United States Environmental Protection Agency

Office of Water
4304T

EPA-822-R-07-010
December 2016

Public Review Draft

Field-Based Methods for Developing Aquatic Life Criteria for Specific Conductivity

U.S. Environmental Protection Agency
Office of Water, Washington, DC

PUBLIC REVIEW DRAFT

Field-Based Methods for Developing Aquatic Life Criteria for Specific Conductivity

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Office of Science and Technology
Washington, DC

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LIST OF ABBREVIATIONS AND ACRONYMS

B-C	background-to-criterion
CCC	criterion continuous concentration
CDF	cumulative distribution function
CI	confidence interval
CMEC	criterion maximum exposure concentration
DO	dissolved oxygen
EMAP	Environmental Monitoring and Assessment Program
EPA	U.S. Environmental Protection Agency
GAM	generalized additive model
HCx	hazardous concentration of the "*x*" centile of a taxonomic sensitivity distribution
LOWESS	Locally Weighted Scatterplot Smoothing
MAHA	Mid-Atlantic Highland Assessment
MAIA	Mid-Atlantic Integrated Assessment
NAPAP	National Acid Precipitation Assessment Program
NRSA	National Rivers and Streams Assessment
NWSA	National Wadeable Streams Assessment
PL	prediction limit
QA/QC	quality assurance/quality control
RBP	rapid bioassessment protocol
R-EMAP	Regional Environmental Monitoring and Assessment Program
S	Siemens
SAB	Science Advisory Board
SC	specific conductivity
TDS	total dissolved solids
TMDL	total maximum daily load
TSS	total suspended solids
USGS	U.S. Geological Survey
WABbase	Watershed Assessment Branch database
WDE	Washington Department of Ecology
WSA	Wadeable Streams Assessment
WVDEP	West Virginia Department of Environmental Protection
XCx	extirpation concentration affecting "*x*" percentage of individuals of a taxon
XCD	extirpation concentration distribution

NOTICES

This public review draft document has undergone two contractor-led external peer reviews as well as a review process within the U.S. Environmental Protection Agency (EPA). Final review by EPA's Office of Science and Technology, Health and Ecological Criteria Division, has been completed and the document has been approved for publication.

This document provides draft methods to assist states and tribes in the development of water quality criteria and other tools to protect aquatic life from effects of elevated ionic concentration as measured by specific conductivity (SC)[1] in flowing waters. States and tribes planning to develop water quality criteria for SC may consider using alternative, scientifically defensible methods. While this document reflects EPA's assessment of the best available science for identifying ambient concentrations of SC in flowing waters that protect aquatic life, it is not a regulation and does not impose legally binding requirements on EPA, states, tribes, or the regulated community, and might not apply to a particular situation based upon the circumstances. EPA may change this document in the future.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use. This document can be downloaded from: https://www.epa.gov/wqc/aquatic-life-ambient-water-quality-criteria.

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[1]This document uses conductivity as a measure of ionic concentration rather than as description of an electrical property of water. As ionic concentration increases, conductivity increases. The terms specific conductivity and specific conductance are often used synonymously in the open literature indicating normalization or measurement at 25°C. Conductivity is a property of water expressed in units of micro-Siemens per centimeter (µS/cm). Conductance of a sample or electrical component is measured as Siemens (S). All measurements in this document refer to specific conductivity, µS/cm at 25°C.

FOREWORD

This document, *Draft Field-based Methods for Developing Aquatic Life Criteria for Specific Conductivity,* provides states and tribes with methods that may be used to develop criteria to protect aquatic life from effects of elevated ionic concentration as measured by specific conductivity (SC) in flowing waters. The EPA tailored these methods to enable derivation of specific conductivity criteria on the scale of Level III ecoregions (Omernik, 1995, 1987) in order to account for natural differences in background ionic concentrations among ecoregions. There are 85 Level III ecoregions in the contiguous United States. Each of the states in the contiguous United States contains 1 to 12 Level III ecoregions within their political boundaries. The EPA is also providing several case studies to illustrate how these draft methods may be applied to different ecoregions with varying background ionic concentrations. The EPA may change the field-based methods and/or provide additional case studies in the future as new scientific information becomes available.

This document is nonregulatory and provides only a scientific assessment of ecological effects. It does not establish or affect legal rights or obligations. It does not establish a binding norm and cannot be finally determinative of the issues addressed. Agency decisions in any particular situation will be made by applying the Clean Water Act and EPA regulations on the basis of specific facts presented and scientific information then available.

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EXECUTIVE SUMMARY

This document describes draft methods that states and tribes may use to derive field-based ecoregional ambient aquatic life criteria for specific conductivity (SC), a measurement of the concentration of ions, in flowing waters. The document also provides four case studies to illustrate how these draft field-based methods may be used to develop criteria in ecoregions with different background ionic concentrations measured as SC and to demonstrate how to assess the applicability of criteria developed for one ecoregion to a different ecoregion. The case studies use field data to demonstrate how to apply the methods described in this document to derive example criteria for SC for flowing waters dominated by calcium, magnesium, sulfate, and bicarbonate ions but not for flowing waters dominated by chloride salts. Elevated ionic concentration measured as SC has been shown to impact aquatic life in a range of freshwater resources. Different mixtures of ions that increase SC are associated with multiple anthropogenic sources, including discharges from wastewater treatment facilities, groundwater recharge affected by climate change, surface mining, oil and gas exploration, runoff from urban areas, and discharges of agricultural irrigation return waters, among others.

The EPA relied on its *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (1985) (EPA/822/R-85/100) and *A Field-Based Aquatic Life Benchmark for Conductivity in Central Appalachian Streams* (hereafter referred to as the "*EPA Benchmark Report*") (EPA/600/R-10/023F), among other documents, to develop the draft field-based method for SC. In the *EPA Benchmark Report*, EPA used a field data set to estimate a numeric SC benchmark for Appalachian streams. The EPA validated the method and the benchmark using an independent data set. In 2011, internal and external reviewers, including EPA's Science Advisory Board (SAB) (U.S. EPA, 2011c), favorably reviewed the analyses and method. This current document uses that same method to estimate a protective criterion continuous concentration (CCC) for chronic (long-term) exposures as well as additional methods to estimate a maximum exposure concentration protective of acute toxicity. This document also provides recommendations for SC criterion duration and frequency.

The EPA typically relies on laboratory toxicity test data for surrogate species as defined in the Agency's *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (U.S. EPA, 1985) for aquatic life criteria development. The draft field-based methods used here were adapted to be consistent with the intent of the Agency's traditional approach to derive aquatic life criteria (U.S. EPA, 1985). The draft field-based methods rely on geographically referenced, paired observations of SC and the presence and absence or abundance of freshwater benthic macroinvertebrate genera from wadeable perennial streams. The case studies that are included to illustrate the method are based on more than 4,000 paired biological (macroinvertebrate) and chemical (SC) field samples from more than 3,000 stations over a 15-year period (1996−2010). An analysis of data for fish from a composite of case study ecoregions demonstrates that the example criteria based on macroinvertebrates are also protective of fish.

For this draft field-based method, the valued resource is the aquatic community. The ecological entities defining the assessment endpoints are macroinvertebrate genera and the measure of effect is extirpation, or effective absence of such genera from a site (the desired attribute is occurrence). Two relationships are derived: one for each macroinvertebrate genus and one for the overall aquatic community. First, a weighted cumulative distribution function (CDF) is developed for each genus to determine the genus extirpation concentration (XC_{95} or 95th centile of the distribution of the occurrences of a genus), the level of exposure above which a macroinvertebrate genus is effectively absent from water bodies in a region or other study area (U.S. EPA, 2011a, 2003). That is, the probability is 0.05 that an observation of a genus would occur above its XC_{95} SC value. Second, the HC_{05} (hazard concentration 5th centile) is developed using a genus-level extirpation concentration distribution (XCD) for the community from the aggregation of the XC_{95} values. This effect threshold is consistent with the intent of EPA's guidelines for aquatic life criteria development (U.S. EPA, 1985), which are designed to protect aquatic animal species (i.e., 95%) in a community.

The HC_{05} is a chronic-duration endpoint and used for derivation of a CCC because it is derived from biological field data that include exposure over whole life cycles and multiple generations of the resident biota. A criterion maximum exposure concentration (CMEC), a level of protection from acutely toxic exposures, is also derived based on stream water chemistry data. The CMEC is estimated at the 90th centile of observations at sites with water chemistry regimes meeting the CCC. The CMEC is the maximum SC level that may occur for a short duration and be protective of 95% of macroinvertebrate genera. Both of these distinct expressions of the example SC criteria would need to be met in order to adequately protect aquatic life.

The type of model used in this draft method, a genus-level XCD, describes how genera in biotic communities in general respond to a stressor (e.g., an ionic mixture dominated by sulfate and bicarbonate salts). This method is based on a distribution of extirpation concentrations and is called the XCD method to distinguish it from other field-based methods. Like the surrogate aquatic taxa that form the minimum data set for laboratory-based aquatic life criteria, the macroinvertebrate taxa included in the case studies are surrogate taxa that represent a potentially exposed aquatic community (U.S. EPA, 1985).

GLOSSARY

Assessment endpoint—An explicit expression of the actual environmental value that is to be protected, operationally defined by an ecological entity and its attribute or characteristics. An assessment endpoint may be identified at any level of organization (e.g., organism, population, community).

Assemblage, stream—A taxonomic or sampled subset of a community as may be collected from a stream.

Background specific conductivity—The specific conductivity (SC) in streams in a region that occurs naturally and not as the result of human activity. Background may also be characterized as a population of minimally affected sites or low SC sites using a weight of evidence.

Benchmark—A dose or concentration of a pollutant that, if exceeded, is expected to produce an adverse effect (called the benchmark response) in one or more assessment endpoints, signifying a decline in water quality or human health.

Bootstrapping—A statistical technique of repeated random sampling from a data set that is often used in environmental studies to estimate confidence and prediction limits of a parameter.

Box plot—A depiction of the 25th, 50th, and 75th quantiles of a distribution as a rectangle with a central line. The two standard deviation range is depicted as "whiskers" extending from the box. Data beyond two standard deviations are indicated by individual circles or dots beyond the whiskers.

Catchment area—The spatial extent of the surrounding landscape that drains into a particular river, stream, or other waterbody.

Chorionic covering—The outermost casing or membranous covering of the egg of various invertebrates.

Community—The full complement of interacting organisms within a defined area of an ecosystem.

Conductivity, specific (or specific electrical conductivity)—A measure of ionic concentration based on the electrical property of water and dissolved ions. As ionic concentration increases, conductivity increases. Standardized measurements in this document refer to specific conductivity, µS/cm (also seen as: µmho/cm) at 25°C.

Conductance, specific—Conductance is the inverse of resistance for a particular sample expressed as Seimens (S) usually at 25°C. In the literature, it is sometimes used synonymously with specific conductivity, but to avoid confusion, the term conductance is not used in this document.

Confounder—An extraneous variable that correlates with both the dependent and independent variable. The presence of confounders can interfere with the ability to characterize a causal relationship.

Criterion continuous concentration (CCC)—An estimate of the highest concentration of a material in surface water to which an aquatic community can be exposed indefinitely without resulting in an unacceptable effect.

Criterion maximum exposure concentration (CMEC)—An estimate of the maximum concentration of a material in surface water to which an aquatic community can be exposed for a short time without resulting in an unacceptable effect. In this document, the CMEC is estimated at the 90th centile of specific conductivity observations that contribute to the annual CCC.

Cumulative distribution function (CDF)—The probabilities that a random variable with a given probability distribution will be found at a value less than or equal to *x*. Weighted CDFs are used to estimate extirpation concentrations of individual genera or species and unweighted CDFs to estimate a SC level that is expected to extirpate 5% of aquatic invertebrate genera.

Ephemeral stream—A stream that flows briefly only in direct response to local precipitation, and whose channel is above the local groundwater table at all times.

Extirpation—The depletion of a population of a species or genus to the point that it is no longer a viable resource or is unlikely to fulfill its function in the ecosystem.

Extirpation concentration—The level above which a genus is effectively absent from its normal habitat. The threshold for extirpation is operationally defined by the level below which 95% of the observations of the genus occur.

Extrapolation—The process of extending the applicability of a model beyond the measured range of the original data set from which the model was derived.

Flowing waters—Inland waters with a unidirectional flow including permanent, intermittent and ephemeral streams.

Generalized additive model—A nonparametric, likelihood-based local regression model that replaces the linear function of a generalized linear model with a locally smoothed additive function.

Hazardous concentration—A concentration threshold that is hazardous for a proportion of taxa. In this document, it is the concentration that is hazardous to 5% of genera calculated as the 5th centile of a taxonomic extirpation concentration distribution.

Intermittent stream—A stream that flows continuously for only part of the time. During low flow there may be dry reaches alternating with wetted, nonflowing reaches. The stream bed may lie below the local groundwater table for at least part of the year.

Interpolate—Process of estimating an unknown value that lies between known values.

Ionic composition—The specific ions dissolved in water. In this document, the ionic composition is used to distinguish water dominated by chloride salts from those dominated by bicarbonate and sulfate salts.

Ionic mixture—An undefined or defined blend of dissolved ions. In this document, the example case studies refer to the most common mixture of ions contaminating U.S. streams, specifically those dominated by calcium, magnesium, sulfate, and bicarbonate ions.

Ionic regulation—The passive and active physiological processes that maintain the ionic composition, pH, and water content of tissues that is necessary for life.

Least disturbed condition—the best available physical, chemical, and biological habitat conditions given today's state of the landscape or the least disturbed by human activities (Stoddard et al., 2006). Contrast with "minimally affected condition."

Major ions—The most common contributors to ionic concentration in surface waters, consisting of the following cations: Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}; and anions: HCO_3^{-}, CO_3^{2-}, SO_4^{2-}, Cl^{-}.

Measure of effect—A measurable ecological characteristic that is related to the valued characteristic chosen as the assessment endpoint and is a measure of biological effects (e.g., survival, reproduction, growth). In this document it is the presence/absence of macroinvertebrate genera along a specific conductivity gradient.

Measure of exposure—A measured or estimated characteristic that is used to characterize the level of exposure to the stressor. In this document, the measure of ionic exposure is specific conductivity.

Minimally affected condition—The physical, chemical, and biological habitat found in the absence of significant human disturbance (Stoddard et al., 2006). Contrast with "least disturbed condition."

Osmoregulation—The physiological control of water content of an organism's tissues to maintain fluid and electrolyte balance within a cell or organism relative to the surrounding environment.

Perennial stream—A stream with continuous surface or shallow interstitial flow year-round, and whose stream bed intersects the local groundwater table throughout the year. Also referred to as a permanent stream.

Produced water—Waters that are produced by oil and gas development, mine dewatering, and related activities (e.g., coal bed methane mining, hydraulic fracturing).

Reference site—Sampling locations that have been identified as minimally affected or least disturbed based on land use, habitat, and water quality characteristics other than specific conductivity.

Salinity—The amount of salts dissolved in water. Traditionally expressed as parts per thousand (‰) or grams of salt per kilogram of water.

Sensitivity analysis—A process that involves changing input values of a model in various ways to see the effect on the output value. The main goal of sensitivity analysis is to gain insight into which assumptions are most critical for model building.

Total dissolved solids (TDS)—A measure of the combined content of all inorganic and organic substances dissolved in water, conventionally expressed as mg/L and operationally defined as those solids that pass through a filter, typically 0.45 μm.

Univoltine—An organism having one brood or generation per year.

Validation—Confirmation of the quality of a model and its results, typically by applying an independent data set.

Valley fill—A headwater valley filled with mining overburden. This practice usually occurs in steep terrain where there are limited disposal alternatives.

Verification—Demonstrating the accuracy of measurements or calculations.

1. INTRODUCTION AND BACKGROUND

This document describes a set of draft methods that states and authorized tribes may use to derive field-based ecoregional ambient aquatic life criteria for ionic mixtures measured as specific conductivity (SC), a measurement of ionic concentration. Four case studies illustrate how these draft methods may be applied to develop such criteria in different ecoregions with different background SC and data sets. The case studies illustrate how these methods may be used to develop criteria applicable to flowing waters dominated by sulfate and bicarbonate salts. Chloride constitutes less than half of the total anions in the case examples. Although the methods may be appropriate for use with other ionic mixtures, the example criteria generally are not appropriate for waters with different ionic compositions (e.g., waters dominated primarily by sodium chloride).

Among the documents the U.S. Environmental Protection Agency (EPA) relied upon to develop the draft field-based method for SC are EPA's *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (U.S. EPA, 1985) and *A Field-Based Aquatic Life Benchmark for Conductivity in Central Appalachian Streams* (hereafter referred to as the "*EPA Benchmark Report*") (U.S. EPA, 2011a). The EPA used an extensive field data set in the *EPA Benchmark Report* to estimate a numeric SC benchmark. The EPA validated the method and benchmark using an independent data set. The *EPA Benchmark Report* provides details on the approach, as well as a causal analysis of the stressor-response relationship and a confounder analysis that explored the potential influence of habitat, water quality factors, other pollutants, and other factors. Internal and external reviewers, including EPA's Science Advisory Board (SAB), reviewed the primary method and derivation of the SC benchmark and validation exercises in 2011 (U.S. EPA, 2011c). Subsequently, the method and results of its application were published (Cormier and Suter, 2013a, b; Cormier et al., 2013a, b, c; Suter and Cormier, 2013). This current draft document uses that method as well as additional methods to estimate a protective maximum exposure concentration, duration, and frequency. It also presents a draft method for assessing applicability of field-based SC criteria developed in one geographic area to another area. In 2014 and 2015, panels of five external experts (selected independently by an EPA contractor) reviewed these additional draft methods.

These draft field-based methods may be used to develop SC criteria on the scale of Level III ecoregions (Omernik, 1995, 1987) in order to take into account natural ecoregional differences in background SC. In some areas, it may be appropriate to derive criteria at a different scale because background conductivity or ionic composition varies significantly across a Level III ecoregion (see Section 6 for an example). There are 85 Level III ecoregions in the continental United States (Omernik, 1995, 1987). SC tends to be low in most eastern and western montane ecoregions (25^{th} centiles of SC <200 µS/cm), intermediate in the midcontinent (200−600 µS/cm), and very high in arid areas (>600 µS/cm) (Griffith, 2014). States and tribes may use this method to derive ecoregional criteria for SC at a level that protects 95% of resident macroinvertebrate genera based on field sampling data from a set of sites within the ecoregion or from another ecoregion, when applicable.

The EPA typically relies on laboratory toxicity test data as defined in the Agency's *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (U.S. EPA, 1985). EPA designed the draft field-based methods described herein to be consistent with the intent of the Agency's traditional approach used to derive aquatic life criteria (U.S. EPA, 1985). Like the Agency's traditional approach, criteria derivation through field-based methods can capture characteristics of the stressor and the ecosystems potentially at risk (e.g., stressor occurrence and distribution, stressor-response relationships).

The structure of this draft document, *Field-based Methods for Developing Aquatic Life Criteria for Specific Conductivity*, is consistent with the EPA's *Guidelines for Ecological Risk Assessment* (U.S. EPA, 1998a; Suter and Cormier, 2008). The assessment begins with a planning phase, termed *Problem Formulation* (see Section 2), in which the stressor of concern is identified, its presence in the environment and potential impacts are described, and assessment endpoints (i.e., specific ecological entities and attributes to be protected and the level of protection to be achieved) are identified. In the case studies, the stressor is a mixture of ions in the form of dissolved bicarbonate and sulfate salts, measured as specific SC, in the field. The endpoint populations are aquatic benthic macroinvertebrates and the measure of effect is extirpation not to exceed 5% of genera. Section 2 serves as the *Problem Formulation* in general and for all four case studies.

In this draft document, the *Analysis Plan* (see Section 3), which is the last step in *Problem Formulation,* is included as a separate stand-alone section. The *Analysis Plan* describes three methods, (1) a field-based method that states may use to directly derive field-based aquatic life criteria for SC (the extirpation concentration distribution [XCD] method), (2) a regression model that can be used to derive criteria from minimally affected background (the background-to-criterion [B-C] model method), and (3) a method to assess the geographic applicability (extent) of the criteria using a weight-of-evidence approach. Section 3 serves as the *Analysis Plan* for this draft method in general and for all four of the case studies that follow in the *Case Study Analysis* sections. Each of the methods considers the causal relationship between exposure to major aqueous ions and the response of macroinvertebrates.

Next, in the *Case Study Analysis* sections (see Sections 4 and 5), the application of the draft XCD method is illustrated by deriving example SC criteria for different ecoregions with ecoregion-specific data sets. These sections describe magnitude, frequency, and duration as well as factors characterizing geographic range (see Case Studies I and II, Sections 4 and 5). Two other case studies demonstrate how to use the B-C regression method that predicts criteria from minimally affected background (see Case Studies III and IV, Sections 6 and 7). In these case studies, there are several factors relevant to determining geographic applicability (spatial extent of the criteria); among the most important are background SC and the composition of the ionic mixture present (ions of bicarbonate and sulfate salts).

Appendices A and B provide supporting materials, including assessments of potential confounding factors, and plots and effect levels for all genera represented in ecoregional XCDs used in the development of the Case Studies I and II (see Appendices A for Case Study I and B for Case Study II). Appendix C discusses the characterization of background SC and the seasonal regime of a region (a condition assessment) and includes a specific example for Case Study II. Appendix D provides the derivation of a B-C regression model that uses minimally affected background SC to calculate a SC criterion that is useful for areas lacking sufficient data to use the XCD method (see application of this model in Case Studies III and IV). Appendix E provides extirpation concentration (XC_{95}) values for the combined data sets used for Case Studies I and II. Appendix F provides results using an alternate measure of the ionic mixture, sulfate plus bicarbonate (as mg/L). Appendix G provides an analysis that shows that some fish in streams are intolerant of high ionic concentrations and that fish are protected by criteria derived by applying the XCD method to benthic invertebrate data.

Data quality reviews of project data sets were conducted to ensure that the data used and the results of the analyses are accurate and complete. When invalid or incorrect data were identified, these data were either corrected or excluded from analyses. Methods for data extraction, data management, model development, and quality assurance/quality control (QA/QC) for this project are described in the Quality Assurance Project Plan, prepared by Tetra Tech, Inc. 2014. Validation and other QA analyses are described as each model or case study are also presented.

2. PROBLEM FORMULATION

This section serves as the *Problem Formulation* for the XCD method in general and for the case studies, which are presented in Sections 4, 5, 6, and 7. *Problem Formulation* begins with identification of the problem (see Section 2.1), the stressor of concern and its sources (see Section 2.2), and a description of how it can be measured (see Section 2.3). In the case examples, the stressor is a mixture of ions in a form dominated by bicarbonate and sulfate salts, measured using SC. The nature of effects (see Section 2.4), and mechanisms and modes of action are described (see Section 2.5). The assessment endpoints and measures of effect are described (see Section 2.6). The organisms are freshwater benthic macroinvertebrates and the measurement of effect is extirpation of 5% of genera. Extirpation is the depletion of an assessment population of a species or genus (in this case, it is the population in a stream) to the point that it is no longer a viable resource or is unlikely to fulfill its function in the ecosystem (U.S. EPA, 2003). Specifically, this effect threshold is defined in this document as the ionic concentration below which 95% of the observations of the genus occur, representing the extreme of an organism's tolerance to an ionic mixture. In the case studies, the ionic mixture as measured by SC is dominated by sulfate and bicarbonate salts, with either calcium and magnesium or sodium and potassium as the cations (U.S. EPA, 2011a). This effect threshold is consistent with the intent of EPA's guidelines for aquatic life criteria development (U.S. EPA, 1985), which are designed to protect aquatic animal species (i.e., 95%) in a community. The *Problem Formulation* section concludes with the rationale for selection of a field-based method for derivation of criteria for the ionic mixture (see Section 2.7).

