11.1
100	b	12.8	8.4	642	11.2
100	c	12.8	8.3	664	11.1
200	a	12.9	8.3	861	11.5
200	b	12.9	8.4	810	11.2
200	c	12.8	8.4	851	11.2
400	a	12.9	8.2	1,237	10.7
400	b	12.8	8.3	1,327	10.9
400	c	12.8	8.4	1,263	11
800	a	12.9	8.3	2,180	10.9
800	b	12.8	8.4	2,110	11.2
800	c	12.9	8.4	1,977	11.1
Spare	—	13	8.4	458	12.2

KCl added, in mg/L	Replicate	Water temperature, in °C	Water pH	Water conductivity, in µS/cm	Dissolved oxygen, in mg/L
			Day 3		
0	a	13	8.3	569	11.4
0	b	12.9	8.3	586	11
0	c	12.8	8.4	518	11.4
25	a	12.9	8.3	608	11.4
25	b	12.8	8.4	584	11.4
25	c	12.9	8.3	624	11.4
50	a	12.9	8.3	686	11.2
50	b	12.8	8.3	685	11
50	c	12.9	8.5	610	11.2
100	a	12.8	8.4	760	11.2
100	b	12.8	8.4	720	10.8
100	c	12.8	8.4	748	11.2
200	a	13	8.4	949	11.7
200	b	13	8.5	907	11.4
200	c	12.9	8.4	919	11.2
400	a	12.9	8.2	1,419	10.9
400	b	12.9	8.3	1,365	10.9
400	c	12.9	8.3	1,335	11.2
800	a	12.9	8.3	2,330	11.1
800	b	12.9	8.4	2,270	11.4
800	c	12.9	8.4	2,150	11.3
Spare	—	13	8.5	407	12.4
			Day 4		
0	a	13.6	8.3	493	10.6
0	b	13.2	8.2	515	10.5
0	c	13.1	8.3	425	11
25	a	13.3	8.3	525	10.9
25	b	13.2	8.3	498	10.9
25	c	13.2	8.3	551	10.6
50	a	13.2	8.2	616	10.7
50	b	13.3	8.1	608	10.7
50	c	13.3	8.4	524	11.4
100	a	13.1	8.3	674	10.9
100	b	13.2	8.3	633	11.1
100	c	13.2	8.3	667	11
200	a	13.4	8.3	868	10.9
200	b	13.3	8.4	824	11
200	c	13.3	8.3	840	11.7
400	a	13.3	8.1	1,341	9.9
400	b	13.3	8.1	1,299	10.5
400	c	13.1	8.3	1,260	10.9
800	a	13.3	8.2	2,220	10.7
800	b	13.2	8.2	2,200	11
800	c	13.3	8.3	1,969	11.1
Spare	—	13.6	8.4	370	11.2

Appendix 3*D*. Water-quality measurements (temperature, pH, conductivity, and dissolved oxygen) collected daily from all experimental tanks for the 96-hour potassium chloride (KCl) toxicity test with Chinook salmon at low baseline water conductivity.

[mg/L, milligrams per liter; °C, degrees Celsius; µS/cm, microsiemens per centimeter; —, not applicable]

KCl added, in mg/L	Replicate	Water temperature, in °C	Water pH	Water conductivity, in µS/cm	Dissolved oxygen, in mg/L
			Day 1		
0	a	13.3	7.9	167	10.7
0	b	12.9	7.9	170	10.6
0	c	12.8	8.2	167	11.1
25	a	12.9	8	205	10.9
25	b	12.8	8	203	11.1
25	c	12.9	8	199	11
50	a	12.9	7.9	255	10.9
50	b	12	8	256	10.7
50	c	12.9	8.1	258	11.5
100	a	12.9	8	350	11
100	b	12.9	8	369	11.2
100	c	12.9	8	357	11.1
200	a	12.9	8	601	11.1
200	b	12.9	8.1	574	11.3
200	c	12.9	8	531	11.6
400	a	13	7.8	1,026	9.7
400	b	12.9	7.8	1,014	10.5
400	c	12.9	8	899	10.9
800	a	12.9	7.8	1,669	10.6
800	b	12.8	7.9	1,732	11.2
800	c	12.9	7.9	1,719	11.2
Spare	—	13	8.2	159	11.5
			Day 2		
0	a	12.9	8.1	178	10.9
0	b	12.9	8	182	10.7
0	c	12.8	8.2	177	11.1
25	a	12.9	8	216	10.9
25	b	12.8	8	211	11.2
25	c	12.8	8	209	10.9
50	a	12.9	8	264	11
50	b	12.8	8	269	10.7
50	c	12.9	8.2	266	11.4
100	a	12.8	8	361	11
100	b	12.9	8.1	379	11.2
100	c	12.9	8	370	11
200	a	12.9	8	605	11.2
200	b	12.9	8.2	582	11.3
200	c	12.9	8.1	549	11.4
400	a	12.9	7.8	1,044	10.2
400	b	12.9	7.9	1,024	10.6
400	c	12.9	8	909	10.9
800	a	12.9	8	1,681	10.7
800	b	12.8	8	1,746	11.1
800	c	12.9	8	1,736	11.1
Spare	—	12.9	8.3	162	11.5