2.1. PROBLEM IDENTIFICATION

Stress from elevated ionic concentration, measured as specific SC, has been shown to cause significant adverse effects on a range of freshwater ecosystems across the Nation (e.g., Cañeda-Argüelles, et al., 2013; Higgins and Wilde, 2005; Kaushal et al., 2013, 2005; Pond et al., 2008; U.S. EPA, 2011a). The sources of ions in surface waters may be natural, reflecting soils and geology, or anthropogenic. The two most common ionic mixtures in streams are those dominated by either chloride anions (Cl^-) or those dominated by bicarbonate (HCO_3^-) plus sulfate (SO_4^{2-}) anions based on mass (Hem, 1985; Griffith, 2014). The field-based methods are illustrated using case examples with flowing waters with ionic mixtures dominated by HCO_3^- plus SO_4^{2-}. Based on mass, Cl^- constitutes less than half of the total anions in the case examples.

2.2. STRESSOR OF CONCERN—SALTS

Ionic stress has been implicated as a cause of biological impairment in aquatic systems throughout the United States (e.g., Findlay and Kelly, 2011; Farag, and Harper, 2012; Dunlop et al., 2015; Boelter, et al., 1992; Higgins and Wilde, 2005; Johnson et al., 2013; Karatayev et al., 2012; Kaushal et al., 2013, 2005; Fritz et al., 2010; Gerritsen et al., 2010; Palmer et al., 2010; Lindberg et al., 2011; Merriam et al., 2011; Pond et al., 2008, Pond, 2010; U.S. EPA, 2011a,b; Bernhardt et al., 2012; Cormier et al., 2013b; Timpano et al., 2011; Zhao et al., 2016). Nationally, sources of salts can be natural from rock formations and soils or can be associated with human activities and may be exacerbated by changes in climate. Sources include coastal salt water intrusion, irrigation, combustion wastes, resource exploration and extraction, demineralization of concrete, runoff from urban areas, inputs from deicing roads, and sewage and industrial waste (Ziegler et al., 2010; Cañeda-Argüelles, 2013) (see Table 2-1). Furthermore, salts from different sources have different ionic compositions. For example, marine evaporite deposits are dominated by NaCl whereas weathering of minerals such as limestone and dolomite produce Ca^{2+}, Mg^{2+}, and HCO_3^- salts (Hem, 1985).

Consistent with the *EPA Benchmark Report* (U.S. EPA, 2011a), these draft field-based methods may be applied for any waters with a defined ionic composition because the toxicity to aquatic organisms is dependent on the ionic composition of the solution (Mount et al., 1997; Mount et al., 2016; Erickson et al., 2016; Zalizniak et al., 2006; van Dam et al., 2010; Dunlop et al., 2005; Soucek and Kennedy, 2005; Bradley, 2009; Evans, 2008a,b; Nelson and Cox, 2005, Johnson et al., 2015). Aquatic organisms are adapted to different ionic regimes and have different tolerances to changes in ionic concentration and composition (Remane, 1971; Bradley, 2009). Although certain species, particularly of fish and Crustacea, have life histories and ionoregulatory adaptations that facilitate movement across a salinity gradient (Belli et al., 2009), most groups have distinct lineages of orders and families that are limited to either freshwater or marine environments (Remane, 1971; Berra, 2007). Outside of the physiological tolerance of a species, the toxicity of salts interferes with ionic regulation, osmoregulation, and acid-base balance (Bradley, 2009; Nelson and Cox, 2005).

Because toxicities of ions differ and because the example criteria are derived with data for streams where Ca^{2+} plus Mg^{2+}, and HCO_3^- plus SO_4^{2-} (i.e., not Na^+ and Cl^-) dominate the ionic composition on a mass basis, the case example criteria are not recommended for locations where Cl^- concentrations are greater than the combined concentrations of HCO_3^- plus SO_4^{2-}. However, the XCD method could be used to derive criteria for other ionic mixtures, including locations dominated by Cl^-.

Application of this XCD method relies on the availability of paired chemical and biological samples taken from waters with similar ionic composition (e.g., sulfate- and bicarbonate-dominated). The sites included in the data sets are screened based on ionic composition (e.g., chloride-dominated sites are removed from the data set in the case examples). However, removing them did not appreciably change the results in the case examples because there were so few sites that were chloride dominant.

2.2.1. Sources of Ions

Most fresh waters in the United States exhibit rock dominance (i.e., ion concentrations characteristic of natural weathering of minerals in the catchment) (Gibbs, 1970; Stallard and Edmond, 1987; Anning and Flynn, 2014), and the anion signature of these waters is usually dominated by HCO_3^- plus SO_4^{2-} (Wetzel, 2001; Griffith, 2014). SC tends to be low in mountainous and forested ecoregions (25th centiles of SC ~50−200 μS/cm) and higher in more arid ecoregions (Griffith, 2014; Anning and Flynn, 2014). Nationally, the dominant cation combination is calcium (Ca^{2+}) plus magnesium (Mg^{2+}) and the dominant anions combination is bicarbonate (HCO_3^-) plus sulfate (SO_4^{2-}) (Griffith, 2014). Exposure of soils and geologic formations to weathering is a natural source of ions (Olson and Hawkins, 2012; Hem, 1985; Pond, 2004; U.S. EPA, 2011b). Factors such as rock texture and porosity, regional structural geology, the degree of fissuring (or fracturing), exposure time, and other factors may influence the composition of water flowing over and percolating through rocks (Hem, 1985). Igneous and metamorphic rocks do not increase the ionic concentration of water flowing over them as much as sedimentary rocks because they are generally more resistant to weathering (Anning et al., 2007). Carbonaceous sedimentary rocks, such as limestone ($CaCO_3$) and dolomite ($CaMg[CO_3]$), are sources of Ca^{2+}, HCO_3^- and Mg^{2+}, while other sedimentary rocks such as those containing gypsum ($CaSO_4 \cdot 2H_2O$) and anhydrite ($CaSO_4$) can be natural sources of SO_4^{2-}, particularly in arid regions (Hem, 1985). Sedimentary rocks and salt deposits associated with evaporation, such as ancient sea-beds, may contain high levels of Na^+ and Cl^-. Natural geologic variability among neighboring watersheds may result in differences in ionic concentration of associated streams. The ionic concentration of surface waters may increase naturally due to evapotranspiration, evaporation, or recharge from groundwater with higher ionic concentrations.

Precipitation (e.g., rain or snow melt) can also affect ionic concentration. SC increases during episodes of below-normal surface flow and decreases during periods of above-normal surface flow. Seasonal patterns can vary greatly with regional climate, with low SC associated with spring rain or during summer from snow-melt. Aerial deposition of wet and dry SO_4^{2-} strips soils of Ca^{2+} and Mg^{2+} and thus directly and indirectly increases SC (Krug and Frink, 1983; Kaushal et al., 2013). Near ocean coastlines, rain and dry deposition may contain more Cl^- from entrainment of aerosols from seawater (Griffith, 2014). Pure water has low SC, due to low concentrations of ions in solution. Surface and ground waters have a wide range of SC, from <50 microsiemens per centimeter (μS/cm), where water quality is dominated by rainfall and rocks are resistant to weathering, to over 200,000 μS/cm for brines (Hem, 1985).

Anthropogenic sources of ions can contribute to changes in both the ionic composition and concentration in freshwater resources. Human activities can increase the ionic concentration of natural waters either directly (e.g., by introducing new ions to freshwater systems) or indirectly (e.g., by changing land use to those that increase delivery of ions to freshwater systems and reduce freshwater input and recharge). For example, industrial, residential, and commercial activities may discharge ion-rich waters to surface water. Reservoirs increase evaporation, thus concentrating ions. Ionic concentration in freshwater systems can also increase as the result of discharges of brines and wastes from combustion effluents or mines, and runoff from treating pavements for icy conditions. Mining practices remove overlying vegetation and use explosives to break up underlying rock, leading to increased ionic leaching from mine overburden as well as from oxidation of exposed minerals such as pyrite (Johnson and Johnson, 2015; Bernhardt and Palmer, 2011; Fritz et al., 2010; Lindberg et al., 2011; Merriam et al., 2011; Palmer et al., 2010; Pond, 2010; Pond et al., 2008; Sams and Beer, 2000). Some mining practices deposit loosely packed spoils comprised of crushed rock overburden into valley fills, where both chemical leaching due to rainfall and direct transport of ions bound to particulate or suspended sediments (mechanical weathering) can result in an increase of major ions in receiving waters (Schlesinger,

1997) (see Figure 2-1). Most mines manage water and wastewater to minimize impacts on water quality.

Climate change can also contribute to increased salinity of freshwater from increased evaporation, intrusion through groundwater, and mobilization of geological salt deposits by changes in aquifer charge and recharge with increased rainfall. Global climate change is often linked to sea-level rises and intrusion of saltwater attributed to changes in pressure, expansion of oceans as water temperatures increase, and glacial melting (Werner and Simmons, 2009). Expansion and creation of estuarine tidal channels over time, from both anthropogenic and natural causes, and compaction of plain lands have been found to contribute to saltwater intrusion (Mulrennan and Woodroffe, 1998). Storm surges and flood tides in which water levels exceed normal high tide levels may also contribute to saltwater intrusion (Zhichang et al., 2001).

Saltwater intrusion has been well documented in coastal areas of the United States (Barlow and Reichard, 2010). Saltwater intrusion most commonly occurs as groundwater is removed and seawater infiltrates aquifers, potentially contaminating drinking water supplies and streams via groundwater discharge. Saltwater intrusion into freshwater systems can also be attributed to or exacerbated by road construction projects and culverts (Stewart et al., 2002).

Waters used for irrigation mobilize salts within the soil and may increase the ionic concentration of surface waters near agricultural fields. Agricultural irrigation return waters contain a variety of salt ions based on the water source, natural chemical composition of the soil, and ions associated with nutrient enrichment (NO_3^-, NH_4^+, and PO_4^{3-}). Ions including Na^+, Cl^-, F^-, Mg^{2+}, and SO_4^{2-} have been shown to mobilize in soils in the western United States leading to increased salinity of adjacent waterways and aquifers (El-Ashry et al., 1985; Leland et al., 2001; Scanlon et al., 2009). These processes are influenced by changes in the amount and patterns of rainfall and changes in climate. Elevated salinity is estimated to affect 10% of the world’s irrigated lands (Duncan et al., 2008) and may increase as climates become more arid.

Salts are commonly used during periods of snow and freezing weather as a method for deicing roadways. The most common deicing agent is rock salt mainly in the form of sodium chloride (NaCl), though other compounds are available, such as calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), potassium acetate (KCH_3C0_2), or calcium magnesium acetate ($CaMg(CH_3CO_2)_4$) (Novotny et al., 2008; Forman and Alexander, 1998). The use of rock salt on snow and ice covered roads has increased salt usage in the United States from 163,000 metric tons in 1940 to more than 23,000,000 metric tons in 2005 (Novotny et al., 2008), primarily in the northern states. As snow and ice melt, salt is transported via surface runoff to lakes and streams, or groundwater via recharge and has been found to increase concentrations of ions in surrounding waters (Blasius and Merritt, 2002; Novotny et al., 2008; Godwin et al., 2003). Water quality impacts can be important because of the greater percentages of pavement in urbanized watersheds. Salinity associated with deicing commonly occurs as seasonal pulses, as materials are applied during freezing conditions and are transported into waterways upon melting. However, in some areas, increased salinity attributed to deicing salts may persist in surface waters due to delayed transport of salts stored in soil and groundwater from previous winters (Jackson and Jobbágy, 2005, Kaushal et al., 2005).

Wastewater treatment plants and industrial discharges can contribute ions to freshwater systems and can dominate water quality in streams and rivers dominated by effluent discharge. Wastewater treatment plants have been shown to increase concentrations of Na^{+}, Cl^{-}, K^{+}. Total Kjehldahl nitrogen (TKN), SO_4^{2-}, and SC downstream of the treatment plant discharge (Andersen et al., 2004). Kaushal et al. (2005) found increasing concentrations of chloride in a long-term study of streams. Echols et al. (2009) measured SC below a point source brine discharge, which ranged from 5,900−18,000 µS/cm. Other industries including food processing, petroleum, and leather production also produce saline wastewaters as a byproduct of production (Lefebvre and Moletta, 2006).

Wright et al. (2011) have identified weathering of cement as a source of Ca^{2+} and HCO_3^{-} in streams draining urban areas. Rose (2007) also found these ions along with others to be elevated in urban subbasins.

Some specific examples of anthropogenic sources of ions illustrated in Figure 2-1 (adapted and updated from Ziegler et al., 2010) and their associated dominant ions are summarized in Table 2-1.

Table 2-1. Examples of ions associated with different anthropogenic sources

Source	Dominant ions	References
Surface coal mining and valley fills associated with mountaintop-removal coal mining Runoff and effluents from conventional coal mining and processing Deep coal mining	Ca^{2+}, Mg^{2+}, HCO_3^-, SO_4^{2-} Ca^{2+}, Mg^{2+}, HCO_3^-, SO_4^{2-} Na^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}	Bryant et al. (2002), Pond et al. (2008), EPA (2011a, b), Griffith et al. (2012) Zielinski et al. (2001), Kennedy et al. (2003), Kimmel and Argent (2010) Thomas (2002), Mayhugh and Ziemkiewicz (2005)
Combustion effluents	Ca^{2+}, Mg^{2+}, HCO_3^-, SO_4^{2-}	Samarina (2007), Ruhl et al. (2012)
Historical industrial sources, such as chlor-alkali plants	Na^+, Cl^-	Echols et al. (2009)
Wastewater treatment plants	Na^+, Cl^-, K^+, TKN, SO_4^{2-}	Paul and Meyer (2001), Andersen et al. (2004)
Sewage and industrial waste discharges	Na^+, Cl^-, NH_4^+, NO_3^-, PO_4^{3-}	Carey and Migliaccio (2009)
Salt water intrusion	Na^+, Cl^-	Barlow and Reichard (2010), Mulrennan and Woodroffe (1998), Barlow (2003)
Produced water from coalbed methane production Produced water from shale gas production (i.e., hydrofracking) Produced water from conventional production of crude oil or natural gas	Na^+, HCO_3^-, Cl^- Na^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^-, K^+, SO_4^{2-}, Br^- Na^+, Cl^-	Brinck et al. (2008), Dahm et al. (2011), Jackson and Reddy (2007), National Research Council (2010), Clark et al. (2001), Veil et al. (2004) Haluszczak et al. (2013), Entrekin et al. (2011), Gregory et al. (2011), Veil et al. (2004) Meyer et al. (1985), Boelter et al. (1992), Veil et al. (2004)
Agricultural runoff, particularly associated with irrigation	Na^+, Mg^+, NH_4^+, Cl^-, F^-, SO_4^{2-}, PO_4^{3-} Ions may vary by region.	El-Ashry et al. (1985), Leland et al. (2001), Bernot et al. (2006), Lerotholi et al. (2004), Lenat (1984)
Road deicing treatments	Na^+, Cl^-, Ca^{2+}, Mg^+	Forman and Alexander (1998), Kelly et al. (2008), Environment Canada and Health Canada (2001), Evans and Frick (2001), Kaushal et al. (2005)
Impervious surfaces and weathering of concrete in urban drainage systems	Ca^{2+}, HCO_3^-, Cl^-	Kelting et al. (2012), Steffy et al. (2004) Wright et al. (2011), Rose (2007)
Dry and wet acid deposition	Ca^{2+}, Mg^{2+}, HCO_3^-, SO_4^{2-}	Kaushal et al. (2013)

2.2.2. Conceptual Model

A conceptual model consists of a written description and diagram that illustrates the relationships between human activities, stressors, and ecological effects on assessment endpoints (U.S. EPA, 1998a). The conceptual model links exposure characteristics with the ecological endpoints important for management goals.

The simplified conceptual model shown here (see Figure 2-1) summarizes natural and anthropogenic sources of ionic loadings in the case example study areas, transport pathways, and potential ecological responses, all of which are described in greater detail in the following sections. Sources are affected by processes or states that can result in delivery of a source to a proximate stressor to the aquatic system. Sources deliver stressors, in this case, dissolved ions to streams. The proximate stressor is the physical, chemical, or biological agent that directly causes one or more biotic responses of concern, in this case, an increase in ionic concentration and/or a change in the relative amounts of ions dissolved in the water. The physical biological exposure is the form or route of exposure or uptake, which is generally direct contact with semipermeable membranes such as gills and internal integument. The physiological mechanism is the molecular, cellular, tissue, or organ system alteration that results from exposure to the stressor. These include changes in ionic concentration, pH shifts, and possibly loss of epithelial integrity. The mode of action is the organismal effect that may reduce fitness and survivorship and increase emigration. The assessment endpoint is the adverse population level of effect, in this case, extirpation. Extirpation is the depletion of a population of a species to the point that it is no longer a viable resource or is unlikely to fulfill its function in the ecosystem (U.S. EPA, 2003). The threshold for extirpation is operationally defined by the level below which 95% of the observations of the genus occur, an XC_{95}. For a more general model showing other sources, such as marine intrusion associated with water withdrawal or fires resulting in ash, see the conceptual model for ionic concentration on the CADDIS website (http://www.epa.gov/caddis/ssr_ion4d.html).

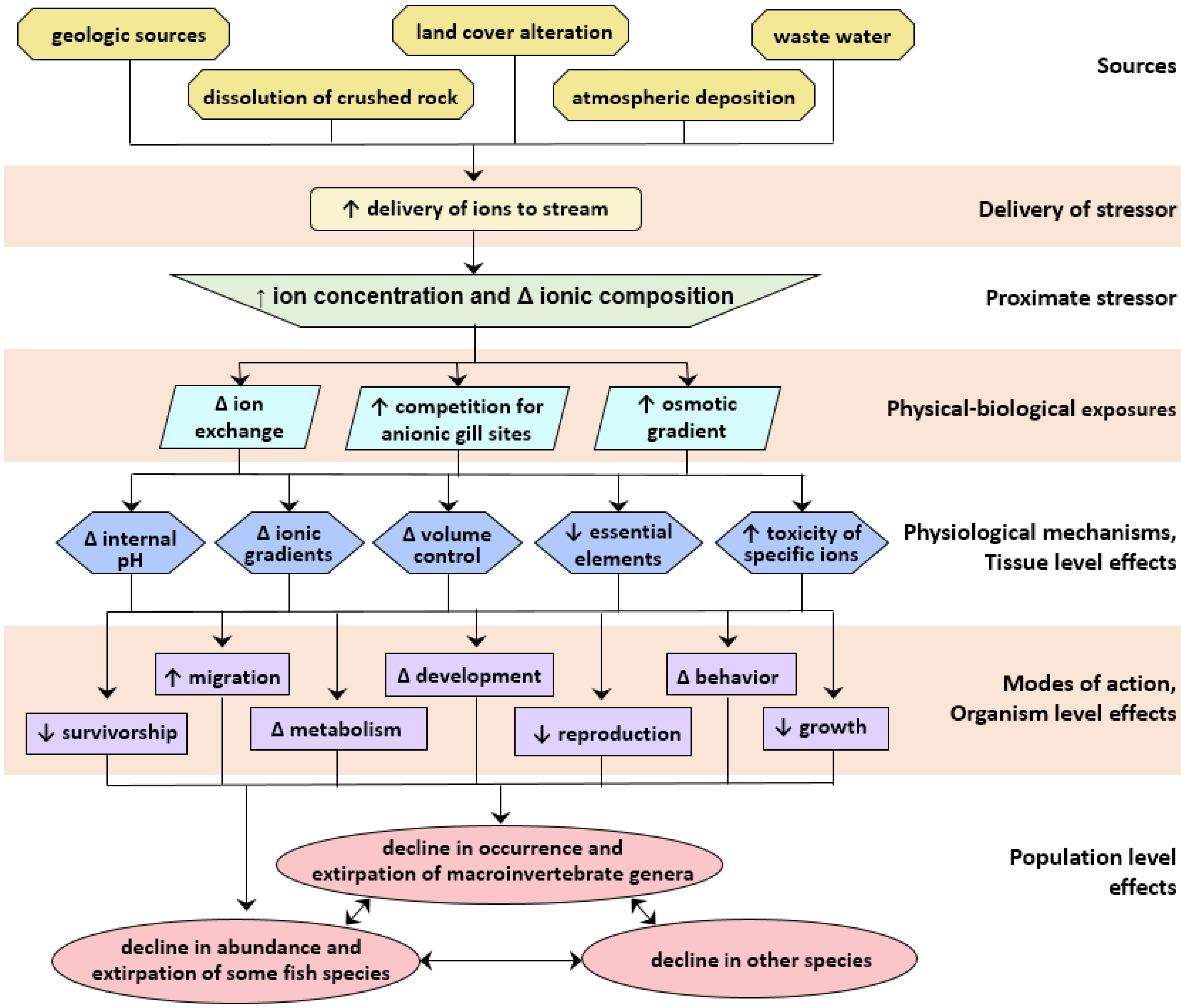

Figure 2-1. Conceptual model showing hypothesized relationships among selected sources of ions and biotic responses to ionic stress by salt intolerant taxa (adapted from Schofield and Ziegler, 2010).
Upward arrows indicate an increase, downward arrows indicate a decrease, and delta symbols indicate a change in the parameter in either direction depending on conditions. Inclusion of a linkage indicates that the linkage can occur, not that it always occurs.

2.2.3. Environmental Transport and Fate of Ions in the Aquatic Environment

The majority of calcium (Ca^{2+}) and magnesium (Mg^{2+}) found in most soils and surface water originates from chemical weathering of common minerals in rock or soils, such as limestones ($CaCO_3$) and dolomites ($CaMg(CO_3)_2$) (e.g., Goddard et al., 2007). Minerals rich in calcite, e.g., apatite ($Ca_5(PO_4)_3(F,Cl,OH)$), can be found in igneous, sedimentary, and metamorphic rocks (e.g., Nezat et al., 2008). In many areas, these calcium and magnesium rich rocks are relatively easily weathered and soluble, with their mobility strongly affected by pH, becoming more mobile with decreasing pH (Likens et al., 1998). In forested catchments, the calcium and magnesium concentrations in surface waters can increase following disturbances, such as deforestation (Likens et al., 1970), and decrease in late successional forest stands relative to early successional forest stands (Hamburg et al., 2003). In general, anions (negatively charged ions) are more mobile than cations (positively charged ions) because they are not bound to negative binding sites on clays. Bicarbonate (HCO_3^-) ions in most soils and groundwater result from chemical weathering of calcareous minerals. Bicarbonate ions are also present in soils as a byproduct of plant and microbial respiration, as well as from the oxidation of organic matter whereby carbon dioxide released in the soil becomes hydrated to form carbonic acid (H_2CO_3) and is then dissociated into bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}), depending on the local soil pH. The relative concentration of HCO_3^- compared to H_2CO_3 and CO_3^{2-} is pH dependent, with HCO_3^- being the dominant form at circumneutral pH. HCO_3^- is readily leached from soils during rainfall. Alkalinity is a measure of HCO_3^- and CO_3^{2-}.