KCl added, in mg/L	Replicate	Water temperature, in °C	Water pH	Water conductivity, in µS/cm	Dissolved oxygen, in mg/L
			Day 3		
0	a	13.2	8	157	10.8
0	b	13.3	8	163	10.8
0	c	13.1	8.2	160	11.3
25	a	13.3	8	195	10.9
25	b	13.1	8.1	197	11.2
25	c	13.1	8	194	11.1
50	a	13.2	7.9	248	11
50	b	13.1	8	251	10.8
50	c	13	8.1	243	11.6
100	a	13.2	8	356	11.1
100	b	12.9	8	353	11.3
100	c	13	8	361	11.2
200	a	13	7.9	547	11.1
200	b	13.1	8.1	536	11.4
200	c	13	8	544	11.8
400	a	13.3	7.8	1,020	10.1
400	b	13.3	7.8	996	10.6
400	c	13.1	7.9	898	11
800	a	13.3	7.9	1,677	10.9
800	b	13.2	7.9	1,747	11.3
800	c	13.1	7.9	1,630	11.4
Spare	—	13	8.2	167	11.5
			Day 4		
0	a	13.1	8.1	175	11
0	b	13	8.1	176	10.9
0	c	12.9	8.1	170	11.4
25	a	13	8	207	11.1
25	b	13	8.1	200	11.4
25	c	13	8	206	11.2
50	a	13	8	257	11.2
50	b	13.1	8	262	11
50	c	13	8.1	251	11.7
100	a	13	8	365	11.3
100	b	13	8	364	11.5
100	c	13	8	375	11.3
200	a	13.1	8	575	11.4
200	b	13	8.1	546	11.5
200	c	13	8	571	11.8
400	a	13.1	7.9	1,048	10.4
400	b	13	7.8	1,019	10.8
400	c	13	8	1,002	11.2
800	a	13.1	8	1,689	11
800	b	13	7.9	1,789	11.4
800	c	13	8	1,660	11.6
Spare	—	13.1	8.2	176	11.6

Appendix 3*E*. Water-quality parameters for a 24-hour potassium chloride (KCl) exposure evaluating physiological impacts on brook trout at high baseline water conductivity (680 microsiemens per centimeter).

[ID, identifier; °C, degrees Celsius; mg/L, milligrams per liter; µS/cm, microsiemens per centimeter; ND, no data available; note that the time=0 conductivity reading is measured before the addition of KCl to the experimental tank whereas the ionized potassium (K^+) measurement is taken following the potassium chloride (KCl) addition]