Sulfate (SO_4^{2-}) ions found in soil and rocks can result from chemical weathering of sulfate minerals, such as gypsum (Mullins and Hansen, 2006) or from chemical weathering of coal deposits (Schlesinger, 1997). Atmospheric deposition can also be a source of sulfate found in soils and is primarily anthropogenic in origin from the burning of fossil fuels (Schlesinger, 1997). Sulfate is readily leachable in soils, and sulfate mobility was found to be positively correlated with rainfall in relatively undisturbed forested watersheds in both Central Pennsylvania (Lynch and Corbett, 1989) and the Georgia Piedmont (Huntington et al., 1994). In the Allegheny River Basin in southwestern Pennsylvania, sulfate concentrations in surface waters draining relatively undisturbed watersheds ranged from 16−20 mg/L (Sams and Beer, 2000).

In addition to runoff, ions can be transported to surface waters through groundwater discharge. Major ions can enter groundwater through dissolution of minerals in soils and rocks during recharge. Particularly during periods of low streamflow, groundwater discharge can be a major contributor of ions to surface waters (Larson and Marti, 1996).

Once mobilized, the majority of major ions that contribute to SC behave conservatively in aquatic systems and are transported in surface water and groundwater to receiving waters.

Although the ions that are the focus of these field-based methods are essential elements for living organisms (within specific ranges), biological uptake does not effectively reduce ionic concentrations in streams (U.S. EPA, 2011b). In addition, Ca^{2+}, Mg^{2+}, SO_4^{2-}, Na^+, and K^+ are not significantly degraded nor adsorbed (U.S. EPA, 2011b). As a consequence, concentrations of the transported major ions tend to increase in receiving waters unless diluted by precipitation or inflow from tributaries with lower ionic concentrations (Johnson et al., 2010; Merriam et al., 2011). An exception is bicarbonate ions, which can be taken up by photosynthetic plants. Geologically bound carbonates (HCO_3^-) are also released into the atmosphere. Vesper et al. (2016) reported a total flux of dissolved organic carbon from two sites near a coal mine that ranged from 13 to 249 kg-C/year (18−364 metric tons of CO_2/year).

2.3. MEASURE OF EXPOSURE

The concentration of a dissolved salt mixture can be measured in a number of ways: as SC, total dissolved solids (TDS), freezing point depression (also referred to as osmotic pressure or osmolarity), refractive index, density, or the sum of the concentrations of individually measured ions. A comparison of the capabilities of these different measurement methods is shown in Table 2-2. The EPA has selected SC as the parameter to represent the measure of exposure for this stressor. SC was selected as the measure of the ionic mixture for these field-based methods because (1) SC is a measure of all ions in the mixture; (2) the measurement technology is fast, inexpensive, accurate, and precise; (3) it measures only dissolved ions; (4) it can be used to provide continuous monitoring records with in situ instrumentation; (5) it is a sensitive measure in dilute waters; (6) it is less influenced by other nonfilterable material such as oils and carbohydrates that may be dissolved in water compared to some measurement methods (e.g., TDS); and (7) it is monitored by most state water monitoring programs at bioassessment sampling sites. Several approved methods for measuring SC are available, including EPA method 120.1 (U.S. EPA, 1982 revised).

SC has been commonly used as a measure of ionic concentration, and as an estimate of major solute concentrations and total dissolved solids concentrations of natural waters (McCleskey, 2011; Ziegler et al., 2010). SC is a measure of a material’s ability to conduct electric current, including natural waters, and is typically expressed in units of microsiemens per centimeter (μS/cm).

Table 2-2. Comparison of methods to measure ionic concentration

Measurement method	All ions?	Speed	Approximate sample range and sample volume	Sample filtration required	Field use	Continuous measure possible	Affected by nonionic constituents
Specific conductivity	Yes	Seconds	Wide range, µL−mL, volume or in situ	No	Yes	Yes	No
Total dissolved solids (gravimetric)	Yes	Days	Requires larger volumes for freshwater	At times	No	No	Yes
Freezing point depression	Yes	Minutes	Wide range, few µL to mL volumes	At times	No	No	Yes
Refractometry	Yes	Minutes	Better suited for higher salinities, µL volumes	At times	Yes	Industrial application	Yes
Densitometry	Yes	Minutes	Better suited for higher salinities, dl volumes	No	Yes	No	Yes
Sum of ion concentrations	Typically major ions only; e.g., Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Cl^{-}, SO_4^{2-}, and HCO_3^{-}	Hours to days	Variable depending on analytical methods	Yes	No	No	No

Because SC predictably increases with increasing ionic concentration, it is used to measure salinity (usually referring to NaCl) or ionic concentration (for any dissolved salts) (Standard Methods #2510 [APHA, 1992]; EPA method 120.1, 0950A [U.S. EPA, 1982]). SC measurements in natural waters indicate the presence of inorganic dissolved solids (e.g., chloride, nitrate, sulfate, bicarbonate, nitrite/nitrate, and phosphate anions and sodium, potassium, magnesium, calcium and iron cations). Electrical currents are carried by both positively charged cations and negatively charged anions—but to differing degrees depending on charge and mobility. Thus, the SC of a mixture depends on the type and concentration of the ions in solution. SC is also dependent on temperature and is known to increase approximately 2% for every 1°C increase in water temperature. The term "Specific Conductivity" indicates the measurement has been standardized to 25°C, a reference temperature (Wetzel, 2001). SC is commonly reported in state monitoring programs, rather than the unstandardized conductivity measurement.

Both specific conductivity and specific conductance are often used synonymously in the open literature indicating normalization or measurement at 25°C. Conductivity is a property of water expressed as μS/cm. Conductance of a sample or electrical component is measured as Siemens (S). All measurements in this document refer to specific conductivity/specific conductance expressed as μS/cm at 25°C as it relates to water samples.

SC is an aggregate measurement of the full ionic mixture of a water sample. The total ionic concentration of natural waters is associated with biological effects. However, waters with similar SC levels may have different ionic compositions, and as a result can have different toxicities to freshwater organisms in the laboratory and in the field (Mount et al., 2016; Zalizniak et al., 2006; Dunlop et al., 2015). Therefore, when using SC as a measure of ionic concentration, it is important to characterize the specific ions and their relative concentrations.

Some states and authorized tribes may want to use an alternative measurement of ionic concentration when developing aquatic life criteria. If a different measure of the ionic mixture is selected as the measure of exposure, the reliability of the measurement should be considered. For example, TDS has greater variability than other methods. If some states and tribes prefer to measure specific ions known to be toxic to aquatic organisms, the interaction of ions within the mixture also needs to be considered. Appendix F provides an example using an alternative measure of exposure for waters dominated by Ca^{2+} and Mg^{2+}, the sum of HCO_3^- and SO_4^{2-} in mg/L.

2.4. NATURE OF THE EFFECT

All tolerances of stressors are determined by the evolutionary adaptations of organisms. The background levels of naturally occurring habitat variables such as temperature, pH, and SC are important determinants of those adaptations. Because aquatic species evolved in unpolluted waters, background levels define aspects of the niche to which the biota of a community is naturally adapted and which it potentially tolerates (MacArthur and Levins, 1967; Colwell and

Rangel, 2009; Peterson et al., 2011; Futuyma and Moreno, 1988; Wiens, 2004). Aquatic species inhabit nearly pure water, estuarine and marine conditions, hypersaline pools, and everything in between (Remane, 1971, Potapova and Charles, 2003; Potapova, 2005; Berra, 2007). In most of the United States, freshwater habitats have very low concentrations of dissolved ions relative to marine systems, so that is the condition to which most freshwater biota are adapted.

Algae, protozoans, zooplankton, and bacteria have all been shown to have SC preferences in freshwater systems (Potapova and Charles, 2003; Potapova, 2014; Bos et al., 1996). Nationally, of 230 soft-bodied algae identified to the lowest practical taxonomic level, 56% had estimated optima <500 μS/cm (Potapova, 2014). Nationally, of 683 diatoms also identified to the lowest practical taxonomic level, 84% had optima <500 μS/cm and 35% did not occur in water >500 μS/cm (Potapova, 2014).

Freshwater benthic macroinvertebrates are extirpated at different ionic concentrations (U.S. EPA, 2011a). In West Virginia, 17% of genera that occur at background SC are extirpated at 500 μS/cm and many more genera decline at that SC (Cormier et al., 2013b). Effects are not limited to Appalachia. In Nevada streams, differences between observed and expected invertebrate communities increased above natural background levels of approximately 300 μS/cm (Vander Laan et al., 2013). Freshwater fish also decline and are extirpated as ionic concentration increases (see Appendix G). Although these data are from waterbodies with a wide range of background SC values, they demonstrate that many species and genera are adapted to particular SC regimes, and many of them are quite low.

The physiological limits of species determine their tolerance ranges, in this case, their potential SC niche with respect to concentrations of a defined ionic mixture (Olson, 2012; Vander Laan et al., 2013). At the extremes of their physiological tolerance, species are less able to develop, grow, and reproduce. A species may not exploit its full tolerance range, because competitor species are better suited for a particular ionic concentration or for other ecological reasons such as predation, parasitism, and habitat requirements. The SC range that is actually inhabited by a species is called a realized niche.

The range of SC conditions varies in natural aquatic systems. Species do not occur where the SC is lower or higher than their SC tolerance. The lowest SC in a freshwater system, therefore, is the lowest possible limit of the potential SC niche (see lower tolerance limit in Figure 2-2). When mineral salts are added to an aquatic system, SC increases, part of the potentially habitable SC niche space is lost, and the size of the realized niche for species adapted to low SC decreases. When the SC is above the physiological tolerance of a species due to natural or anthropogenic causes, it does not persist and the species is extirpated.

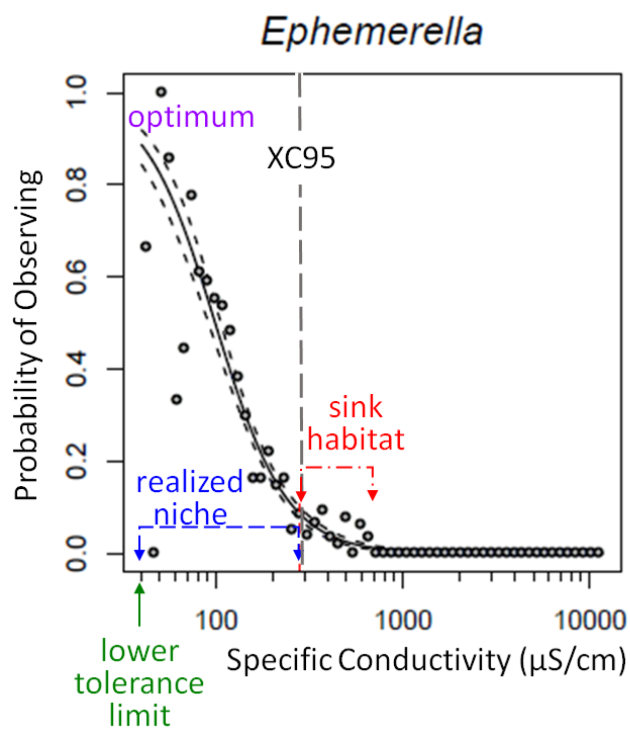

Figure 2-2. A species' (or genus') realized niche is defined by its lower and upper limits of occurrence. In this case, the lower tolerance limit is less than or equal to the lowest specific conductivity (SC), which is the lower limit of occurrence. The XC_{95} represents the upper tolerance limit. Approximately 5% of observations of a taxon are assumed to occur in sink habitats where a population cannot persist without immigration from source habitats. A species or genus optimum is the environmental condition most easily tolerated both physiologically and competitively and can be estimated by the conditions where the taxon is most often observed. The optimum SC may be estimated at the maximum probability of observing the taxon from a generalized additive model, shown here to be the minimum SC. The example involves the genus *Ephemerella* which is comprised of several species of mayflies.

The upper tolerance limit of a species is estimated by its XC_{95} (see Figure 2-2). Extirpation is the depletion of a population of a species to the point that it is no longer a viable resource or is unlikely to fulfill its function in the ecosystem (U.S. EPA, 2003). The occurrences of benthic invertebrate species at locations with a SC greater than their XC_{95} value are believed to represent sink habitats (Pond et al., 2014). Sink habitats are those locations where occurrence of species is primarily the result of immigration from locations with low SC termed source habitats from which immigrants originate. They are “sinks” in the sense that immigrants have low success in establishing sustainable populations in those locations.

These phenomena have practical application. The proportion of species or genera extirpated as a result of increased SC in an ecoregion can be determined and is the basis for the XCD method.

Several other predictions can be made from niche theory. Species with niches that limit them to low SC water are not expected to occur where low SC water does not occur. The source of high SC could be natural or due to anthropogenic inputs (Cormier et al., 2012, Coffey et al., 2014). For example, in an ecoregion lacking streams <400 µS/cm, any species with an upper tolerance limit <400 µS/cm SC would not be expected to occur because there is no habitat for them. As a corollary, where there is a low SC habitat in an ecoregion, species tolerant to low SC will occur.

The relationship between ambient SC levels and SC tolerances of species that are present has at least two practical implications. First, it is inappropriate to set criteria below natural background for a location. Second, the lower limit for any XCD in any given ecoregion cannot be lower than the natural background of the ecoregion. In practical terms, this shifts the origins of XCDs and their 5^{th} centiles toward higher SC (graphically to the right) as the background SC increases. Hence, when XCDs from regions of low to high natural background are simultaneously plotted on the same graph, the curves progress to the right. (For an example, see the XCDs in Appendix D, Figure D-3). Therefore, the background SC of an ecoregion is strongly associated with a predictable extirpation of 5% of species or genera. This relationship between background SC and the proportion of extirpation can be used to predict the SC that will extirpate 5% of species or genera in an ecoregion solely based on ecoregional background (see Section 3.7.2 and Appendix D, Figure D-4).

2.5. MECHANISMS AND MODES OF ACTION

The measure of effect for these field-based methods is extirpation (U.S. EPA, 2011a). The three most likely modes of action for extirpation of a genus or species are the population-level processes mortality, emigration, and failure to recruit (Rubach et al., 2011; Williams and Hynes, 1976; Clements and Kotalik 2016). The sections below discuss some physiological mechanisms of action through which SC acts on organisms and on the processes that constitute the potential modes of action.

2.5.1. Physiological Mechanisms

In exposures to elevated ionic concentrations, physiological stress could cause mortality or drift (a process in which invertebrates emigrate by releasing the substrate and allowing themselves to be carried downstream). The stress occurs because the freshwater organisms cannot maintain or need to use more energy to maintain their internal ionic concentration and pH with altered ionic composition and concentration, and water volume in waters with very high ionic concentration. The mechanism of action is believed to be due to adverse ionic gradients formed by the concentration and relative proportions of ions. For all freshwater organisms, microbes, plants and animals alike, ionic concentration is higher inside an organism than in freshwater. To concentrate and maintain the internal ion concentration, organisms have evolved many interrelated strategies. One cannot describe the specific action of toxicity of one ion or pH without considering all the others (Zhang and Wakamatsu, 2002 Griffith, 2016; Bradley, 2009; Evans, 2008a, b; Wood and Shuttleworth, 2008; Nelson and Cox, 2005; Marshall, 2002; Hille, 2001; Smith, 2001; Thorp and Covich, 2001; Komnick, 1977; Sutcliffe, 1962). For example, Na^+ and Cl^- concentrations are much higher inside organisms than in freshwater. One mechanism used by invertebrates and fish to concentrate Cl^-, an anion with a negative charge, is to exchange Cl^- for a nonmineral anion waste product (CO_2) that is produced during metabolism of sugar. An enzyme, carbonic anhydrase, rapidly and reversibly catalyzes water and CO_2 to HCO_3^- and H^+. HCO_3^- concentrations are higher inside the organism and lower in the water. This concentration gradient is favorable for the exchange of Cl^-. However, a cation also needs to be removed from the organism or else H^+ will accumulate and cause acidosis. Acidosis causes complex cellular reactions and affects function of cellular organelles that lead to many adverse effects including death (Gesser and Poupa, 1983; Vafai and Mootha, 2012). Freshwater animals exploit this increased concentration of H^+ by exchanging it for another cation, such as Na^+. Thus, Na^+ and Cl^- are concentrated inside organisms relative to freshwater. However, when the HCO_3^- concentration in freshwater is high, the concentration gradient does not favor movement of HCO_3^- out of the organism and other ions are not readily brought into the organism. Because this anion-cation exchange mechanism uses waste CO_2, it requires less energy to maintain. Low-energy regulation of ions that depend on favorable HCO_3^- concentrations can be supplemented by adenosine triphosphate-dependent transport of ions as ion concentrations increase outside the organism and concentration gradients become less favorable for passive or low energy transport. The inability to regulate internal ionic concentrations or the greater energy demand for ion regulation may causes stress resulting in death, drift, reduced growth, or reduced reproduction, but definitive cellular studies for most aquatic organisms are lacking.

Organisms use many strategies to minimize loss of ions and the exclusion of water (see references in previous paragraph). At the interface between water and the organism's surface, epithelial tissue integrity is essential. Cell membranes are a barrier to water because they are hydrophobic bilayers of lipids. The membranes are selective for the ions and direction of movement using proteinaceous ion channels, ports, and carriers (for a review see Griffith, 2016). Between the cells making up the epithelial pavement, ultrastructural features called tight junctions hold adjacent cells together and complete the epithelial barrier restricting water and ion movement into or out of the organism. External Ca^{2+} helps maintain tight junctions (Gonzales and McDonald, 1992; Smith et al., 2005; Brown and Davis, 2002). There is some evidence from human studies of the gut that SO_4^{2-} may interfere with tight junctions causing loss of epithelial integrity but the physiological interactions of SO_4^{2-} have not been well studied in freshwater organisms. Note that ion concentrations in freshwater are always less than inside the animal and do not cause loss of water from the animal. Rather, loss of epithelial integrity can lead to excess water or loss of ions. This is a key difference between marine and freshwater organisms.

In summary, the full complement of anions and cations, including others not described here, need to be maintained by organisms. There is an extensive literature on ionoregulation of cations and anions. The higher concentration of ions inside organisms compared to freshwater provides opportunities to use ionic gradients for ionoregulation. Acid-base regulation is linked to the production of hydrogen ions involved in ionoregulation. Because useful gradients are dependent on low concentrations of ions in freshwater, relative amounts of each ion, not necessarily any individual ion, accounts for toxic effect.

2.5.2. Mortality, Growth, and Reproduction

Death of juvenile aquatic invertebrates exposed to different ionic concentrations has been demonstrated in the laboratory (Echols et al., 2010; Kennedy et al., 2003, 2004, 2005; Lasier and Hardin, 2010; Merricks et al., 2007; Mount et al., 1997; Wang et al., 2013, Kunz et al., 2013, Bringolf et al., 2007). Sublethal effects reported from laboratory studies include reduced growth, reproduction (Johnson et al., 2015), early emergence (Nietch et al., 2014), and premature release of unionid glochidia (Gillis, 2011). When death of an entire population occurs, the area remains depopulated until recolonized by aerial dispersion and egg-laying (oviposition) (Smith et al., 2009) or by organisms floating downstream (drift) from refugia at upstream reaches or tributaries to the depopulated stream reach (Williams and Hynes, 1976; Pond et al., 2014).

2.5.3. Emigration

Emigration occurs when organisms vacate a stressful environment after being challenged with a noxious stimulus or lack of food or other resources. In numerous studies, benthic invertebrate drift is induced within minutes of exposures to a range of stressors in natural and artificial streams (Svendsen et al., 2004; Wood and Dykes, 2002). Stress induced drift and avoidance behaviors have been shown to occur with salts, toxic chemical spills, floods, pesticides, drought, sediment, low dissolved oxygen (DO), heat, and organic pollution (Wood and Dykes, 2002; Svendsen et al., 2004; Crossland et al., 1991; Doeg and Millage, 1991; Wallace, 1990; Brittain and Eikeland, 1988; Sheehan and Winner, 1984; Geckler et al., 1976; Waters, 1966, 1972, 1995). In independent studies, colonized substrates were exposed to continuous flowing treatments of ionic mixtures (Clements et al., 2014, 2016; Nietch et al., 2014). The studies showed increased drift, reduced numbers of taxa, and other effects. Drift is more likely to occur when there is an abrupt change in environmental conditions rather than a slow change that allows organisms to physiologically adapt. For example, after a moderate increase in ionic concentration, some aquatic insects synthesize more ionic channels for ionic regulation (Wichard et al., 1973; Sutcliffe, 1974; Komnick, 1977).

2.5.4. Failure to Recr'uit

Development begins with gamete production. Fertilization during the terrestrial phase of the life cycle occurs internally in most aquatic insects and is unlikely to be affected by aqueous ions. At oviposition, contact with freshwater causes the swelling and formation of an extrachorionic coating that is necessary for adherence of the eggs of some invertebrates to substrates in the stream (Percivale and Whitehead, 1928). Eggs oviposited into water with higher specific SC do not form the adhesive coating (Percivale and Whitehead, 1928) and the eggs are washed downstream and presumably perish (Gaino and Bongiovanni, 1992). Ionic gradients that initiate biological changes are also necessary to permit propagation of a fertilization potential over the surface of some eggs and to allow successful embryonic development and hatching in some species such as fish (Jaffe, 1991; Coward et al., 2002). Similarly, toxicity tests with fathead minnow larvae were more sensitive during the transitional period from embryo development to hatching (Wang et al., 2016a). Mesocosm experiments with mayflies also indicated greater vulnerability during early life stages and during emergence to winged adults (Clements and Kotalick, 2016; Nietch et al., 2014).

2.5.5. Community Interactions

Increased competition, predatation, or parasitism have been suggested as possible modes of action leading to loss of some species and an increase in salt-tolerant taxa where ions are elevated (Olson, 2012; Olson and Hawkins, 2012; Micieli et al., 2012; Wood-Eggenschwiler and Barlocher, 1983). These processes may affect the benthic invertebrate communities that form at different ionic concentrations.

2.6. ASSESSMENT ENDPOINTS AND MEASURES OF EFFECT

2.6.1. Assessment Endpoints

Assessment endpoints represent the actual environmental value to be protected. They are defined by an ecological entity (e.g., species, community, or other entity) and attributes, (e.g., survival, growth, and reproduction) (U.S. EPA, 1998a). In the development of water quality criteria for SC, the entities are aquatic biotic communities and the attribute is protection of all but a small fraction of species from extirpation.

The relevant ecological entities for these field-based methods are macroinvertebrate assemblages, which are characterized by their taxonomic composition at the genus level. Macroinvertebrates were selected because they are susceptible to ionic stress, they are important to stream function and ecosystem integrity, they provide numerous ecosystem services that benefit humans, they can be found in all types of streams, and they are intrinsically valuable aquatic life forms (Suter and Cormier, 2015). Furthermore, because macroinvertebrates constitute the great majority of multicellular species in streams and have a wide range of sensitivities, they are excellent indicators of adverse effects on ecological processes and on the larger aquatic community. For these reasons, all states and many tribes monitor aquatic macroinvertebrates to assess the health of the aquatic community (U.S. EPA, 2002).

The most commonly recognized contribution of aquatic macroinvertebrates is that they are food for larger invertebrates and fish and other vertebrates, including recreationally important fish species (Allan, 1981; Richardson, 1993; Sweka and Harman, 2008; Hitt and Chambers, 2014), amphibians (Burton, 1976; Wallace et al., 1997), insectivorous bird species (Nakano and Murakami, 2001; Gray, 1993; Epanchin et al., 2010), bats (Clare et al., 2011), and mammals. However, the overall function of freshwater aquatic ecosystems is also dependent on macroinvertebrates (Hooper et al., 2005; Cardinale, 2011). Macroinvertebrates improve water quality through forest and stream nutrient retention (Newbold et al., 1983, 1982; Wallace and Webster, 1996; Huryn and Wallace, 2000; Evans-White et al., 2005), aid in leaf litter decomposition (Wallace and Webster, 1996), and remove pathogens and nuisance periphyton blooms by filtering and grazing (Wallace and Merrit, 1980; Yasuno et al., 1982; Hall et al., 1996). Because macroinvertebrates provide many ecosystem services, it is well understood that stream macroinvertebrate diversity and abundance are important indicators of overall stream condition (Carter et al., 2006; Resh, 1995), and many stream monitoring programs and stream condition indices rely on macroinvertebrate sampling metrics (Gerritsen et al., 2000; Pond et al., 2008; U.S. EPA, 2002).

These field-based methods can be used to develop ecoregional criteria that are fully protective of aquatic life. Many freshwater insects are among the most salt-intolerant organisms relative to other taxa, including crustaceans such as crayfish and daphnids, fish, and amphibians (compare Appendices A.4 and B.4 with Appendix G of this report). Recent studies suggest that mussels in the family Unionidae are acutely salt-intolerant (Kunz et al., 2013; Wang et al., 2016a, b), particularly during early (glochidia and juvenile) life stages (Bringolf et al., 2007; Gillis, 2011; Wang et al., 2016a, b).

Fish also are adversely affected by ionic stress (see Appendix G, Stauffer and Ferreri, 2002; Kimmel and Argent, 2010; Mount et al., 1997; Kennedy et al., 2005, 2004, 2003; Harper et al., 2012; Farag and Harper, 2012, Hopkins and Rousch, 2013, Hitt and Chambers, 2014). EPA's assessment of fish in Appendix G indicates that they are sensitive to ionic stress but are extirpated at slightly higher SC levels than macroinvertebrates. Therefore, fish are expected to be protected by criteria based on macroinvertebrate data. More complex organisms (e.g., fish) generally have a greater ability to regulate internal ionic concentrations and water volumes than simpler organisms, such as benthic invertebrates (Dunlop et al., 2005). Fish also have greater mobility and may be able to more readily migrate from high SC sites to more habitable areas (e.g., Goldstein et al., 1999; Woodward et al., 1997, 1995).

In sum, macroinvertebrates are a critical component of ecological integrity, provide numerous ecosystem services, and appear to be a salt-intolerant ecological taxonomic group; therefore, they are used as an assessment endpoint for these field-based methods.

2.6.2. Measures of Effect

The measures of effect for these field-based methods have been selected to be consistent with the intent of the conventional laboratory-based method for developing aquatic life criteria (U.S. EPA, 1985). Two relationships are derived from the paired SC and macroinvertebrate field data: one for each macroinvertebrate genus and one for the overall macroinvertebrate community in the study area. First, a cumulative distribution function (CDF) is developed for each genus[2] to determine its genus XC_{95}, the SC level above which a genus is effectively absent from water bodies in a region (U.S. EPA, 2003). It is defined in this method as the 95th centile of the distribution of occurrences of a macroinvertebrate genus. In other words, the probability of observing a genus above its XC_{95} SC value is 0.05; i.e., if a genus is observed at 100 sites, only 5 sites would be expected to have SC above the XC_{95}. XC_{95} values that are uncertain or unmeasured within the exposure range are noted and generally do not influence the hazardous concentration (HC_{05}) because their estimated XC_{95} values are greater than those genera in the 5th centile. Second, the HC_{05} is developed using a genus-level XCD for the macroinvertebrate community from the aggregation of the XC_{95} values.

[2]Conventionally, species have been aggregated to the genus level. However, effect levels may be different for species within a genus due to niche partitioning afforded by naturally occurring causal agents such as dissolved ions. (Remane, 1971; Suter, 2007). Hence, an apparently salt-tolerant genus may contain both salt-intolerant species and tolerant species. Analyses with fish species indicate that the range of XC_{95} values within a genus can be quite broad and the empirical genus-level XC_{95} tends to represent the maximum XC_{95} of the species in the data set (see Appendix G).

One key difference from laboratory-based methods used to develop aquatic life criteria for chronic exposures is the measure of effect. In the XCD method, the measure of effect is genus extirpation (population-level) rather than an effect at the organism level. In the laboratory-based method, the measures of effect represent survival, growth, or reproduction (U.S. EPA, 1985). Because the example ecoregional criteria are based on field data for a large number of macroinvertebrate genera across many sites across a broad SC gradient, the EPA anticipates that a reasonable level of protection of the overall aquatic community will be provided if all except a small fraction (i.e., 5%) of sampled macroinvertebrate genera from the region are protected. In their review of the *EPA Benchmark Report*, the EPA SAB stated that this approach provides a degree of protection comparable to or more protective than a conventional water quality criteria based on conventional chronic toxicity testing (U.S. EPA, 2011c).

The genus-level XCD used in the XCD method represents the response of genera in biotic communities in general to a stressor (e.g., an ionic mixture dominated by sulfate plus bicarbonate). XCDs do not require that the species or genera be the same in all applications or at all locations (Posthuma et al., 2001; Cormier and Suter, 2013a; Cormier et al., 2013a). Similarly, the genera that form the minimum data set for laboratory-based aquatic life criteria are not intended to match any particular community; rather, they are surrogate taxa that represent any potentially exposed freshwater community (U.S. EPA, 1985). In the same way, the distribution of genera in the XCDs used in the XCD method (e.g., see Section 4.2) represent all stream communities from a similar background ionic concentration exposed to a similar ionic mixture. All of the macroinvertebrate taxa used to develop an XCD may not occur at any one site in an ecoregion.

Because this approach relies directly on paired observations of in situ measurements of SC and benthic invertebrate assemblage information, the potential adverse effects of ionic stress on all life stages is considered in the context of other complex relationships (e.g., food web dynamics) and aquatic ecosystem processes. The measures of effect (i.e., XC_{95} and HC_{05}; see Sections 3.1.2 and 3.1.3) are considered chronic-duration endpoints because the field data reflect exposures over whole life cycles and multiple generations of the resident biota (see Table 2-3). A field-based method to directly develop acute criteria for SC is not yet available due to a lack of field data with sufficiently high temporal resolution (e.g., daily measurements of SC paired with macroinvertebrate sampling for at least 1 year). However, these field-based methods include a method that uses within-site variability of SC levels to derive a criterion maximum exposure concentration (CMEC) that will protect aquatic life from acutely toxic exposures (see Section 3.2). The CMEC differs from a criterion maximum concentration (U.S. EPA, 1985) because it is not calculated using laboratory or field data showing a direct relationship between SC and an acutely toxic biological response. However, the protectiveness of the CMEC was corroborated with field biological and SC data in Case Examples I and II (see Appendices A and B).

Table 2-3. Summary of assessment endpoints and measures of effect used in this field-based method to derive a criterion continuous concentration (CCC) and criterion maximum exposure concentration (CMEC) for specific conductivity

Stressor of concern	Measure of exposure
Mixture of ions (e.g., ($[HCO_3^-] + [SO_4^{2-}]) > [Cl^-]$	Specific conductivity
Assessment endpoints for the aquatic community	**Measures of effect**
Occurrence of macroinvertebrate populations	Chronic XC_{95} (genus-level effect) Chronic HC_{05} (assemblage-level effect)

XC_{95} = Extirpation concentration, the SC value below which 95% of the observations of a genus occur.

To summarize, for these field-based methods, the valued resource is the aquatic community, characterized by the macroinvertebrate populations that occur at a site. The ecological entities defining the assessment endpoints are populations of macroinvertebrates (aggregated to genera) and the measure of effect is extirpation (the desired attribute is occurrence). Macroinvertebrate populations are appropriate assessment entities because they occur in all but the poorest-quality streams, they are important to ecosystem structure and function, they are highly diverse, they are common forms of aquatic life, and they are affected by many different agents including ionic stress. Extirpation is the depletion of a population of a species or genus to the point that it is no longer a viable resource or is unlikely to fulfill its function in an assessed ecosystem. Extirpation of genera is an appropriate attribute for these methods that rely upon analyses of field data to determine population-level effect thresholds. Aquatic life criteria developed using these field-based methods are set at a SC level that protects

95% of resident macroinvertebrate genera that occur at reference sites in the relevant ecoregion. Aquatic communities are expected to be resilient to these effects and support the overall integrity and function of the aquatic ecosystem if the derived ecoregional criteria (magnitude, duration and frequency) are not exceeded.

2.7. SELECTION OF A FIELD-BASED METHOD

The EPA typically relies on laboratory toxicity test data for the development of aquatic life criteria (U.S. EPA, 1985). To the extent laboratory toxicity tests have been performed on similarly mixed proportions of these major ions, these tests with commonly tested laboratory species have not indicated sensitivity at the concentrations associated with loss of macroinvertebrate genera in the field (U.S. EPA, 2011a). Although it is impractical to replicate in the laboratory the range of taxa, conditions, effects, or interactions that occur in natural streams, a number of recent toxicity studies have begun to bridge the gap between physiological studies and field observations (Mount et al., 2016; Erickson et al., 2016; Wang et al., 2016a; Kunz et al. 2013). Also, mesoscosm studies have begun to identify the modes of action that likely lead to extirpation in the field and corroborate effects observed in the field (Clements and Kotalik, 2016; Nietch et al., 2014).

Analyses of field data show the reduced presence of many benthic macroinvertebrate genera at increasing SC levels. The associations between SC and benthic macroinvertebrate occurrence observed in the field have been assessed as causal (e.g., see Section 2.2 of this document; [e.g., Gerritsen et al., 2010; Palmer et al., 2010; Lindberg et al., 2011; Merriam et al., 2011; Pond et al., 2010, 2008; U.S. EPA, 2011a, b; Bernhardt et al., 2012; Cormier et al., 2013b, c; Timpano et al., 2014; Dunlop et al., 2015; Zhao et al., 2016]). Furthermore, the field data used in this analysis represent exposures to regionally representative assemblages of taxa and life stages at levels and proportions of ions under realistic physical and chemical conditions. In this case, field data can be used to directly assess ecologically-relevant measures of effects, such as extirpation of genera in aquatic communities as a result of exposure to these ionic mixtures in the field. Protection of a diverse assemblage of benthic macroinvertebrates is protective of stream communities and aquatic life.

For these reasons, the EPA concluded that a field-based approach is appropriate for developing SC criteria where there are sufficient data for analysis.

3. ANALYSIS PLAN: FIELD-BASED METHODS TO DEVELOP SPECIFIC CONDUCTIVITY CRITERIA

This section presents field-based methods that may be used to derive SC water quality criteria for the protection of aquatic life where sufficient data are available. The methods describe how to derive a criterion continuous concentration (CCC) to protect against effects from chronic exposure (see Section 3.1) and a CMEC to protect against effects from acute exposure (see Section 3.2). The methods also describe how to estimate duration (see Section 3.3) and frequency (see Section 3.4), how to assess causation (see Section 3.5), and how to determine the applicable geographic range of criteria (see Section 3.6). Because the primary method (the XCD method) requires large, paired chemical and biological data sets which are not available for all ecoregions in the United States, EPA developed another method which uses a model to calculate a CCC for SC. This method is useful for Level III ecoregions where sufficient paired biological data are lacking (see Section 3.7). Section 3 serves as the *Analysis Plan* for the case studies in Sections 4, 5, 6 and 7. Some ecoregion-specific examples are included in this section to illustrate key concepts and methods.

3.1. DERIVING A CRITERION CONTINUOUS CONCENTRATION (CCC)

The XCD method requires a field data set with paired in situ measurements of SC and benthic macroinvertebrate survey results for streams in the study area. The field SC measurements allow for the development of exposure-response relationships across a SC gradient. The inclusion of high quality and impaired sites in the data sets assures a range of SC (exposure) for characterizing changes in taxa occurrence (response). In aggregate, the in situ field measurements of SC from many sites from different times of the year represent the variability over a year.

Using this method, two relationships are derived from the field data: one for each macroinvertebrate genus and one for the overall macroinvertebrate assemblage in the study area. First, a weighted CDF is developed for each macroinvertebrate genus to determine each XC_{95}. Second, the HC_{05} is developed using a genus XCD for the macroinvertebrate community from the aggregation of the XC_{95} values (an example is provided in Figure 3-1).

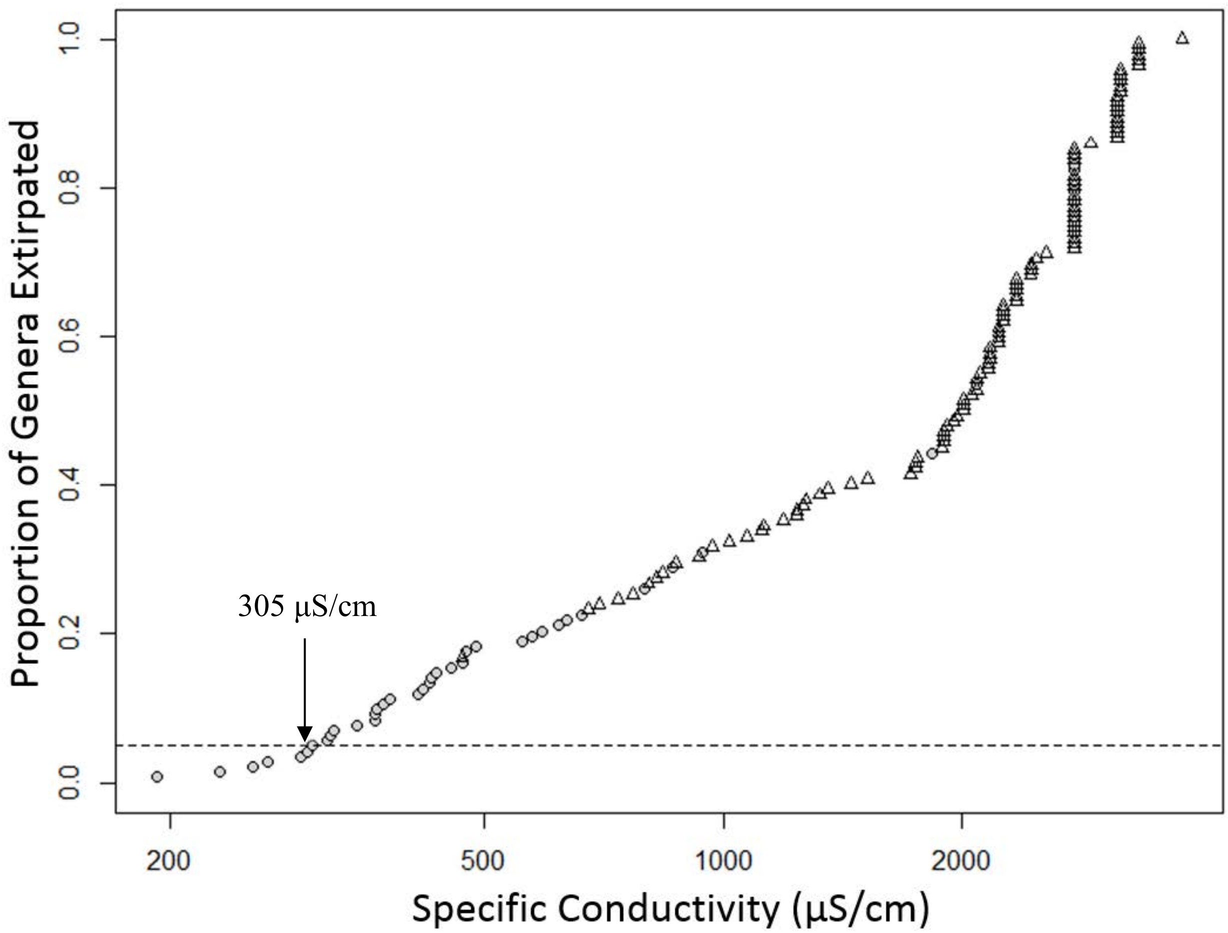

Figure 3-1. Example of a genus extirpation concentration distribution (XCD) depicting the proportion of genera extirpated with increasing ionic concentration measured as specific conductivity (SC). Each point on the XCD plot represents an extirpation concentration (XC_{95}) value of one genus arranged from the least to the most salt-tolerant. XC_{95} values that were defined as greater than values are indicated by triangles. The 5th centile of the XCD is shown as a dotted horizontal line. The 5th centile hazardous concentration (HC_{05}) is the SC at that intercept of the XCD and the 5th centile line. In this example, the HC_{05} is 305 µS/cm.

Several methods were evaluated prior to selection of a weighted CDF model to estimate an XC. They included models of XC from logistic regression, a generalized additive model (GAM), unweighted CDF, and other options. A weighted CDF model was selected because the HC_{05} value fell within the range of the other methods, and it was computationally simple. Weighting normalizes the distribution of samples taken across the SC gradient. The weighted CDF does not assume any particular shape to the distribution and does not fit a function to the data points. Outliers were not identified or removed because little was known about the sustainability of populations in high SC water or the movement of salt-intolerant taxa from biological sources to sinks. However, at least one study indicates that apparent outliers may represent transient occurrences of genera drifting from low SC to a high SC stream reach (Pond et al., 2014). Removal of the outliers or using the area under a fitted curve such as a GAM generally yields lower XC_{95} values and a lower HC_{05}. As new information arises, the method for modeling the XC_{95} may be updated. The EPA SAB endorsed EPA's selection (U.S. EPA, 2011c) of the weighted CDF model for this field-based method (U.S. EPA, 2011a). The statistical package *R*, Version 2.12.1 (December 2010), was used for all statistical analyses in the Case Studies (*R*-Development Core Team, 2011). The program "R" is open-source and open-access computational software that runs on Microsoft Windows, Apple MacOS, and UNIX platforms. The calculations can also be performed with a hand-held calculator or with a spreadsheet such as Microsoft Excel.

Different forms of the exposure-response relationships (i.e., decreasing, unimodal, increasing, and no relationship) are expected given the nature of the ions and the physiology of a macroinvertebrate genus. For example, many ions are required for survival and are beneficial at low levels but elicit toxic effects at high levels; such a stressor-response relationship is expected to have a unimodal distribution (see Appendix A.3, *Isonychia*). In the ascending (left) limb, requirements for ions are increasingly being met; in the descending (right) limb, toxicity is increasing. However, many empirical exposure-response relationships for ions do not display both limbs of the distribution. For example, some may show: (a) only the descending portion of the curve because none of the observed SC levels are sufficiently low to show elemental deficiency for the taxon (see Appendix A, *Ephemerella and Leuctra*); (b) only the ascending portion because none of the observed SC levels are sufficiently high to show toxicity for the taxon (see Appendix A, *Cheumatopsyche*); or (c) no trend at all because the optimum is more of a plateau than a peak so it extends across the range of observed SC levels (see Appendix A.3, *Brilla*).

The steps involved in selecting, characterizing, and analyzing macroinvertebrate sampling field data to derive a CCC are depicted in Figure 3-2 and described in the following sections.

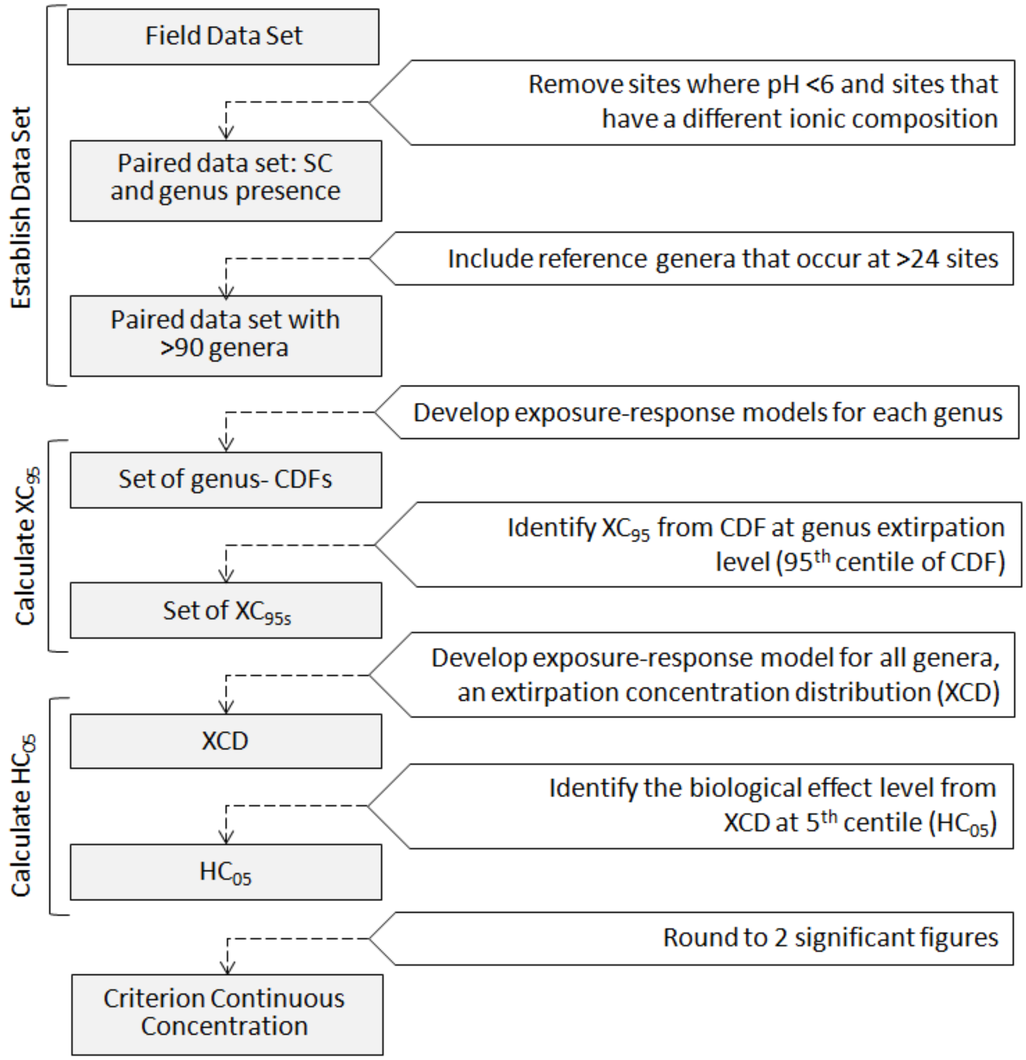

Figure 3-2. Main steps in the derivation of a chronic specific conductivity (SC) criterion using the extirpation concentration distribution (XCD) method. Rectangular boxes on left are products and pentagonal boxes on right are operations performed on those products. In the Case Studies (see Sections 4, 5, 6 and 7), example SC criteria are derived using data from sites in a defined area that have an ionic composition dominated by sulfate plus bicarbonate anions. Cumulative distribution function (CDF), 95th centile extirpation concentration (XC_{95}), 5th centile hazardous concentration (HC_{05}).