Time=0 (Start)						Time=24 hours (Terminus)				
Tank ID	Water temperature, in °C	Water conductivity, in µS/cm	Water pH	Dissolved oxygen, in mg/L	K^+, in mg/L	Tank ID	Water temperature, in °C	Water conductivity, in µS/cm	Water pH	Dissolved oxygen, in mg/L
0-A	13.4	577	8.6	10.6	1	0-A	13.5	481	8.5	10.6
0-B	13.5	574	8.6	10.3	1	0-B	13.5	476	8.5	11
0-C	13.4	495	8.5	10.4	1	0-C	13.5	415	8.4	9.9
0-D	13.4	571	8.7	10.4	2	0-D	13.5	482	8.5	10.4
0-E	13.5	574	8.4	9.8	1	0-E	13.6	491	8.4	9.5
0-F	13.5	491	8.6	10.4	1	0-F	13.6	411	8.4	10.5
0-G	13.5	569	8.5	9.7	1	0-G	13.6	492	8.4	9.6
0-H	13.6	503	8.4	13.6	ND	0-H	13.5	455	8.5	10.9
0-I	13.5	541	8.5	11.2	ND	0-I	13.6	510	8.5	11.6
0-J	13.5	584	8.4	10.2	ND	0-J	13.6	559	8.5	10.3
0-K	13.5	597	8.4	10.2	ND	0-K	13.6	560	8.4	9.9
0-L	13.6	558	8.4	9.7	ND	0-L	13.7	534	8.3	9.6
0-M	13.6	561	8.5	10.2	ND	0-M	13.7	544	8.4	9.9
0-N	13.6	596	8.4	9.4	ND	0-N	13.7	568	8.4	9.4
200-A	13.5	636	8.7	9.9	80	200-A	13.6	892	8.6	10.1
200-B	13.5	610	8.6	10.1	75	200-B	13.6	852	8.5	9.6
200-C	13.5	561	8.6	10.3	81	200-C	13.6	820	8.5	9.7
200-D	13.4	404	8.5	10.4	77	200-D	13.6	720	8.4	9.8
200-E	13.4	460	8.5	10.4	78	200-E	13.6	780	8.5	10.2
200-F	13.5	410	8.4	10.2	77	200-F	13.5	749	8.5	10.2
200-G	13.4	531	8.5	10.2	80	200-G	13.4	885	8.6	9.6
200-H	13.6	522	8.6	10.4	ND	200-H	13.7	860	8.6	10.6
200-I	13.6	523	8.6	10.4	ND	200-I	13.7	860	8.5	10.4
200-J	13.6	555	8.5	9.2	ND	200-J	13.6	948	8.3	9.6
200-K	13.5	550	8.4	10.3	ND	200-K	13.6	914	8.5	10.1
200-L	13.4	555	8.3	9.6	ND	200-L	13.5	954	8.3	9.6
200-M	13.5	513	8.5	10.1	ND	200-M	13.5	899	8.5	10.2
200-N	13.4	495	8.5	10.4	ND	200-N	13.5	871	8.5	10.4

Appendix 3*F*. Water-quality parameters for a 24-hour potassium chloride (KCl) exposure evaluating physiological impacts on brook trout at low baseline water conductivity (150 µS/cm).

[ID, identifier; °C, degrees Celsius; mg/L, milligrams per liter; µS/cm, microsiemens per centimeter; Note that the T=0 conductivity reading is measured before the addition of KCl to the experimental tank whereas the ionized potassium (K^+) measurement is taken following the potassium chloride (KCl) addition]