3.1.1. Establishing the Data Set

3.1.1.1. *Information Sources*

The data sets are developed at an ecoregional scale to account for natural differences in background SC levels found in different ecoregions, and QA/QC of the data sets are described.

Several data sets were used to illustrate the method in the case studies, details of which are provided in Sections 4, 5, 6, and 7. In addition to the description of field data, information available in the scientific literature is considered in the development of ecoregional SC criteria, particularly for assessing causation and confounding factors (see for example Appendices A and B in U.S. EPA, 2011a; Cormier et al., 2013b; Suter and Cormier, 2013) and to support recommendations for duration and frequency of the criteria. Relevant literature related to the characteristics of the receptor organisms, the ions of interest, and the potential confounding agents are also considered, much of which is presented in Section 2 (*Problem Formulation*) of this document and also in the precursor reports and manuscripts (U.S. EPA, 2011a; Cormier et al., 2013b; Suter and Cormier, 2013).

3.1.1.2. *Selection and Adequacy of Data Sets*

Developing aquatic life criteria for SC using the XCD method requires a large data set with certain characteristics. The adequacy of the data set can be judged by the following attributes (U.S. EPA, 2011c):

- Measurements of the agent(s) are paired in space and time with biological sampling;
- High-quality (i.e., minimally affected) sites are included in the data set;
- Background SC levels are similar throughout the region (see Section 3.7.1);
- Characteristics of the agent (i.e., ionic composition) are similar across the region for the paired data (i.e., other mixtures may occur but they are analyzed separately);
- Some biological sampling occurs when salt-intolerant genera are likely to be collected (e.g., March through June in Appalachia) and where they are likely to occur (e.g., leaf packs, riffles);
- The exposure gradient is broad enough to include no effects, weak effects, and strong effects;
- Data are available to evaluate potential confounding factors; and
- An independent data set or statistical models are available to validate the criteria.

Inclusion of many genera and a representative proportion of salt-intolerant genera help to ensure that the XCD model is representative of the aquatic community. A sensitivity analysis of data sets has determined that a reliable HC_{05} can be determined from 90−120 genera and 500−800 sites, based on the stability of the HC_{05} value as those variables increased (U.S. EPA, 2011a; see Sections 4 and 5 of this report). Samples taken throughout the year reduce biases from seasonal SC regimes and seasonal occurrence of some genera. For example, samples taken only in dry seasons when SC tends to be higher would likely bias results toward more salt-tolerant genera and to maximum SC exposures rather than an annual average.

3.1.1.2.1. Sample size

The number of observations of a genus can affect the reproducibility of the XC_{95} and the HC_{05} (Cormier et al., 2013a). Similarly, the number of genera affects the reproducibility of the HC_{05} (U.S. EPA, 2011a; Cormier et al., 2013a) and the number of genera depends on the overall number of samples in the data set and individuals in the sample that are identified to genus.

For the example case studies, EPA estimated XC_{95} values using the XCD method with genera that were observed in ≥25 samples in the ecoregion (Cormier et al., 2013a) because estimations of the 95th centile with <25 observations are less robust. The recommended number of genera in an XCD using this method is 90 (Cormier et al., 2013a). For a sampling protocol that identifies 200 individuals in a sample, the adequate number of sampling stations in an ecoregion using this method is about 500 (U.S. EPA, 2011a). However, if more individuals are identified in each sample, e.g., 300 or a full count of individuals, then fewer sampling stations may be needed to obtain XC_{95} values for 90 or more genera (see Appendix G in U.S. EPA, 2011).

The effect of selecting a minimum number of observations of a genus for calculating an XC_{95} value can be visualized in several ways. For example, for a range of genus sample sizes (e.g., N = 5−60), the HC_{05} is calculated and the number of genera in the XCD is enumerated. The resulting HC_{05} values and number of genera versus minimum number of samples are plotted. As taxa with fewer occurrences are excluded from the XCD, the number of genera decreases and the HC_{05} increases (see Figures 4-11 and 5-11). To ensure representation of salt-intolerant taxa and reasonable accuracy of the HC_{05}, the minimum number of samples is chosen that maximizes the number of taxa in the XCD while minimizing the variance in the XC_{95} and resulting XCD near the 5th centile. The number of observations of a genus is also chosen to provide sufficient occurrences to estimate the genus XC_{95} values while minimizing bias due to eliminating salt-intolerant genera that have few occurrences. A minimum sample size of 25 maximizes the number of taxa that are included without having to extrapolate beyond the range of the data set (Cormier et al. 2013a, on-line supplemental material). Therefore, a sample size of 25 was utilized in the case studies, and in general, is a recommended minimum sample size for this method.

The effect of sample size on the HC_{05} and its confidence bounds can be estimated using a bootstrapping technique (see Section 4.5 and 5.5). Bootstrapping is a statistical technique of repeated random sampling from an empirical data set that is often used in environmental studies to estimate confidence limits of a parameter (Newman et al., 2001, 2000). This is akin to having different samples to compare results and fidelity of the model. A similar method is used to calculate confidence bounds on the HC_{05} values (described in Section 3.1.3.1). Using this technique, a data set of a selected sample size with replacement is randomly selected from the original set of samples. Next, the XC_{95} for each genus is calculated from the bootstrap data set by the same method applied to the original data, and the HC_{05} is calculated. The uncertainty in the HC_{05} value can be evaluated by repeating the random sampling and HC_{05} calculation numerous times (e.g., 1,000 times) for each selection. The distribution of 1,000 HC_{05} values is used to generate two-tailed 95% confidence bounds on these bootstrap-derived values. The whole process is repeated for a selected sample size ranging from 100 to the full data set of all samples. The mean of all bootstrapped HC_{05} values, the numbers of genera used for the HC_{05} calculation, and their 95% confidence bounds are plotted to show the effect of sample size. The number of samples at which the HC_{05} values reach an approximate asymptote (500−800) suggests the minimum sample size (500) for a data set (see Figures 4-12 and 5-12).

3.1.1.2.2. Treatment of multiple samples from a particular site

Multiple samples collected from the same site can provide valuable information especially when they are from different seasons (when SC may be different and different genera may be present), but they can be problematic if they introduce a bias (e.g., if extremely low or high SC sites are more likely to be sampled repeatedly).

In the case studies, most of the sites were sampled only once, but a portion of them were sampled more than once (see Sections 4.1 and 5.1). To assess for a potential bias, a simple inverse weighting scheme can be applied (e.g., if a site is sampled twice, each observation is weighted 0.5). In the example case studies, this weighting scheme did not substantially change the magnitude of the HC_{05} values; therefore, all data were used with no weighting. In cases where geographic distribution of sites and/or SC levels affects the HC_{05}, then some form of correction may be warranted, such as random selection of only one sample per site. In the case studies, sites were fairly evenly distributed; therefore, no weighting was performed for replicates.

3.1.1.2.3. Stressor identity

The stressor identity in this case is the proportion of constituent ions, characterized on the basis of the field data set. The stressor of concern in the example case studies is an ionic mixture dominated by sulfate (SO_4^{2-}) plus bicarbonate (HCO_3^-) (see Section 2.2). As a result, for these example criteria, sites with an ionic mixture dominated by chloride (i.e., those where the concentration of HCO_3^- plus $SO_4^{2-} \leq Cl^-$, in mg/L) are removed from the data set. The ionic mixture in the example case studies (see Section 4, 5 and 7) are dominated by the cations on a mg/L basis by ($[Mg^{2+}] + [Ca^{2+}]) > [Na^+]$ and one by ($[Mg^{2+}] + [Ca^{2+}]) < ([Na^+] + [K^+]$) (see Section 6). Alternatively, other data can be removed to focus on other mixtures or salts (e.g., NaCl).

3.1.1.2.4. Ambiguous taxa

The XCD method uses genus-level taxonomic identification. This method does not mix data of lower or higher levels of taxonomic identification. However, species-level taxonomic identification can be used when it is available and the number of species is sufficient for constructing an XCD (see Appendix G for an example). Data records with ambiguous taxonomic identification or family-level or higher identification (i.e., no genus-level identifications) are excluded from the data sets.

3.1.1.2.5. Exclusion of genera from extirpation concentration distribution (XCD)

This method is for freshwater systems, and therefore, estuarine and marine genera were not included in the XCD. One way to ensure that only freshwater organisms are represented in the XCD is to only include genera that are present at a minimum of one freshwater reference site. The selection of reference sites is beyond the scope of this document (Stoddard et al., 2006; Herlihy et al., 2008; Whittier et al., 2007b; Hawkins, et al. 2010; U.S. EPA, 2011d; Environment Canada, 2012). For the example case studies, the reference sites used in analyses were identified by the sampling organization, but only after EPA reviewed the reference site selection criteria (see Sections 4 and 5). When reference sites were not identified by the sampling organization, all genera were used (see Appendix D).

3.1.1.2.6. Confounding factors

Field observations are uncontrolled, largely unreplicated, and may not be randomized; as a result, they are subject to confounding. Confounding is the appearance of apparently causal relationships that are due to noncausal correlations. Noncausal correlations and the inherent noisiness of environmental data can obscure true causal relationships. Reducing confounding as much as possible is recommended by identifying potential confounding variables; determining their contributions, if any, to the relationships of interest; and eliminating their influence when possible and as appropriate based on credible and objective scientific reasoning.

A method to assess the potential effect of confounders is described in the *EPA Benchmark Report* (U.S. EPA, 2011a) and in Suter and Cormier (2013). The analysis of potential effects on the model by potential confounders used a weighted scoring system to evaluate ten types of evidence that determined whether the observed relationship between benthic macroinvertebrate community composition and SC was affected by other factors. The weighted scoring system was based on work by Hill (1965) and Cormier et al. (2010) and is described in detail in Appendix B of EPA (U.S. EPA, 2011a) and in Suter and Cormier (2013). As described in the *EPA Benchmark Report*, the potential for other stressors to affect the XCD model was evaluated using a weight-of-evidence assessment that considered habitat quality, organic enrichment, nutrients, deposited sediment, pH (low and high), selenium, temperature, lack of headwaters, catchment area, settling ponds, dissolved oxygen, and metals (see Appendix B in U.S. EPA, 2011a). Overall, the analyses showed that the effects attributed to increased ionic concentration were not due to other stressors.

In these analyses (U.S. EPA, 2011a), only one of the assessed factors (pH <6) was identified as a likely confounder, so samples with pH <6 were removed from the data set to minimize the influence of acidity and associated dissolved metals. Due to the toxicity at low pH, unless shown to the contrary, sites with pH <6 are excluded from data sets prior to analysis.

Also, when using this method, the EPA recommends that at least one sequence of analyses be used to evaluate the effect of confounding on the XCD model. For example, potential confounding can be evaluated using multiple regression analyses followed by modification of the data set to control for the strongest potential confounding factors and then calculating the HC_{05}. If the HC_{05} is not appreciably altered by this data set manipulation, the full data set can be used. If the HC_{05} is appreciably altered, the criterion data set may be modified to minimize that factor's effect on model prediction. The model is accepted if the confidence bounds of the original and new HC_{05} overlap. Examples for confounding analyses and reducing effects of confounding can be found in Case Studies I and II in Appendices A.2 and B.2, respectively.

There are two common means for reducing the influence of confounders. First, sites with a confounder can be removed from the data set, thus reducing its influence on the XC_{95} estimates and XCD model. For example, the EPA removed samples with low pH in the case study examples (see Appendices A.2.3 and B.2.3). Secondly, the effect of a confounder can be minimized by normalizing the influence of a confounder with appropriate weighting. The EPA used this approach to assess the influence of temperature and season in the case study examples and these methods could be used to adjust for confounders if necessary (see Section 3.1.4, and Appendices A.2.3 and B.2.3). Removing samples from the data set can reduce the number of species or the range of exposures of the stressor of interest, thus affecting the reliability of the estimates. Therefore, it is important to evaluate whether the manipulation of the data set improves the accuracy of the HC_{05}. Each case is different, and professional judgment is recommended.

3.1.1.3. *Quality Assurance/Quality Control (QA/QC)*

Information is reported about the specific methods used to choose sampling locations, to sample water SC and macroinvertebrates, and to assure data quality. Some considerations include whether standardized quantitative or semiquantitative techniques are used for macroinvertebrate sampling, the mesh size used in the field and lab for sampling and sorting, whether samples are subsampled, and if so, what percentage was subsampled. The data set description and metadata include sampling dates; total number of chemical, physical, and biological samples from distinct locations (and total samples); sampled years; stream types represented by samples; and ecoregions represented by sites. Annual sampling when salt-intolerant genera are likely to be present is usually sufficient and avoids damage to the habitat and stream biota due to repeated sampling during the year. If sampling occurs more than once per year from the same location, the use or restriction of repeat samples is described. Similarly, a full description or literature references are provided for the chemical and physical parameters that are used in any analysis (see an example in Section 4.1). Additional information regarding QA/QC and other critical technical elements of a robust biological assessment program (e.g., taxonomic resolution, sample collection, sample representativeness, sample processing, data management, and professional review) can be found in EPA's technical assistance document*, Biological Assessment Program Review: Assessing Level of Technical Rigor to Support Water Quality Management* (U.S. EPA, 2013a).

3.1.2. Calculating Genus Extirpation Concentrations (XC_{95})

For each genus meeting the data-selection conditions, a CDF is constructed that is weighted to correct for any potential bias from the unequal distribution of sampling of sites across the range of logarithm10 transformed SC values. This weighted CDF represents the proportion of observations of a genus with respect to increasing exposure levels. The extirpation effect threshold for a genus is 95% of the total occurrences of the genus. The two exposure levels bracketing the 95^{th} centile are linearly interpolated to give an XC_{95} for a genus.

In the case examples, all calculations are performed using logarithm base 10 (log10) transformed SC values. Variables are routinely log transformed when applying the field-based methods. Because environmental data are usually skewed, log transformation normalizes the data so that normality assumptions are not violated. Log transformation also tends to increase equality of variance, increase the linearity of relationships, and makes plotted relationships clearer.

First, equally-sized bins are defined to compute weights for each sample. Bin size depends on the data set and is based on balancing the requirements of sufficient observations in a single bin to define the proportion and a sufficient number of bins to define the form of the response. The effect of bin size can be analyzed by developing a series of HC_{05} values using different bin and sample sizes. In general, 40 to 60 bins usually gives acceptable results. For example, for Case Studies I and II, the width of each bin is 1/60th of the range of the log10 transformed SC values. Thus, each bin was assigned a width equal to 0.017 (1/60 bins) multiplied by the range of the log10 transformed SC values within the data set (for examples see Figures 4-6 and 5-6).

Next, the bins are weighted to ensure that sites in bins with many observations are not overly influential. The assigned weight for each sample within a given bin is $w_i = 1/n_i$, where n_i is the number of samples in the *i*th bin. The value of the weighted cumulative distribution function, *F(x)*, is computed using the following equation for each unique observed value of the agent *x* associated with observations of a particular genus:

$$F(x) = \frac{\sum_{i=1}^{N_b} w_i \sum_{j=1}^{M_i} I(x_{ij} < x \text{ and } G_{ij})}{\sum_{i=1}^{N_b} w_i \sum_{j=1}^{M_i} I(G_{ij})} \tag{3-1}$$

where

x_{ij} is the stressor value in the j^{th} sample of bin *i*,
N_b is the total number of bins,
M_i is the number of samples in the i^{th} bin,
G_{ij} is true if the genus of interest is observed in j^{th} sample of bin *i*,
I is an indicator function that equals 1 if the indicated conditions are true, and 0 otherwise,
W_i is the assigned weight of a sample within the i^{th} bin, $w_i = 1/M_i$.

The XC_{95} value is defined as the stressor value corresponding to *F(x)* = 0.95. Equation 3-1 is an empirical cumulative distribution function, and the output is the proportion of observations of the genus that occur at or below a given exposure level. See Figure 3-3 for examples of weighted CDFs. Figure 3-3 shows weighted proportions of samples with *Epeorus* and *Nigronia* present at or below the indicated SC value (μS/cm). The XC_{95} is the SC at the 95th centile of the weighted CDF (vertical dashed line). Within the observed ranges of SC, the weighted CDFs of some genera demonstrate a response to SC (e.g., *Epeorus*) as shown by a steep slope and asymptote well below the maximum exposures. Conversely, genera unaffected within this SC range (e.g., *Nigronia*) have a steady increase over the entire range of measured exposure and do not reach a clear asymptote.

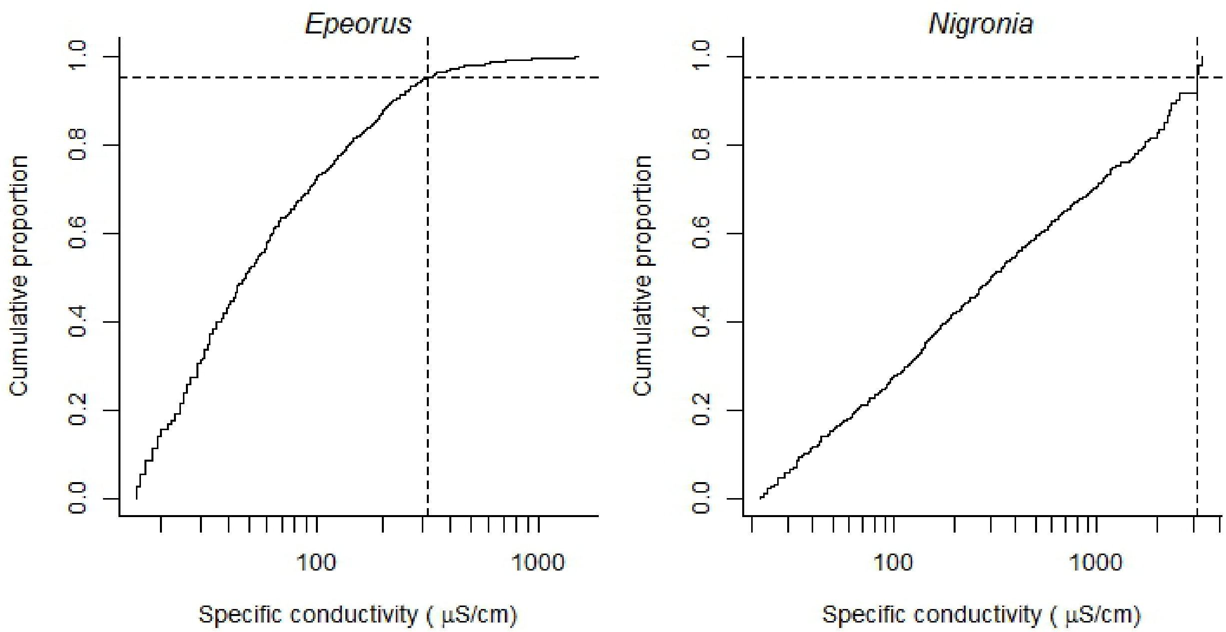

Figure 3-3. Examples of weighted cumulative distribution functions (CDFs) and the associated 95th centile extirpation concentration values.
The plot for *Epeorus* rises steeply and reaches a plateau, indicating that the genus is affected by increasing specific conductivity (SC). The plot for Nigronia increases linearly without reaching an asymptote, indicating that this genus is not as affected by changes within the tested range of SC.

This method for calculating the XC_{95} will generate a value even if the genus is not extirpated. For example, the occurrence of *Nigronia* changes little with increasing SC (i.e., the cumulative distribution is linear [see Figure 3-3]). Because of the data distributions, not all 95th centiles correspond to extirpation, and some imprecisely estimate the extirpation threshold. The weighted CDFs (see Figure 3-3, Appendices A and B) and scatter plots (see Figure 3-4, Appendices A and B) should be visually inspected for anomalies; if there is no clear trend in the response or if the response does not include extirpation, the XC_{95} can be given a qualifying assignation such as either approximately (~) or greater than (>) the calculated value. The assignation of > or ~ does not affect the HC_{05} if the values are above the 5th centile, but it alerts users of the uncertainty of the XC_{95} values (see Section 3.1.2.1).

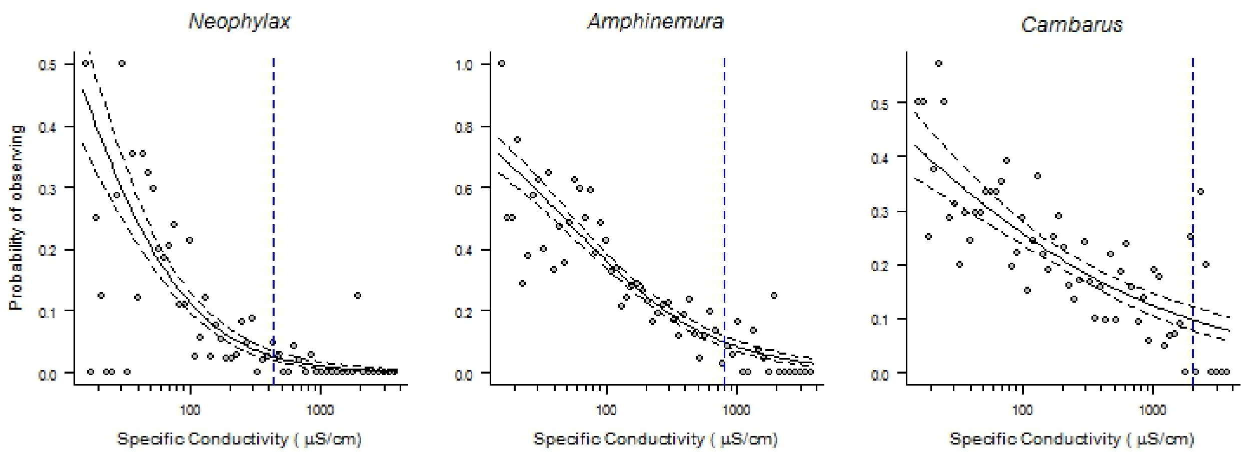

Figure 3-4. Examples of extirpation concentration (XC_{95}s) for three genera listed as being definitive (*Neophylax*), approximate (*Amphinemura*), and greater than (*Cambarus*). In this example, the probability of observing a genus is the proportion of sampled stations in a SC bin with the genus present based on taxonomic identification of 200 individuals per sample. Qualifications based on the slopes of the confidence bounds as described above. For *Neophylax*, both upper and lower 90% confidence limits intersect *x*-axis, for *Amphinemura* only the lower intersects, and for *Cambarus* neither intersect. The vertical line is the XC_{95} calculated from the weighted cumulative distribution functions.

3.1.2.1. *Assigning Qualifying Designation to Extirpation Concentration (XC_{95}) Values*

The uncertainty bounds of a GAM are used to indicate the confidence in the calculated XC_{95} and whether the value is greater than the tested range. A GAM is not used to estimate the XC_{95}. In order to examine the trend of taxa occurrence along the SC gradient, a GAM is used to model the likelihood of a taxon being observed with increasing SC (Hastie and Tibshirani, 1986). In the example, the probability of observing a genus is the percentage of sampled stations in a given SC bin with the genus present based on taxonomic identification of 200 individuals per sample (see Figure 3-4).

Three typical distributions of observational probabilities are shown in Figure 3-4. A GAM is similar to a regression model except that it iteratively fits a line to the data using a scatterplot smoother which can use any function, not only a straight line or polynomial. The form of the fit is automated and the final shape of the fitted line is useful for revealing nonlinear covariate effects. In this analysis, the GAM shows whether a genus is increasing, decreasing, or unchanging along the SC gradient. The 90% confidence interval (CI) of the GAM is used to judge whether a decreasing model identifies the XC_{95}, approximates the XC_{95}, or is not measured within the exposure range of the data set, which is indicated as being greater than (>) the estimated value. In the figure, the solid line is the mean smoothing spline fit, and the dots are the mean observed probabilities of observing a genus within a defined SC range, estimated as the proportion of samples within each SC bin. The SC at the vertical dashed line is the estimated XC_{95} previously calculated from the weighted cumulative distributions such as depicted in Figure 3-3.

If the GAM mean fitted curve at maximum SC is approximately equal to 0 (defined as less than 1% of the maximum modeled probability), as in the *Neophylax* example in Figure 3-4, then the XC_{95} is listed without qualification (*Neophylax* XC_{95} is listed as 434 µS/cm without qualification; Appendix A.4). If the GAM mean fitted curve at maximum SC is greater than 0, but the lower 5% confidence limit is approximating to 0 (<1% of the maximum mean modeled probability), as in the *Amphinemura* example in Figure 3-4, then the value is listed as approximate (*Amphinemura* XC_{95} ~805 µS/cm). If the GAM lower 5% confidence limit is greater than 0, as in the *Cambarus* example in Figure 3-4, then the XC_{95} is listed as a greater than value (*Cambarus* XC_{95} > 1,974 µS/cm). The assignations of greater than (>) and approximately (~) does not affect the HC_{05}. They are provided to alert users of the uncertainty of the XC_{95} values for other uses such as comparison with toxicity test results or with results from other geographic regions.

3.1.3. Calculating the Community-Level Effect Estimate Hazardous Concentration (HC_{05})

The XCDs are cumulative distribution plots of XC_{95} values for each genus relative to SC level (see Figure 3-1). The XCD is useful for visualization, but not necessary to calculate the HC_{05}. The cumulative proportion for each genus P is calculated as $P = R/(N + 1)$, where R is the rank of the genus and N is the number of genera. Relatively tolerant genera coded as ~ or > are included. They are reported as ‘‘approximate” or “greater than’’ values. Their inclusion assures that N is the correct number of genera, but they do not otherwise contribute substantially to the HC_{05} because they fall in the upper portion of the XCD. The HC_{05} is derived by two-point interpolation between the XC_{95} values bracketing $P = 0.05$ (i.e., the 5^{th} centile of modeled genera). For an example of an XCD cumulative distribution plot of XC_{95} and HC_{05} derivation, see Figure 4-7.

3.1.3.1. *Validating the Effect Estimate Hazardous Concentration (HC_{05}) by Bootstrapping*

The EPA's Science Advisory Board (U.S. EPA, 2011c) recommended using bootstrapping as one way to validate the XCD model and estimate uncertainty around the HC_{05} values. This method generates distributions and confidence bounds for each genus in the first step and propagates the statistical uncertainty of the first step through the later steps in which the XCD is created and the HC_{05} is estimated (see Figure 3-5). A data set of the same sample size as the original data set is randomly selected with replacement from the original set of samples (Efron and Tibshirani, 1993). The XC_{95} for each genus that occurs at least once in the original data set's reference sites and in more than 24 sampled sites is calculated from the bootstrap data set by the same method applied to the original data. The XC_{95} for each genus is stored and later used to estimate the confidence bounds of each genus' XC_{95}. Then, the HC_{05} is calculated and stored. The uncertainties in the XC_{95} and HC_{05} values are estimated by repeating the sampling and calculations 1,000 times. The distribution of 1,000 HC_{05} values is used to generate two-tailed 95% confidence bounds on these bootstrap-derived values (see Figure 4-13 for an example). The particular genera and the number of genera in any bootstrapped XCD differ in each bootstrapped sample of sites; therefore, the number of bootstrapped XC_{95} values of genera may be more or less than the number of genera in the original data set. The distribution of 1,000 XC_{95} values for each genus is also used to generate two-tailed 95% confidence bounds on these bootstrap-derived XC_{95} values. See Appendices A.3 and B.3 for example 95% confidence bounds for each genus.

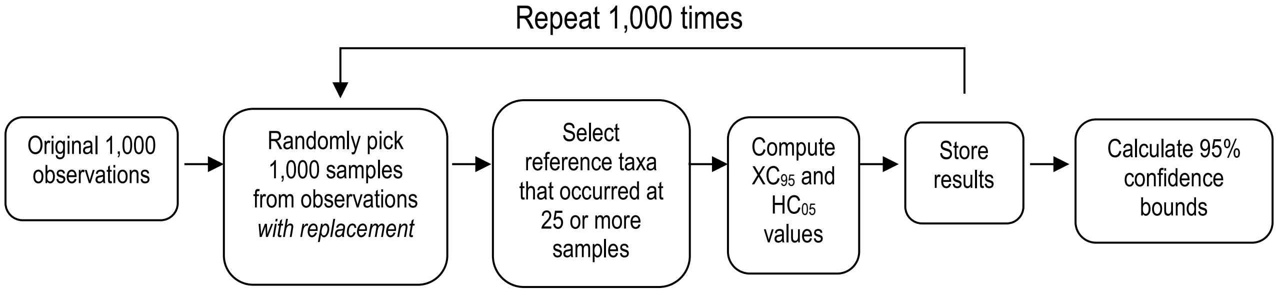

Figure 3-5. Diagram from *EPA Benchmark Report* depicting the process for estimating uncertainty. Bootstrapping is a resampling of the original data set to create 1,000 new data sets and from each of the 1,000 data sets, the extirpation concentration (XC_{95}) values are calculated. After each run, an extirpation concentration distribution (XCD) is made and a hazardous concentration (HC_{05}) estimated. This process is repeated until there are 1,000 HC_{05} values and then the confidence limits of the HC_{05} are estimated. The number of samples varies depending on the data set. The same resampling process is used to evaluate the effect of different sample sizes, exclusion of genera using different database selection criteria, or other parameter choices (see Section 3.1.1.2).

Confidence bounds represent the potential range of HC_{05} values using the XCD approach based on the data set. Conceptually, these confidence bounds may be thought to represent the potential range of HC_{05} values that one might obtain by returning to the field and resampling the same set of streams. The contributors to this uncertainty include measurement variance in determining SC and sampling variance in the locations for monitoring, collecting, and enumerating organisms. These also include variance due to differences in stream reaches, weather, and other random factors.

The confidence bounds do not address potential systematic sources of variance such as differences in sampling protocols. The contributions of those sources of uncertainty, in addition to the sampling uncertainty, can best be evaluated by comparing the results of independent studies. One estimate of that uncertainty may be provided by comparing the all-year HC_{05} values derived from the region for which criteria is being derived to another comparable region. Even if data are obtained in different areas by different agencies using different laboratory processing protocols, the HC_{05} values may be similar.

In the *EPA Benchmark Report*, the HC_{05} value was validated by an independent data set which had a similar background SC, and the values differed by less than 5% (see Appendix G in U.S. EPA, 2011a). Large data sets for ecoregions from more than one sampling organization are rare and so EPA has provided this bootstrapping method (Newman et al., 2000) as an alternative method to validate the XCD model as suggested by the SAB in their review of the *EPA Benchmark Report* (U.S. EPA, 2011c).

3.1.4. Assessing Seasonality, Life History, and Sampling Methods

The seasonality of life-history events such as emergence of aquatic insects can affect the probability of detecting a species because eggs and early instars are not collected by most sampling methods. As an illustration, in the example cases in Sections 4 and 5, annual insects (univoltine) that emerge in the spring, although present, are less likely to be detected in the summer, when coincidently, SC levels increase in some streams (e.g., due to decreased flow). In other locations, this pattern may be different. For example, high mountain systems may be affected by melting snow pack. Seasons may shift based on latitude. Also, sampling restricted to a season can bias the estimate of natural background or effect estimates (i.e., XC_{95} values). Both high-concentration and low-concentration periods should be represented when the salt-intolerant genera are collected in order to ensure that the tolerated range is evaluated. These periods may vary by time of year among regions, and among years (based on climate variability) for any given region. Professional experience with the SC regimes and the life cycles of vulnerable species is required when assessing whether a data set is suitable for using the XCD method.

Because the hydrologic and SC regime and the natural history of salt-intolerant taxa vary by region, the potential effect of sampling date on the form of the XCD model may be assessed using several methods. As an illustration, in the example cases in Sections 4 and 5, the HC_{05} using the spring (March−June) only data set was compared with the HC_{05} based on the full-year data set for the ecoregion. If the spring HC_{05} is within the confidence bounds of the full-year data set, then the full annual data set can be used (see example in Appendix A, Figure A-8); if this is not the case, further correction for a confounding factor may be necessary as described below for the example using sampling date.

A scatter plot and regression model can be developed to evaluate the mean relationship between measurements of SC at the time of the biological sample and annual mean SC (for an example, see Appendix A, Figure A-9). The annual geometric mean SC values are calculated from at least six water samples collected before biological samples were taken. At least one spring (when salt-intolerant taxa can be collected) and one summer macroinvertebrate sample is recommended in order to increase the likelihood that salt-intolerant taxa will be included in the data set. On the *x*-axis is the SC when biological samples are collected and on the *y*-axis is the annual geometric mean value during that rotating year for a site. A Model II regression is fitted to the data because the two SC measurements are uncertain. [A Model I regression using least squares is not used because it underestimates the slope of the linear relationship between the variables when they both contain error (Legendre, 1998)]. The mean relationship between measurements of SC at the time of the biological sample and annual mean SC is supported if the relationship approaches 1:1, and prediction for the annual mean from the regression model is within the confidence bounds of the HC_{05}.

A third approach to account for seasonal variability involves adjusting SC results collected at the time of biological sampling to estimate annual mean SC values. In situations where the SC tends to be lower in the spring than in the summer, the effect of seasonality on the HC_{05} is evaluated by converting the instantaneous biological sample SC into an annual mean value based on monthly weighting factors. Then, the XC_{95} and HC_{05} values are estimated. The average weighting factors for each month can be calculated from the previous sampling year. One way to do this is to select a subset of stations where multiple SC measurements are taken within a rotation year (e.g., from July to the following June). For each site, the annual mean SC is calculated using the monthly measurements. Then, the weights for each month are calculated as a ratio of the annual mean SC to the observed SC at each site on the day of biological sampling. Next, the average weight within each month is calculated. Finally, for the data set used to develop the XCD and the HC_{05}, the SC on the day the biological sample was collected is multiplied by the weighting factor for that month to yield the estimated annual SC for each site. The resulting products are considered the annual mean SC at each station in that rotation year, adjusted by month. The weighting factors vary slightly for different months in different data sets (see Appendix A, Table A-2 for an example). This approach may be adapted to different seasonal SC patterns, as appropriate.

If the confidence bounds of the weighted HC_{05} overlap the confidence bounds of the unweighted HC_{05}, the unweighted model is accepted. For example, the HC_{05} values in Case Studies I and II vary by less than 3%, suggesting that the impact of sampling date (seasonality) is minor for these data sets. As a result, seasonally unweighted XCDs are used for the assessment in these case studies. In general, the use of unweighted XCDs is easier and requires fewer data points. However, all data sets may not yield similar results, and thus this method provides a way to evaluate the influence of season and also provides a method for normalizing for sampling date when necessary.

3.2. DERIVING A CRITERION MAXIMUM EXPOSURE CONCENTRATION (CMEC)

A CMEC is defined as the SC level that protects aquatic life from acutely toxic exposures. The CMEC analysis described here estimates the 90th centile of observations at sites with water chemistry regimes for sites meeting the CCC. It is not directly estimated from paired biological and water chemistry during acute exposures. However, if sufficient data are available (e.g., daily measurements of SC paired with macroinvertebrate sampling), a protective criterion maximum concentration could be estimated from the maximum concentration in a year prior to the observation of salt-intolerant genera at a site. An example of this type of analysis is provided in Appendices A.3 and B.3, but such data sets are rare. Even for the case studies using the large data sets in Ecoregions 69 and 70, there are only modest amounts of data to estimate such a criterion maximum concentration in this manner.

Using only water chemistry measurements and a previously determined CCC, a CMEC is estimated such that where the CCC is attained, 90% of observations are likely to be less than the CMEC. The steps involved in selecting, characterizing, and analyzing SC (chemistry) sampling data to derive a CMEC for flowing waters in the study area are described below (see Figure 3-6) and example derivations in Sections 4.2.2 and 5.2.2.

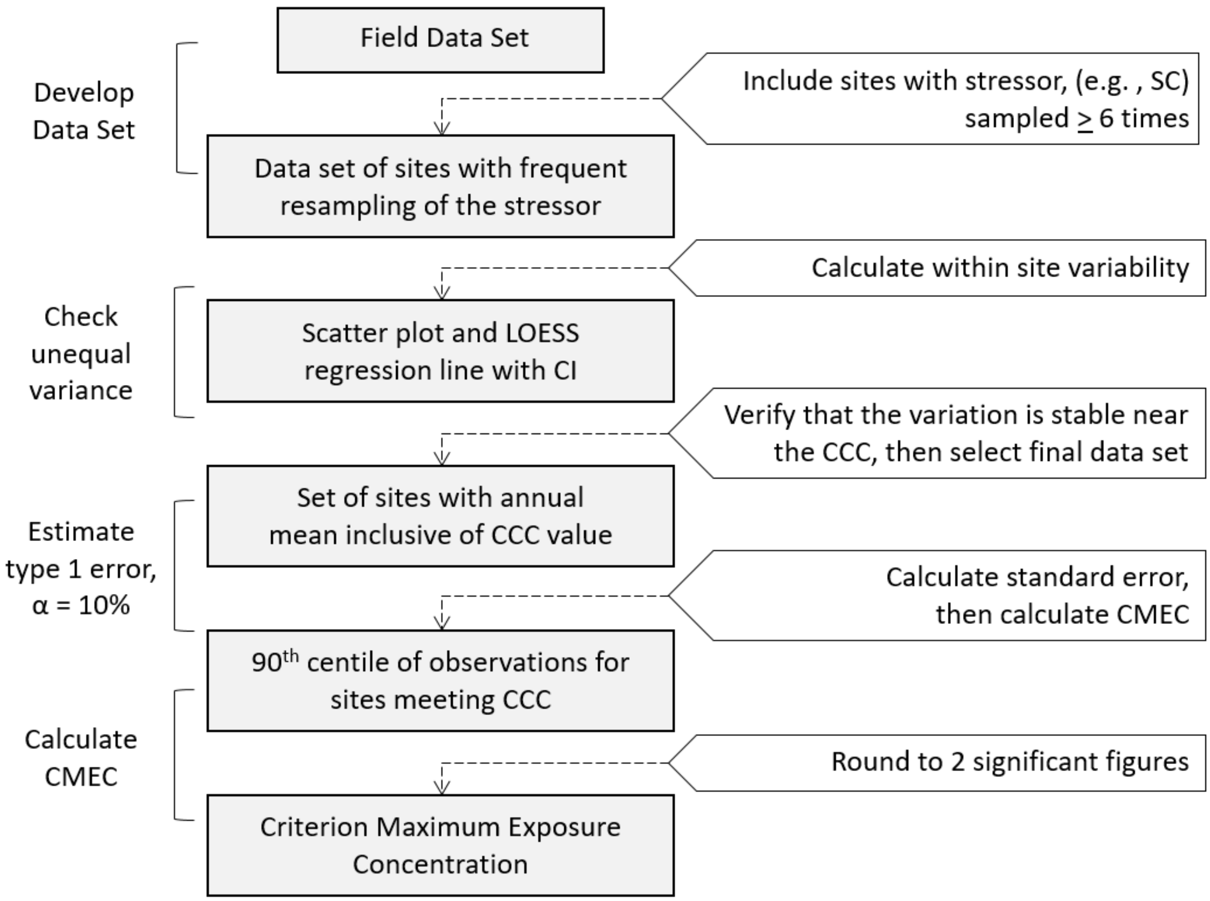

Figure 3-6. Main steps in the derivation of a criterion maximum exposure concentration (CMEC) based on field water chemistry data. Rectangular boxes on left are products and pentagonal boxes on right are operations performed on those products. A locally weighted scatterplot smoothing (LOWESS) estimates a line for a scatter plot by iteratively calculating many nonparametric regression models using local approximations (linear polynomial) from neighboring points.

The CCC is expressed as an annual geometric mean of the SC values measured from a sampling station in a particular region. Because SC values vary spatially and temporally, it is expected that the maximum SC values (or the CMEC) at any given station may be estimated by incorporating both among-station (spatial), and within-station (temporal) variability. Using the mean SC at the CCC and the variance of a SC distribution, a centile near the maximum, such as the 90th centile, for that distribution can be estimated. Thus, where the CCC is met within the region, only 10% of the observations (grab samples) would be predicted to exceed the CMEC.

To do this, a subset of frequently sampled sites is developed. For Case Studies I and II, a representative sample set was identified in which *n* stations (*j* in *1,.., n* stations) were sampled at least six times (sample size k_i, i^{th} in *1,…,* k_i observations) on a rotating yearly basis (in the case example from July to June). The preferred data set would have multiple SC measurements evenly distributed throughout the year. A minimum of one sample during the low SC season (e.g., March−June in Appalachia), and one sample in the high SC season (e.g., July−October in Appalachia) may be sufficient to capture temporal variability. As with the derivation of the CCC, a range of exposures that leads to adverse effects on the most salt-intolerant taxa needs to be represented in the data set and there needs to be assurance that there is no bias in the sampling within that range. The grand mean and standard deviation of this data set are calculated. The CMEC can be calculated at the 90th centile of the distribution from log values of SC in the region from this equation:

$$CMEC = 10^{(\bar{X}+z_{\alpha}*\sigma_r)} \quad (3\text{-}2)$$

Where $\bar{X}$ is the proposed annual geometric mean value limit for all stations ($\bar{x}_j$) (i.e., the CCC), z_{α} is the one-tail critical value for the 90th centile of a normal distribution (α, 10%), σ_r is the total residual standard deviation, i.e., the square root of the standard deviation. The CMEC is calculated based on eq 3-2 with $\bar{X}$ equal to log10 of the CCC for the ecoregion. For example, if the grand mean of all sites is 310 μS/cm, and the standard deviation is 0.243 μS/cm, and the z_{α} at 90th centile is 1.28, then the estimate of 634.1 μS/cm is rounded to two significant figures resulting in a CMEC of 630 μS/cm (see eq 3-3).

$$10^{\log10(310)\,+\,1.28*0.243} = 630\ \mu S/cm \quad (3\text{-}3)$$

3.3. ESTIMATION OF CRITERIA DURATION

The water quality standards handbook (U.S. EPA, 1983) describes duration as follows:

> The quality of ambient water typically varies in response to variations of effluent quality, stream flow, and other factors. Organisms in the receiving water are not experiencing constant, steady exposure but rather are experiencing fluctuating

exposures, including periods of high concentrations, which may have adverse effects. Thus, the EPA's criteria indicate a time period over which exposure is to be averaged, as well as an upper limit on the average concentration, thereby limiting the duration of exposure to elevated concentrations.

Because this field-based approach relies directly on paired in situ measurements of SC and benthic invertebrate assemblage composition, the potential adverse effects of ionic stress on all life stages are considered in the context of other complex relationships (e.g., food web dynamics) and aquatic ecosystem processes. The measures of effect (i.e., XC_{95} and HC_{05}) are considered chronic-duration endpoints because the field data reflect exposures over whole life cycles and multiple generations of the resident biota (see Table 2-3).

The EPA typically recommends an averaging period of 4 days for a CCC, which may be appropriate for some field-derived criteria (U.S. EPA, 1985). Important considerations for estimating duration are the temporal resolution of the biological data set and the seasonal window for observing salt-intolerant genera (typically early in the year). Based on available field data, salt-intolerant macroinvertebrate genera may be exposed to a range of SC levels greater than the CCC throughout the year and often for more than 4 days (see example in Figure 3-7). For example, biological samples collected once annually (as in Case Studies I and II in Sections 4 and 5) represent the average stream chemistry and macroinvertebrate assemblage information over the course of 1 year. In cases where samples were collected on an annual basis the EPA recommends a duration of 1 year for CCCs for SC derived using the XCD method unless there are sufficient data to support an alternative duration.

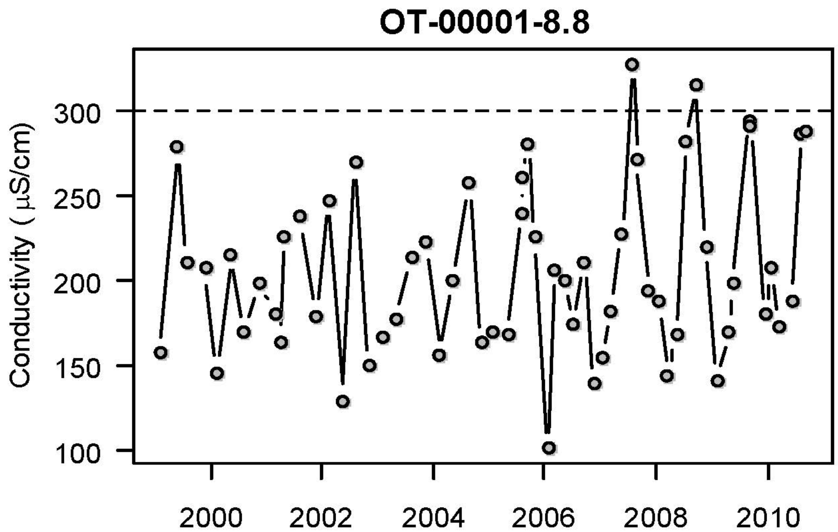

Figure 3-7. Typical specific conductivity (SC) pattern of a stream with annual mean SC well below 310 µS/cm. Aquatic life is typically exposed to a range of SC levels throughout the year.