Time=0 (Start)						Time=24 hours (Terminus)				
Tank ID	Water temperature, in °C	Water conductivity, in µS/cm	Water pH	Dissolved oxygen, in mg/L	K^+, in mg/L	Tank ID	Water temperature, in °C	Water conductivity, in µS/cm	Water pH	Dissolved oxygen, in mg/L
0-A	13.6	154	7.9	11.2	1	0-A	13.5	164	8	11.6
0-B	13.6	154	8	11	0	0-B	13.6	165	8.1	11.6
0-C	13.6	155	8	10.5	0	0-C	13.6	166	8	11.2
0-D	13.6	154	7.9	10.5	0	0-D	13.6	165	8.1	11.3
0-E	13.7	164	7.9	10.2	0	0-E	13.6	170	8	11.3
0-F	13.7	156	7.8	10.3	0	0-F	13.7	165	8	11.2
0-G	13.7	154	7.8	10.2	0	0-G	13.7	167	8	10.8
0-H	13.5	160	8	10.7	0	0-H	13.6	166	8.1	11
0-I	13.6	159	8.1	10.7	0	0-I	13.7	166	8.1	11.1
0-J	13.6	161	8	10.1	0	0-J	13.7	169	8	9.7
0-K	13.6	159	8.1	9.9	0	0-K	13.7	170	8	10.6
0-L	13.7	159	8	10.3	0	0-L	13.8	167	8.1	11.2
0-M	13.7	158	8	10.6	0	0-M	13.8	165	8	11.1
0-N	13.7	160	8	10.3	0	0-N	13.8	170	8	11.2
200-A	13.7	154	8.1	10.2	93	200-A	13.7	520	8.1	11.2
200-B	13.7	156	8	10.7	90	200-B	13.7	550	8.1	11.2
200-C	13.6	158	8.1	10.3	91	200-C	13.6	550	8	10.9
200-D	13.6	154	8.1	10.1	92	200-D	13.6	553	8	10.6
200-E	13.5	157	8.1	10.8	88	200-E	13.5	491	8	10.6
200-F	13.5	162	8.1	10.6	90	200-F	13.5	468	8.1	10.8
200-G	13.5	162	8.1	10.5	91	200-G	13.4	478	8	10.2
200-H	13.7	159	8	10.5	92	200-H	13.8	540	8	11.5
200-I	13.7	159	8	10.6	94	200-I	13.7	539	8	10.6
200-J	13.6	159	8	10.2	90	200-J	13.7	532	8	10.2
200-K	13.5	159	8	10.2	93	200-K	13.6	534	8	10.4
200-L	13.6	160	8	9.7	90	200-L	13.7	547	8	10
200-M	13.5	160	8	10.4	98	200-M	13.6	562	8	10.6
200-N	13.4	160	8	9.6	98	200-N	13.5	557	8.2	10.6

Appendix 3*G*. Water-quality parameters for a 10-day potassium chloride (KCl) exposure for the evaluation of physiological impacts on Chinook salmon.

[ND, no data available; K^+, ionized potassium; °C, degrees Celsius; mg/L, milligrams per liter; ppm, parts per million]

Trial day	Water temperature, in °C	Water conductivity, in µS/cm	Water pH	Estimated dissolved oxygen, in mg/L	Total ammonia nitrogen, in mg/L	Nitrate, in mg/L	K^+, in mg/L
0 milligrams per liter KCl (control)							
0	12.1	298	7.9	10.7	0	ND	1
1	12.7	309	7.9	10.5	0	ND	1
2	ND	ND	ND	ND	ND	ND	ND
3	12.2	311	7.9	10.5	0	ND	1
4	11.6	314	7.8	10.5	0	ND	1
5	ND	ND	ND	ND	ND	ND	ND
6	12.6	316	7.9	10.5	0	ND	1
7	12.8	316	8	10.5	ND	ND	1
8	12.2	319	8	10.5	0	4	1
9	12.6	321	7.9	10.5	ND	ND	1
10	11.9	322	8.1	10.5	0.05	5	1
200 ppm KCl exposure							
0	11.8	287	7.8	10.5	0	2	1
1	11.8	693	8	10.5	0	4	74
2	ND	ND	ND	ND	ND	ND	ND
3	11.7	698	7.8	10.5	0	4	70
4	11.9	701	7.8	10.5	ND	ND	ND
5	11.8	699	7.8	10.5	0	5	74
6	11.9	706	7.8	10.5	ND	ND	ND
7	12	707	7.9	10.5	0	6	71
8	ND	ND	ND	ND	ND	ND	ND
9	12	712	7.9	10.5	0	4	82
10	11.7	716	7.8	10.5	1	4	82

Appendix 4. Behavioral and morphological changes observed among acute toxicity tests for Chinook salmon and brook trout.

[These changes were observed among both species and in various potassium chloride (KCl) test concentration groups and observation times, as noted. None of these changes were statistically significant (one-way analysis of variance [ANOVA]); $p<0.05$; mg/L, milligrams per liter]

Species	Abnormality	KCl concentration at effect, in mg/L	Duration of exposure at onset, in days	Total number of fish
Brook trout	Hyper-excitable swimming	50, 100	1	3
	Tail undulation at rest	50	1	1
Chinook salmon	Mildly elevated respiratory rate	25, 50, 400	1+	6
	Hyper-excitable swimming	0, 25, 50, 100, 200, 800	1+	24
	Mildly lethargic swimming	800	4	1
	Deformed/damaged opercle	100, 200, 400	1+	9
	Coughing	25	1	1
	Hypopigmentation	200	4	1
	Hyperpigmentation	400	4	1

Appendix 5. Histological changes noted among brook trout and Chinook salmon in the 96-hour acute toxicity testing.