The duration for the CMEC in Case Studies I and II is based on a literature review of the rate of onset of critical biological responses and the sampling duration used in the field data set used to establish the CMEC. Although reproductive effects (see Section 2.5) may occur rapidly following exposure, they occur only during distinct temporal windows that vary with species (life history). Increased drift (benthic invertebrates floating downstream), in contrast, can occur any time a spike in exposure occurs. In numerous studies, increased drift may be induced within minutes of stressful exposures in streams and in artificial test channels (Svendsen et al., 2004; Wood and Dykes, 2002). Most ecological studies describe drift as a part of the natural history of dispersal and colonization, but disturbance has also been identified as a cause of drift (Svendsen et al., 2004; Wood and Dykes, 2002; Crossland et al., 1991; Doeg and Millage, 1991; Wallace, 1990; Brittain and Eikeland, 1988; Sheehan and Winner, 1984; Geckler et al., 1976; Waters, 1995, 1972, 1966). In a study that induced drift from the addition of sodium chloride (NaCl), the onset of drift occurred within 15 minutes, and on average, the greatest occurrence of drifting genera took place within 4 hours (Wood and Dykes, 2002). In that study, prior to the addition of salt, SC was 110 µS/cm (River Holmes). During three trials, drift occurred at maximum total dissolved solids (~110 mg/l or SC ~157 µS/cm) and not at the lower concentration (~85 mg/L or SC ~121 µS/cm). In stream mesocosm studies with HCO_3^- and SO_4^{2-} salts, Clements and

Kotalik (2016) reported an approximate doubling of drift within 24 hrs (196 µS/cm). Avoidance and drift behaviors, in general, typically occur quickly following most noxious stimuli.

Unlike laboratory studies, maximally tolerated SC exposures were not measured. Rather, the CMEC is the 90th centile of observations measured at sites meeting the CCC. Therefore, the duration parameter is based on the rate of onset of drift described in published field experiments; the recommended duration for the CMEC is 1 day.

3.3.1. Summary of Recommended Duration for Criterion Continuous Concentration (CCC) and Criterion Maximum Exposure Concentration (CMEC)

The temporal resolution of the biological data set and the seasonal window for observing salt-intolerant genera are key considerations when estimating the duration for the CCC and CMEC for SC. In cases where sampling occurs once annually, as in Case Studies I and II provided in Sections 4 and 5, the recommended durations for the CCC and CMEC are 1 year and 1 day, respectively.

3.4. ESTIMATION OF CRITERIA FREQUENCY

The water quality standards handbook (U.S. EPA, 1983) describes frequency as follows:

> To predict or ascertain the attainment of criteria, it is necessary to specify the allowable frequency for exceeding the criteria. This is because it is statistically impossible to project that criteria will never be exceeded. As ecological communities are naturally subjected to a series of stresses, the allowable frequency of pollutant stress may be set at a value that does not significantly increase the frequency or severity of all stresses combined.

The frequency with which criteria may be exceeded depends on the rate of recovery of the biotic community. In general, if the interval between exceedances is less than the time to recovery, impairment is perpetuated. Time to recovery may be estimated mechanistically from the life histories of the organisms involved or empirically from field studies of stream community recovery.

In this case, to estimate the interval between extirpation and reestablishment of a reproducing population, the EPA adapted a list of potential factors that may affect recovery time of stream organisms (Wallace, 1990). Although these considerations were originally outlined with respect to pesticide exposure, they are a reasonable list of factors to consider for any stressor. The revised considerations are listed below with corresponding numbers from Wallace (1990):

1a. Magnitude of the initial stressor load

1b. Adverse effect of the stressor

1c. Areal extent of continued inputs of the stressor

2. Spatial scale of the disturbance

3. Persistence of the stressor at the site

4. Vagility of the populations (ability to move and disperse) influenced by exposure

5. Timing of contamination in relation to life history stage

6. Position within the drainage network

For the purposes of estimating frequency, the concentration of the initial stressor load (see Item 1a) is greater than the magnitude of an annual average CCC or a daily CMEC. The initial adverse effects (see Item 1b) are extirpation resulting from drift and failure to recruit. Because insect colonization (the predominant invertebrate group in streams) is sometimes possible via aerial dispersal, frequency was estimated for conditions where the effect of areal extent (see Item 1c) or spatial scale of the disturbance (see Item 2) is minimal. However, on a case-by-case basis, if the disturbance by ionic concentration is spatially extensive, the frequency recommendation for these criteria might not be protective (Smith et al., 2009; Lindberg et al., 2011; Bernhardt et al., 2012). Wallace (1990) defines persistence (see Item 3) as continuation of exposure in the environment after cessation of new inputs. Because salts are highly soluble in water, they are flushed downstream (in flowing waters) when loading stops, and therefore, they are not persistent chemicals in the sense defined by Wallace (1990) although lag times can be long when contaminated groundwater flows to surface waters. Although intermittent releases of water with high specific SC result in intermittent exposures, the aquatic impacts appear to be long term owing to persistent exposures (U.S. EPA, 2011b; Pond et al., 2014; Evans et al., 2014; Williams, 1996; Feminella, 1996; Delucchi, 1988; del Rosario and Resh, 2000). Recolonization rates from upstream sources or connected tributaries that provide a source of drifting juveniles was not used to estimate frequency so as to provide a conservative estimate and because in some situations, such as headwater streams, there may not be an upstream source of recolonizing juveniles (see Item 6). Using these conditions, the life history of salt-intolerant benthic invertebrates (see Item 5) is considered with respect to recolonization potential (vagility) (see Item 4) from aerial dispersal of adults and oviposition.

3.4.1. Recovery Rates in Literature Reviews

The frequency parameter for this method is estimated from ecosystem recovery rates following disturbance as reported in the literature. In this case, frequency is an estimate of the period of time between macroinvertebrate extirpation and recovery (reestablishment) of the population. The estimate of time to recovery is based on life cycles and natural history. The assessment is supported by a literature review of the recovery of aquatic macroinvertebrates following chemical and nonchemical-induced effects in 31 nonflowing systems (lentic) and 111 flowing (lotic) systems reviewed by Niemi et al. (1990) and more than 12 streams reviewed by Wallace (1990). Niemi et al. (1990) indicated that recovery time was less than 3 years *except* when (1) the disturbance resulted in physical alteration of the existing habitat, (2) residual pollutants remained in the system, or (3) the system was isolated and recolonization was suppressed. The frequency estimated for SC criteria applies when the three factors listed above are not operative; that is, physical alteration has not taken place, pollutants are flushed from the system, and colonization is possible.

Ionic regimes may be long lasting. In a study of 15 valley fills, Pond et al. (2014) found that SC remained elevated 11−33 years after reclamation. Despite good instream habitat, nearly 90% of these streams exhibited biological impairment. Valley fill sites with higher index scores were near unaffected tributaries, an indication that drifting colonists accounted for the presence of sensitive taxa. Based on 137 valley fills, Evans et al. (2014) estimated that it would take approximately 20 years to potentially attain SC levels <500 μS/cm after initiation of valley-fill construction. These two studies underscore the fact that although recovery can occur within 3 years when the exposure no longer exists, some streams may take decades to return to levels that salt-intolerant genera can tolerate and maintain viable populations.

Wallace (1990) also indicated that the definition of recovery was inconsistently applied in the scientific literature and in most cases true recovery was not attained within the study interval;

that is, some species had recolonized but the original assemblage was altered. Many other studies of invertebrate recovery times from single events, such as toxic chemical spills, floods, pesticides, drought, and organic pollution, indicate recovery times of a few months to several years (e.g., Kattwinkel et al., 2012; Molles, 1985; Minshall et al., 1983; Heckman, 1983; Fisher et al., 1982; Hynes, 1960; Mebane et al., 2015). However, in some situations, biological communities may have faster recovery times (Wood and Dykes, 2002) if the exposure duration is short and if there are upstream sources for recolonization. In a more recent review of 200 studies with pesticide exposures, Kattwinkel et al. (2012) reported that migration from upstream uncontaminated areas is a main driver for recovery and that recolonization varied with generation time and source of migrants; however, upstream sources of colonizers may not be present (e.g., in headwater streams). Faster recovery times were related to drift from external sources (Caquet et al., 2007; Liess and Schulz, 1999) or untreated refugia (Brock et al., 2009). Salt-intolerant univoltine species (life cycles of 1 year) do not recover within 1 year after exposure (Liess and Beketov, 2011; Liess and Schulz, 1999). Overall, most studies reported that recovery took two or more generations and as many as five generations even with upstream sources that can recolonize by drift. Furthermore, there is a trend of longer population recovery time with increasing generation time; species with long generation cycles often take longer for population recovery.

Most of the macroinvertebrate genera sensitive to ionic stress have a univoltine generation time (1-year life cycle), and therefore, their recovery time is likely to be longer compared to multivoltine genera (having less than 1-year life cycles). Where recolonization by juveniles drifting from upstream refugia is not possible, aerial dispersal from nearby streams would be necessary to reestablish populations of aquatic insects; in this case, recovery may take longer than 3 years. The frequency recommended for this method assumes there are either sources from upstream or airborn dispersal for recolonization.

In summary, if the concentration of major ions in a stream can be returned to levels that are capable of supporting aquatic life, and if the physical habitat is suitable, and if there are opportunities for recolonization from an upstream source and/or through aerial dispersal from nearby streams, then the allowable recommended frequency of exceedance for criteria derived using this method is once every 3 years. If any of these conditions are not met, then the frequency parameter is expected to be more than 3 years, because the stream lacks the ability to recover within that span of time.

3.4.2. Life History Considerations

Often, more than 90% of benthic invertebrates in streams are insects. Several of the most salt-intolerant benthic insects (e.g., mayflies, stoneflies, caddisflies, and true flies) have a 1−2 year life cycle with emergence, mating, and early development occurring in the spring months (Merritt and Cummins, 1996; Brittain, 1982; Clifford, 1982). Hypothetically, if a univoltine genus is extirpated in the first year and in the following spring migrating insects laid eggs, offspring from the colonizers would be large enough to be observed in the collections the following year (i.e., 2 years after the initial extirpation event). Assuming that a recovered population required two reproductive seasons (Liess and Beketov, 2011; Liess and Schulz, 1999), the earliest measurable recovery would be the year after that, or 3 years after the initial extirpation event. The genetic diversity of the population founded by a few colonists may be low (i.e., the founder effect) and as a result the population may be less resilient.

Gastropods, amphipods, isopods, and crayfish tend to be more tolerant to ionic stress (see Appendix E); these taxa would likely remain provided that SC levels did not exceed their predicted extirpation concentrations. Extirpation of most noninsect benthic invertebrates is not expected to occur if the yearly average is <960 μS/cm, the XC_{95} of the most salt-intolerant crustacean based on values calculated using a combined data set from Case Studies I and II (U.S. EPA, 2011a, see Appendix D). The natural history of fish suggests that they may be able to recolonize quickly due to their greater mobility; however, immigration may be limited for some species because they are endemic to specific drainages or there may be barriers to emigration (Hitt and Chambers, 2014; see Appendix G). Unionid mussels were not evaluated by the EPA, but some field and laboratory studies suggest that Unionidae are also salt-intolerant (Price et al., 2014; Gillis, 2011; Wang et al., 2013, Kunz et al., 2013). If immigration of fish is restricted or if less mobile species such as mollusks and crayfish are extirpated, their recolonization could take much longer than 3 years, or may require reestablishing a colonizing population by stocking (Wallace, 1990).

3.4.3. Summary for Field-Based Frequency for Criterion Continuous Concentration (CCC) and Criterion Maximum Exposure Concentration (CMEC)

If SC criteria derived using this method are exceeded, it is expected that at least 5% of macroinvertebrate genera will be extirpated, and many more genera will have been exposed to levels that reduce their occurrence (U.S. EPA, 2011a). However, recovery is expected to occur in 3 years if the following conditions are met: (1) the SC regime returns to a yearly average below the CCC, (2) there are nearby streams with low SC supporting a diverse community, and (3) there is an upstream source of colonizers or the flight or recolonizing distance is within the dispersal range of genetically diverse, reproducing adult colonizers. This frequency recommendation is based on consideration of the life history of insects that are able to recolonize a site by drifting from upstream sites or aerially dispersing from a nearby stream, and published studies of recovery of stream communities. If any of these conditions are not met, the time necessary for community recovery (and thus, the allowable frequency of exceedance) would likely be longer than 3 years.

3.5. ASSESSING CAUSATION

Field studies can generate statistical relationships between environmental attributes and biological responses, but those relationships are not necessarily causal. Epidemiologists evaluate whether an apparent relationship is causal by weighing evidence of causation in terms of lists of considerations (Norton et al., 2015). General causation between SC and macroinvertebrate occurrences was previously assessed (U.S. EPA, 2011a; Cormier et al., 2013b) and therefore does not need to be repeated. Many other studies have corroborated that assessment for the particular ionic mixture, Ca^{2+}, Mg^{2+}, HCO_3^- and SO_4^{2-}, and for different salt mixtures, e.g., Na^+, K^+, HCO_3^- and SO_4^{2-}, and Na^+ and Cl^- (see Section 2.2). A new general causal assessment is recommended when it is uncertain whether an agent, for example a newly synthesized chemical or novel mixture, can or has ever harmed aquatic life. If a new causal assessment is warranted, EPA recommends using epidemiological methods to demonstrate that the agent or mixture can and does cause extirpation at concentrations using the method described in the *EPA Benchmark Report* (U.S. EPA, 2011a; Cormier and Suter, 2013b). The causal assessment methodology does not compare the relative importance of ionic-induced impairment with other known stressors such as metal toxicity, stream bed erosion and siltation, or eutrophication (U.S. EPA, 2011b; Gerritsen et al., 2010). Effects from these stressors are likely to occur and do occur in any given ecoregion and at times concurrently with increased ionic inputs. Rather, the causal assessment is designed to determine whether the addition of ions to streams can and does cause extirpation of aquatic life.

Although causal assessments of most ionic mixtures do not need to be repeated, it is good practice to evaluate the predictive performance of the XCD model, that is, how well the model characterizes the modeled relationship, e.g., SC and extirpation. See Section 3.1.1.2.6 for analytical approaches for assessing potential confounders.

In summary, the causal relationship between elevated ionic concentration and extirpation of macroinvertebrates can be assessed using an approach modified from Hill's (1965) considerations (for complete details see Appendix A in U.S. EPA, 2011a; Cormier et al., 2013b). Hill's approach for establishing a probable causal relationship has been adapted for ecological applications (Cormier et al., 2010; Fox, 1991; Suter et al., 2001; U.S. EPA, 2000b). Based on that assessment, the body of evidence indicates that the loss of macroinvertebrate genera occurs where SC is high even when potentially confounding causes are low, but is rare when SC is low. Furthermore, there are sources of ions that increase stream SC in the region, and aquatic organisms are directly exposed to these ions. Physiological laboratory studies indicate that ionic gradients in high SC streams would not favor the exchange of ions across gill epithelia and that physiological functions of organisms are affected by elevated ionic concentration. Some genera, composite metrics, and assemblages are affected at sites with higher SC, while others are not. Laboratory studies using moderately salt-intolerant species and ionic compositions relevant to the study area support ionic stress as a cause of extirpation; and increased exposure to ionic stress affects macroinvertebrate abundance and diversity based on field observations. More recently, mesocosm studies have corroborated adverse effects at similar exposures (Clements and Kotalik, 2016).

The causal assessment confirmed that the mixture of ions in streams with elevated SC and neutral or somewhat alkaline waters can and is causing the extirpation of salt-intolerant genera of macroinvertebrates as well as in low pH systems (U.S. EPA, 2011a; Cormier et al., 2013b). The relative SC level of waters with a similar ionic composition, rather than any individual constituent of the mixture, is implicated as the cause of impairment (see Section 2.5.1). The causal relationship describes how *Ephemeroptera* and similarly salt-intolerant invertebrates, in general, respond to ionic stress and does not require that the species or genera be the same in all applications or at all locations. Although the specific constituents of the ionic mixture were not individually assessed, the cause of impairment is attributable to one or more of the primary constituents of the mixture. Therefore, based on the causal assessment (U.S. EPA, 2011a), it is expected that SC levels sufficient to cause extirpations would occur with a similar salt mixture containing HCO_3^-, SO_4^{2-}, Ca^{2+}, and Mg^{2+} in other regions. Furthermore, based on other studies (see Section 2.2), different salt mixtures also cause extirpation, e.g., Na^+, K^+, HCO_3^- and SO_4^{2-}, and Na^+ and Cl^-.

3.6. ASSESSING WATERBODY APPLICABILITY

3.6.1. Stream pH

Due to the nature of ions and pH, it is important to consider the potential impact of pH on the XCD. Acidity (e.g., associated with acid mine drainage, atmospheric deposition and other sources) and potentially associated dissolved metals could affect the field-based XC_{95} values and the XCD model. As a result, unless shown to the contrary, it is recommended that sites with pH <6 be excluded from data sets prior to analysis.

In Case Studies I and II provided in Sections 4 and 5, sites with pH <6 were excluded from the data set prior to analysis. Therefore, the case studies were developed without the influence of pH, analogous to controlling for confounders in a laboratory test. Nevertheless, field data show that even below pH 4.5, high SC was a stronger predictor than acidity on the occurrence of *Ephemeroptera* (see Appendix B in U.S. EPA, 2011a). A contingency table showed that *Ephemeroptera* were observed at low pH unless SC was high. Also, calculating the HC_{05} using the data set from the *EPA Benchmark Report* (U.S. EPA, 2011a), with and without the inclusion of low pH sites, yielded very similar results (295 µS/cm for all sites compared to 288 µS/cm pH <6 sites removed). Therefore, although EPA recommends the removal of pH sites from the data set prior to analysis, there is evidence to suggest that the derived criteria are applicable to all streams regardless of pH.

3.6.2. Waterbody Type

Another important consideration when it comes to applicability of the field-based approach is waterbody size and type. The EPA recommends analyzing the effect of catchment size on the XCD model and documenting the decision, rationale, and supporting analyses for applicable water body types for SC criteria derived using this method. For example, in Case Study I, the data used to derive the CCC are from perennial streams with catchments that range from 0.34 to 17,985 km^2. Literature reviews and analyses (described below) were performed in the Problem Formulation phase of this assessment to determine relevant (applicable) waterbody types. As a result, all stream types and sizes were included in the data sets for these case studies.

Although the field data used in the case studies were only collected from perennial streams, available information from the open literature indicates that many of the macroinvertebrate taxa persist in intermittent and perennial channels, albeit at different densities and for varying amounts of time. For example, Grubbs (2010) assessed the relationship between stream-flow permanence and macroinvertebrate community structure along temporary and perennial hydrologic gradients in forested headwater streams in a Cumberland Plateau watershed in the Kentucky River Basin. Grubbs found that the vast majority (91 out of 108) of macroinvertebrate taxa were observed in both the perennial and temporary channels. Macroinvertebrate taxa have many adaptations to survive temporary dry periods including egg diapause, nymph aestivation, and nymph migration into hyporheic zones (the area beneath a streambed, where shallow groundwater and surface water mix) or intermittent pools (Datry, 2012). Macroinvertebrates may use temporary stream resources for portions of their life cycle (e.g., nursery habitat) and move downstream as they get older and larger and conditions require emigration to areas of greater flow (De Jong and Canton, 2013; Feminella, 1996; Stout and Wallace, 2003). These studies suggest that temporary streams are used, at least for a portion of their life cycle, by many of the macroinvertebrate taxa considered in the XCD method.

Discharge to ephemeral streams ultimately affects downstream intermittent/perennial streams (via gravity and flow through the tributary system during precipitation events). As a result, addressing SC in upstream ephemeral streams is often critical to ensuring that downstream aquatic life is not exposed to harmful levels of SC above the criteria.

Although intermittent and perennial streams are likely to have similar SC regimes, larger catchments may not have the same background SC as smaller streams owing to hydrological contributions from different geologies or other factors. Options include limiting the use of derived criteria to the range of sampled catchments represented in the data set, developing criteria for different stream classes, or demonstrating that there is no difference due to catchment size.

In the *EPA Benchmark Report*, larger streams (catchment areas >155 km^2) were originally screened from the data set that was used because sampling methods might differ for nonwadeable streams (U.S. EPA, 2011a; Flotemersch et al., 2006). However, a subsequent analysis indicated that 25 of the 30 most salt-intolerant genera based on derived XC_{95} values for Ecoregions 69 and 70 (see Appendix E) were documented in these larger rivers (see also Appendix B in U.S. EPA, 2011a). Inclusion of the data from large streams did not significantly change the magnitude of the HC_{05} (289 µS/cm) compared to the HC_{05} without data from larger systems (295 µS/cm). Additional analyses support that result. An analysis of 3,115 sites (3,736 samples total: 1,661 in Ecoregion 69 and 2,075 in Ecoregion 70) with drainage areas up to 17,986 km^2 suggests that SC and drainage area are very weakly correlated ($r^2 = 0.044$, see Figure 3-8). These are neither random samples nor reference streams and may not represent natural background. The apparent background SC, estimated as the 25th centile of probability sites, for streams draining areas >155 km^2 in Ecoregion 69 and 70 are 148 and 188 µS/cm, respectively; both of these estimates are within the confidence bounds for estimated background SC using the example criterion-derivation data sets. Therefore, the example ecoregional criteria in the case studies are relevant for all stream sizes. However, professional judgment is warranted when applying the example criteria to streams crossing ecoregional boundaries and stream catchments draining >1,000 km^2 because they are less well represented in the data sets (see Figure 3-8). For example, great rivers such as the Ohio and Mississippi Rivers were not represented in the data set, and they cross many ecoregional boundaries.

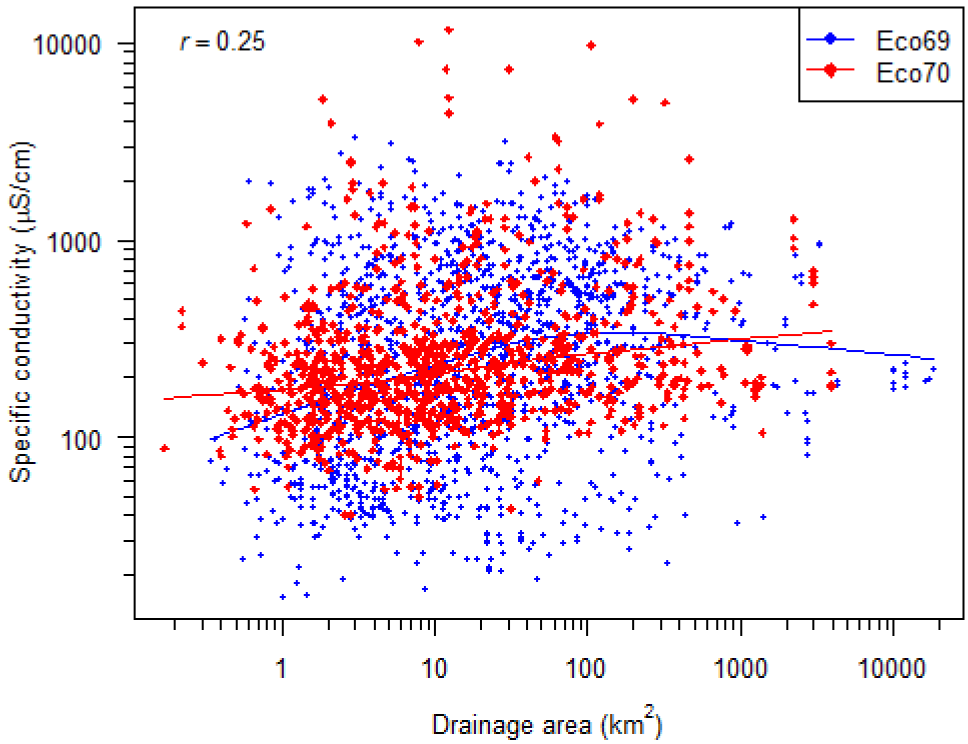

Figure 3-8. Correlation of specific conductivity and drainage area up to 17,986 km^2, Spearman's *r* = 0.25. This analysis shows a very weak correlation between specific conductivity and drainage area and supports inclusion of data from all stream sizes in the data set for example criteria derivation. The fitted lines are the locally weighted scatterplot smoothing (LOWESS, span = 0.75, linear polynomial) for each data set.