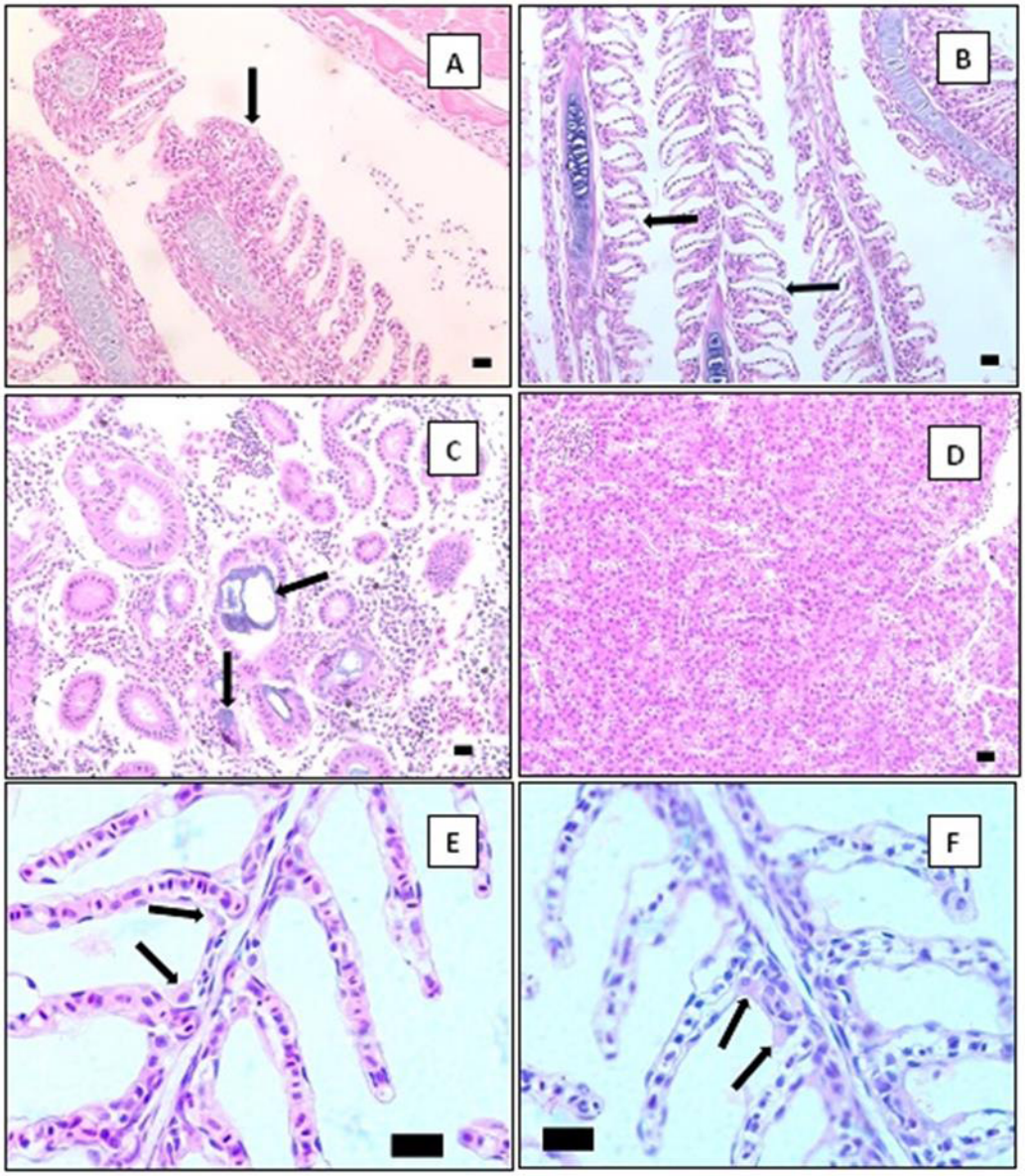
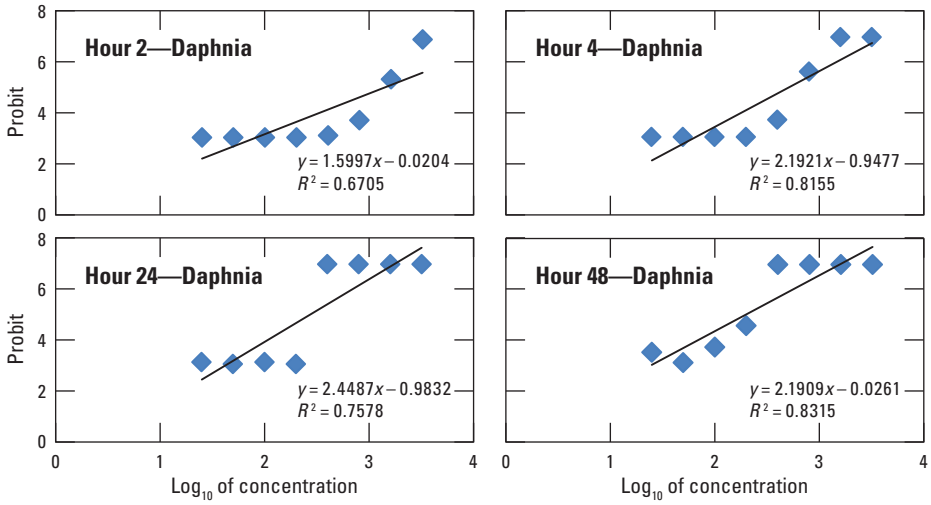


Figure 5-1. None of the changes were significantly different in frequency of occurrence among the test groups and all were noted incidentally with no clinical indication of disease among the fish: *A*, Epithelial lifting of gill lamellae (Chinook salmon); *B*, Gill epithelial hyperplasia along distal lamellae (brook trout); *C*, Mineral deposition in tubule lumen with the excretory kidney; *D*, Hepatocellular vacuolation; *E*, Interlamellar chloride cells along the gill epithelium in a brook trout; *F*, Interlamellar chloride cells along the gill epithelium in a Chinook salmon. Black arrows indicate the histological change/finding and black scale bars within each photograph represent 20 microns length.

Appendix 6. Log probit analysis calculation of the potassium chloride lethal concentration (KCl LC_{50}) concentrations for daphniid toxicity trials.

[Probit analyses are displayed as graphs and tabular data for the LC_{50} calculations at 2 hours, 4 hours, 24 hours, and 48 hours]



Concentration	Log_{10} of concentration	Percent dead	Corrected percent	Probit	Calculated LC_{50}, in milligrams per liter	Log_{10} of concentration at probit=5
			Hour 2			
25	1.397940009	0	2.5	3.04	1,375.111772	3.138338438
50	1.698970004	0	2.5	3.04		
100	2	0	2.5	3.04		
200	2.301029996	0	2.5	3.04		
400	2.602059991	3	3	3.12		
800	2.903089987	10	10	3.72		
1,600	3.204119983	63	63	5.33		
3,200	3.505149978	97	97	6.88		
			Hour 4			
25	1.397940009	0	2.5	3.04	516.7054103	2.713243009
50	1.698970004	0	2.5	3.04		
100	2	0	2.5	3.04		
200	2.301029996	0	2.5	3.04		
400	2.602059991	10	10	3.72		
800	2.903089987	73	73	5.61		
1,600	3.204119983	100	97.5	6.96		
3,200	3.505149978	100	97.5	6.96		
			Hour 24			
25	1.397940009	3	3	3.12	277.5996773	2.443418957
50	1.698970004	0	2.5	3.04		
100	2	3	3	3.12		
200	2.301029996	0	2.5	3.04		
400	2.602059991	100	97.5	6.96		
800	2.903089987	100	97.5	6.96		
1,600	3.204119983	100	97.5	6.96		
3,200	3.505149978	100	97.5	6.96		
			Hour 48			
25	1.397940009	7	7	3.52	196.8249084	2.294080058
50	1.698970004	3	3	3.12		
100	2	10	10	3.72		
200	2.301029996	33	33	4.56		
400	2.602059991	100	97.5	6.96		
800	2.903089987	100	97.5	6.96		
1,600	3.204119983	100	97.5	6.96		
3,200	3.505149978	100	97.5	6.96		

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