3.7. METHODS FOR APPLICATIONS TO NEW AREAS

Not all areas of the country have sufficient water chemistry and biology data to derive criteria for SC by the XCD method of calculating XC_{95} and HC_{05} values. For such cases, the EPA is providing alternative methods that geographically extend results of the primary XCD method (see Section 3.1). One alternative method extends criteria developed in one area to other areas within the same ecoregion. This method is termed background matching. A second alternative method estimates criteria for new areas with different background SC using a background-to-criterion regression model. Both of these methods rely on the estimated background SC.

The feasibility of applying a conductivity benchmark outside its area of derivation was considered by the EPA SAB in their review of the *EPA Benchmark Report* (see Section 3.7

Transferability of the Method to Other Regions in U.S. EPA, 2011c). In general, the SAB concluded that the numeric benchmark was applicable to the regions where the field data were collected and could be applicable to other areas where sufficient data allow for evaluation of applicability of the benchmark. Since then, the EPA has developed an understanding of the mechanistic relationship between background SC and the extirpation of salt-intolerant invertebrates. This relationship allows the EPA to relate HC_{05} values from one area to another based on background SC.

Background SC in a region and the associated HC_{05} are expected to be strongly related based on ecological and evolutionary theory and the observed responses of invertebrates to major ions (see Section 2.4). The most salt-intolerant invertebrates occur in streams with the lowest background SC (see Appendix D). As SC increases, the most salt-intolerant species are adversely affected and ultimately cannot persist. As a result, where regional background SC is higher, those taxa adapted to low SC are absent, and the SC level that is protective of 95% of taxa (HC_{05}) is higher.

The EPA developed the B-C method using 24 field XCDs from ecoregions with background SC ranging from 22 to 626 μS/cm. Relatively salt-intolerant genera, as indicated by low XC_{95} values, occupy habitats in each region with the lowest ionic concentration. When both are log-scaled, the increase in background SC is linearly related to the HC_{05}. This regular and biologically relevant relationship between background SC and the HC_{05} confirms that the lower portion of the XCDs are similar in similarly exposed communities even though the represented genera may differ among ecoregions. The relationship between background SC and the HC_{05} identified from the XCD is sufficiently strong to identify a CCC for areas with sufficient stream chemistry data but little or no paired biological data within an ecoregion or for new ecoregions (see Appendix D).

The association between background SC and the HC_{05} was used to develop the background matching approach and the approach using the B-C method described in Sections 3.7.1 and 3.7.2, respectively.

3.7.1. Application within an Ecoregion—Background Matching

If paired SC and biological data are not available for a new area within an ecoregion, the background SC may be used to assess applicability of the derived CCC to the new area using a technique called background-matching. Regional background SC is defined here as the range of SC naturally occurring in waters that have not been affected by human activity. Background SC is important to water quality protection because it represents the ionic concentrations to which organisms in the region are naturally adapted. Minimally affected waters with low SC play a particularly important role by diluting polluted downstream waters and serving as refugia for salt-intolerant organisms. The background-matching approach is demonstrated in Case Studies I and II (see Sections 4 and 5) for the new areas within an ecoregion that were not included in the original example criterion-derivation data sets.

In this discussion, the phrase, original area, refers to the geographic area from which the data are obtained to develop SC criteria using the XCD method. The phrase, new area, refers to a geographic area within the same Level III ecoregion that was not represented in the criterion derivation data set. When applying field based SC criteria developed with data from the original area to a new area, the background SC levels and the ion composition should be similar in both areas. For instance, the example criteria are derived with data for streams where the ionic mixture is dominated on a mass basis by ($[SO_4^{2-}] + [HCO_3^-]) > [Cl^-]$.

The relationship between background SC and the HC_{05} identified from the XCDs is sufficiently strong to identify a HC_{05} for areas without biological sampling within an ecoregion or for new ecoregions. This B-C regression model was developed using biological data paired with SC data from waters with ionic mixtures dominated by calcium, magnesium, sulfate and bicarbonate ions and where background SC did not exceed 626 μS/cm. Therefore, the model is most appropriate for waters with similar ionic characteristics. The model has not been thoroughly tested and professional judgment is required for places where on a mass basis the major ions are $([HCO_3^-] + [SO_4^{2-}]) < [Cl^-]$ or $([Ca^{2+}] + [Mg^{2+}]) < ([Na^+] + [K^+])$. In particular, the B-C model is not appropriate for waters dominated by NaCl (Haluszczak et al., 2013, Entrekin et al., 2011; Gregory et al., 2011; Veil et al., 2004) or road salt (Forman and Alexander, 1998; Kelly et al., 2008; Environment Canada and Health Canada, 2001; Evans and Frick, 2001; Kaushal et al., 2005). The B-C model may also be defensible for ionic mixtures dominated by sodium, sulfate and bicarbonate ions (Brinck et al., 2008; Dahm et al., 2011; Jackson and Reddy, 2007; National Research Council, 2010; Clark et al., 2001; Veil et al., 2004). This is because the toxicity of these mixtures are more similar to that of calcium, magnesium, sulfate and bicarbonate ions than the toxicity of NaCl (Mount et al., 2016; Kunz et al., 2013; Soucek and Dickinson, 2015).

In the background-matching approach, the background for the ionic mixture of the new area is compared with the background of the original area. If the 95% CI of the background SC of the new area overlaps with the 95% CI of the background in the original area, the original criterion is considered applicable. If the CIs for the two areas do not overlap, then a dichotomous decision tree is used to guide further evaluations (see Figure 3-9). The dichotomous decision tree for assessing the applicability of criteria from the original area to a new area of an ecoregion may require a weight-of-evidence assessment described in detail in Appendix C, calculation of an HC_{05} using a regression model described in Section 3.7.2 and Appendix D, or collection of sufficient data to derive a different HC_{05} for the new area.

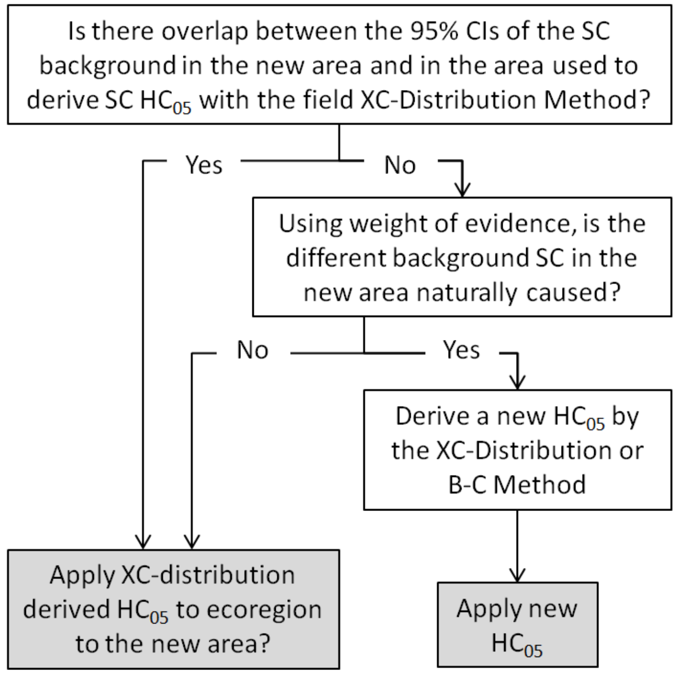

Figure 3-9. Method for selecting a criterion continuous concentration (CCC) for a new area within an ecoregion using minimally affected background. The hazardous concentration of the 5th centile of a taxonomic extirpation concentration distribution (HC_{05}) from a field derived extirpation concentration distribution (field XCD) is one that has been previously developed using a large data set. The background-to-criterion (B-C) method uses a regression model to predict a criterion and confidence interval (CI) from background specific conductivity (SC).

Portions of the same ecoregions in different political jurisdictions are expected to have similar characteristics with respect to the primary factors that control background SC (Hem, 1985, Griffith, 2014, Olson and Hawkins, 2012, see Section 2.1). These primary factors are underlying geology, physiography, and climate; secondary factors include soils and vegetative cover (Olson and Hawkins, 2012; Griffith, 2014; Hem, 1985). Because Level III ecoregions were delineated based on similar considerations (Omernik, 1987), the SC regime and ionic composition of dissolved salts in streams within an ecoregion tend to be similar throughout. However, there may be situations where it is not appropriate to apply criteria derived for the ecoregion to a particular stream reach. For example, naturally lower or higher concentrations of ions may occur due to subecoregional differences such as cross boundary influences, glacial melt, salt springs, highly soluble rock, or other natural sources.

3.7.1.1. *Obtaining a Data Set*

The first step in the background-matching approach is to assemble the data sets from sampled sites that are distributed across the full range of SC conditions in the new area. All else being equal, the larger the data set, the more reliable the estimate of background SC. Next, sites with qualitatively different ionic mixtures are removed from the data set. In the example case studies, chloride-dominated sites are removed from the data set so that background SC is estimated only for sites dominated by sulfate and bicarbonate (i.e., $([HCO_3^-] + [SO_4^{2-}]) > [Cl^-]$ in mg/L). For three of the example case studies, Sections 4, 5, and 7, the dominant cations are Ca^{2+} and Mg^{2+}. For the example case study, Sections 6, the dominant cation is Na^+.

3.7.1.2. *Estimating Background Specific Conductivity (SC)*

If minimally affected background SC is not known, it can be estimated from field data that are representative of SC throughout the year. In particular, the data set should not be biased toward seasonal extremes by sampling only during seasons of freshets or droughts. Background SC may be estimated as a proportion of a regional sample of sites or a sample of reference sites that are judged to be among the best within a region (U.S. EPA, 2011a).

Regional samples from a random or probability-based design (Stevens and Olsen, 2004) include all waters within the sampling frame, including impaired sites. To characterize the minimally affected streams in a regional sample, the 25th centile is conventionally used (U.S. EPA, 2000a). However, when land cover modification (or other anthropogenic disturbance) is pervasive, selection of a centile lower than the 25th may be justifiable.

When estimating background concentrations using minimally affected reference sites, it is conventional to use only the lower 75% of reference values (U.S. EPA, 2000a). One indication of the need for a different centile is when reference sites have a broad range of SC values suggesting that the reference condition contains some sites with anthropogenic disturbance, or that the sites are not classified to partition natural variability (e.g., headwaters draining through limestone glacial till into an area of weathered bedrock). An expanded list of possible considerations is provided in Appendix C. When there is great confidence in the quality of reference sites, a 90th centile may be used.

When there are sufficient good quality reference sites, the regional and reference methods yield similar background estimates (NYSDEC, 2000, TDEC, 2000, U.S. EPA, 2000a). But, in general, estimation based on a random sample of the region tends to yield a more accurate estimate of current background when there are sufficient data to characterize the full distribution of SC in the region. Unlike the selection of reference sites, a random sample does not depend on the original intent of data collection or the judgement of the data collectors (Whittier et al., 2007a, b).

After estimating background SC for both the original and new areas using the method in this section, the background-matching approach requires estimating their variability so that confidence intervals can be calculated. The confidence interval for a background SC estimate can be calculated using a bootstrapping technique. Bootstrapping is a statistical resampling technique that is often used in environmental studies to estimate confidence limits of a parameter. This bootstrapping application involves randomly resampling the original water chemistry data set 1,000 times with replacement, storing the 1,000 data sets, calculating the background for each data set, and then estimating the 95% CI for the mean of the set of 1,000 background values generated by the bootstrapping procedure. This is similar to the procedure described in Section 3.1.3.1.

3.7.1.3. *Background-Matching Approach*

Once the means and confidence limits on the background SC in the original area and new area have been estimated, they can be compared to determine whether they sufficiently match using the decision criteria depicted in Figure 3-9.

1. If the 95% CI of the background SC values from the new area overlaps with the 95% CI of the background SC values from the original area, then apply the XCD derived HC_{05} for that ecoregion throughout the new area.

2. If the 95% CIs do not overlap, then use a weight-of-evidence approach to determine whether the background in the new area represents natural or anthropogenic sources as described in Appendix C.

3. If the difference in 95% CIs is due to anthropogenic alteration, then apply the XCD derived HC_{05} from the original area to the new area.

4. If the difference in SC is naturally caused by geology, climate, or other natural factors, then derive a new HC_{05} for the new area using a sufficiently large and appropriate data set from the new area (see Section 3.1) or calculate an HC_{05} based on background SC for the new area using the B-C regression method (see Section 3.8).

For example, the decision matrix was used to determine the applicability of XCD SC criteria from an original area to a new area using this background-matching approach (see Example Case Studies in Sections 4 and 5).

3.7.1.4. *Options When Background in New Area is Different than in Original Area*

If the estimated background SC is higher or lower in the new area and the ion composition is similar compared to the original area, two possible causes should be considered. First, differences may be due to natural geological factors (e.g., higher SC due to salt springs, lower SC due to glacial melt, or other differences due to natural geological features) or to climatological factors. In these situations, criteria for the new area can be developed using either the XCD method or the B-C regression method. Second, differences may be due to widespread anthropogenic changes that have increased the apparent background (e.g., due to irrigation, agriculture, impervious surfaces, resource extraction, or acid deposition, etc.). In this second situation, the criteria developed for the original area may or may not be applicable to the new area.

To distinguish between these two possibilities, the cause of the apparent background can be evaluated in a weight of evidence. Considerations may include analysis based on geology, land use, ionic signatures and known inputs, historical and recent trend analysis, atmospheric sources, discontinuities in background across political boundaries, identification of high and low SC anomalies, stream size and connectivity, data set characteristics, sampling methods, and biological evidence of past and present observation of susceptible genera (see Appendix C).

3.7.1.5. *Summary of Background Matching Method*

The original criteria are ***applicable*** to a new area in the same region if the background SC is not different from the background of the original area (i.e., overlapping 95% CIs) and the ionic mixture is the same (e.g., in the case studies, ($[HCO_3^-] + [SO_4^{2-}]) \geq [Cl^-]$ in mg/L). The original criteria are ***not applicable*** to the new area if the background for the ionic mixture is different owing to natural causes or if the ionic mixture is different. If the background SC is higher or lower than the original background, a new criterion may need to be developed for the new area. A weight-of-evidence analysis can be done to evaluate whether the difference in background SC is due to natural causes. When possible, an independent data set should be used to corroborate the estimates of background SC.

3.7.2. Developing a Criterion Using Background-to-Criterion Regression Method

Because large sets of paired chemical and biological data are not available for all ecoregions in the United States, the EPA developed a model to calculate a CCC using the background SC of an ecoregion. The B-C regression method can be used in a new ecoregion or a new area within an ecoregion with a different SC regime (e.g., at a scale smaller than Level III ecoregions).

The relationship between minimally affected background SC and HC_{05} for 24 Level III ecoregions was characterized using least squares linear regression (see Figure 3-10). The relationship between background SC and HC_{05} was modeled and the association was strong ($r = 0.93$), as was expected given the importance of the concentrations of major ions in defining the tolerance of species (see Section 2.4). The relationship between background SC and HC_{05} is sufficiently reliable for identifying a CCC for areas without biological sampling within an ecoregion or for new ecoregions (see Appendix D).

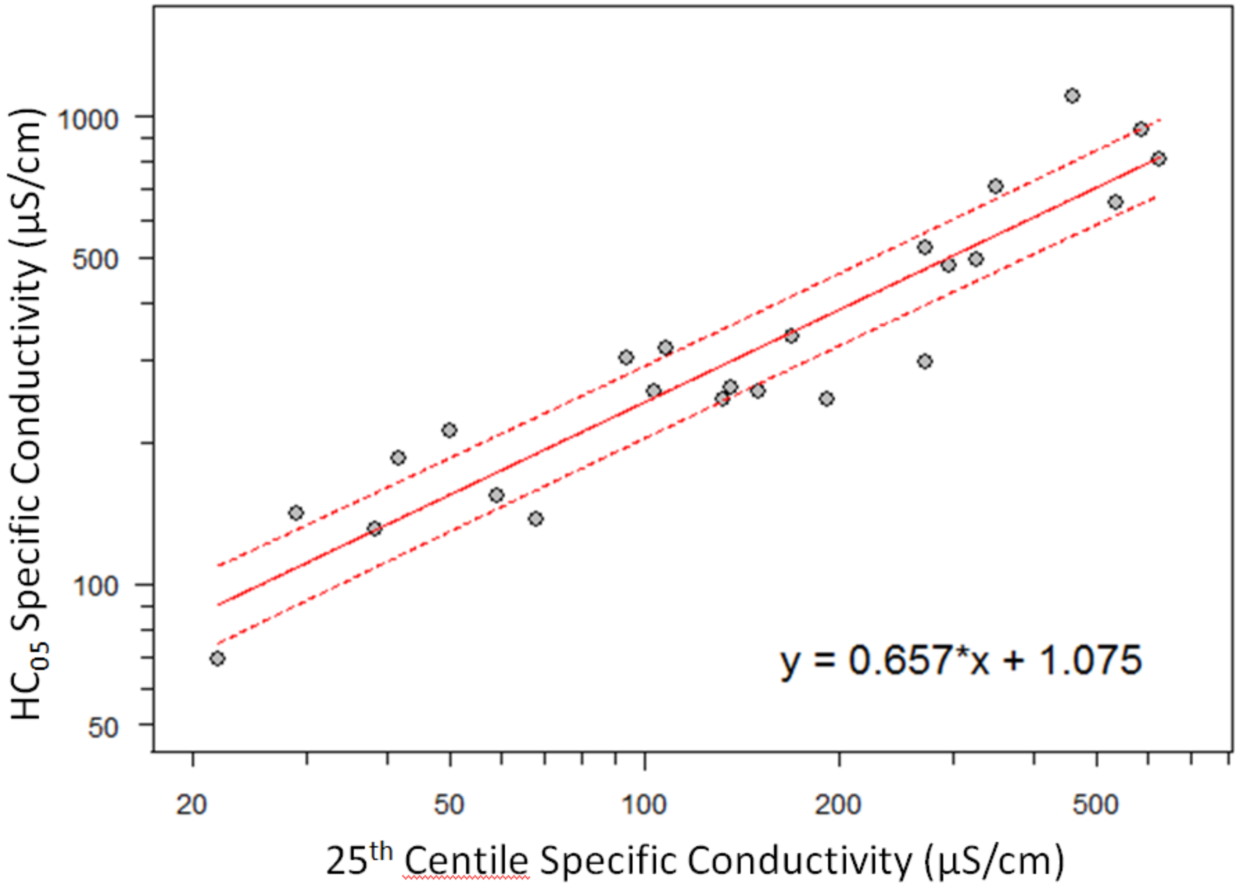

Figure 3-10. Empirical model of the 5th centile of a hazardous concentration (HC_{05}) and background specific conductivity (SC) estimated at the 25th centile for 23 distinct ecoregions (24 data sets). Solid line is the log10-log10 normal regression line; therefore, *x* and *y* are log10 expressions. Dotted lines demarcate the 50% prediction intervals, that is, the 50% probability that any new HC_{05} would plot within those bounds and only 25% below the lower prediction limit (PL). The regression coefficient $R^2 = 0.87$.

The B-C regression method shown in Figure 3-10 was derived using independent data sets from 24 ecoregions (see Appendix D). First, SC XC_{95} values were estimated. From these, 24 genus-level XCDs were constructed and HC_{05} values derived. Those HC_{05} values were regressed against the estimate of background SC for each ecoregion. In an ecoregion with low background SC, very salt-intolerant taxa are represented. In an ecoregion with a moderate background SC, taxa with an XC_{95} greater than the moderate background are likely to survive and contribute to the XCD, whereas salt-intolerant taxa with XC_{95} values less than the moderate background are not likely to contribute to the XCD. As XCDs are developed for ecoregions with increasingly higher background SC levels, each XCD begins at a higher background SC, and thus the most salt-intolerant genera in an ecoregion occur at progressively higher SC levels. This association is evidence that where low ionic concentration waters are present in an ecoregion, organisms that are specialized for that niche are likely to inhabit them; and, where low ionic concentration waters are not present, salt-intolerant species are not likely to occur. The resulting B-C regression model provides a convenient method to predict an HC_{05} from the minimally affected or least disturbed background SC of an ecoregion. Descriptions of the derivation of the regression model, the data sets used, and the individual XCD models are presented in Appendix D.

The central tendency of a regression model is more robust than any single measurement. For the purpose of model development, data requirements were relaxed relative to those for calculating a HC_{05} using the XCD method (i.e., fewer than 90 genera across 500 sites) (see Appendix D for a description of data requirements for the B-C method). Individually, many of the 24 HC_{05} values used to develop the B-C method have not been subject to analyses needed for development of a CCC and should be considered as provisional. For example, the HC_{05} estimates used in this model were not supported by full confounding analyses, as is described in the *EPA Benchmark Report* (U.S. EPA, 2011a). However, the true HC_{05} value is expected lie between the upper and lower 50% prediction limit (PL). Values in Appendix D, Table D-3 are provisional with a good degree of confidence owing to the larger sample sizes (>60 samples) used to estimate background SC. Table D-4 lists ecoregions with background estimates based on modest survey data sets ($N = 20-60$ samples) and would benefit from additional sampling to confirm the calculated background SC and the calculated HC_{05}. Table D-5 lists ecoregions where the data set may represent fewer than 25% minimally affected streams and therefore are protective of aquatic life in least disturbed streams. Table D-6 lists ecoregions that may not be served by the B-C method because the ionic mixture is likely to be different (e.g., chloride dominated), the estimated natural background SC exceeds the range of the model, and/or there were fewer than 20 samples available. In all cases, the EPA recommends using the largest data set possible to estimate background SC, understanding and accounting for areas with different (higher or lower) background SC, and performing independent calculations to derive HC_{05} values.

3.7.2.1. *Using Background to Calculate a Hazardous Concentration of the 5th centile (HC_{05}) of a Taxonomic Extirpation Concentration Distribution (XCD)*

The HC_{05} for a defined geographic area or ecoregion without a sufficient data set or without suitable biological data may be calculated using the B-C method based on the background SC of that area or region. The decision tree for calculating a CCC from minimally affected background is shown in Figure 3-11. Equations 3-1 and 3-4 can be used to calculate the mean HC_{05} and eq 3-5 calculates the lower 50% PL for the area or region. Sections 6 and 7 provide examples that use the decision tree to develop example criteria for 2 ecoregions in the West, one with low and one moderately high background SC.

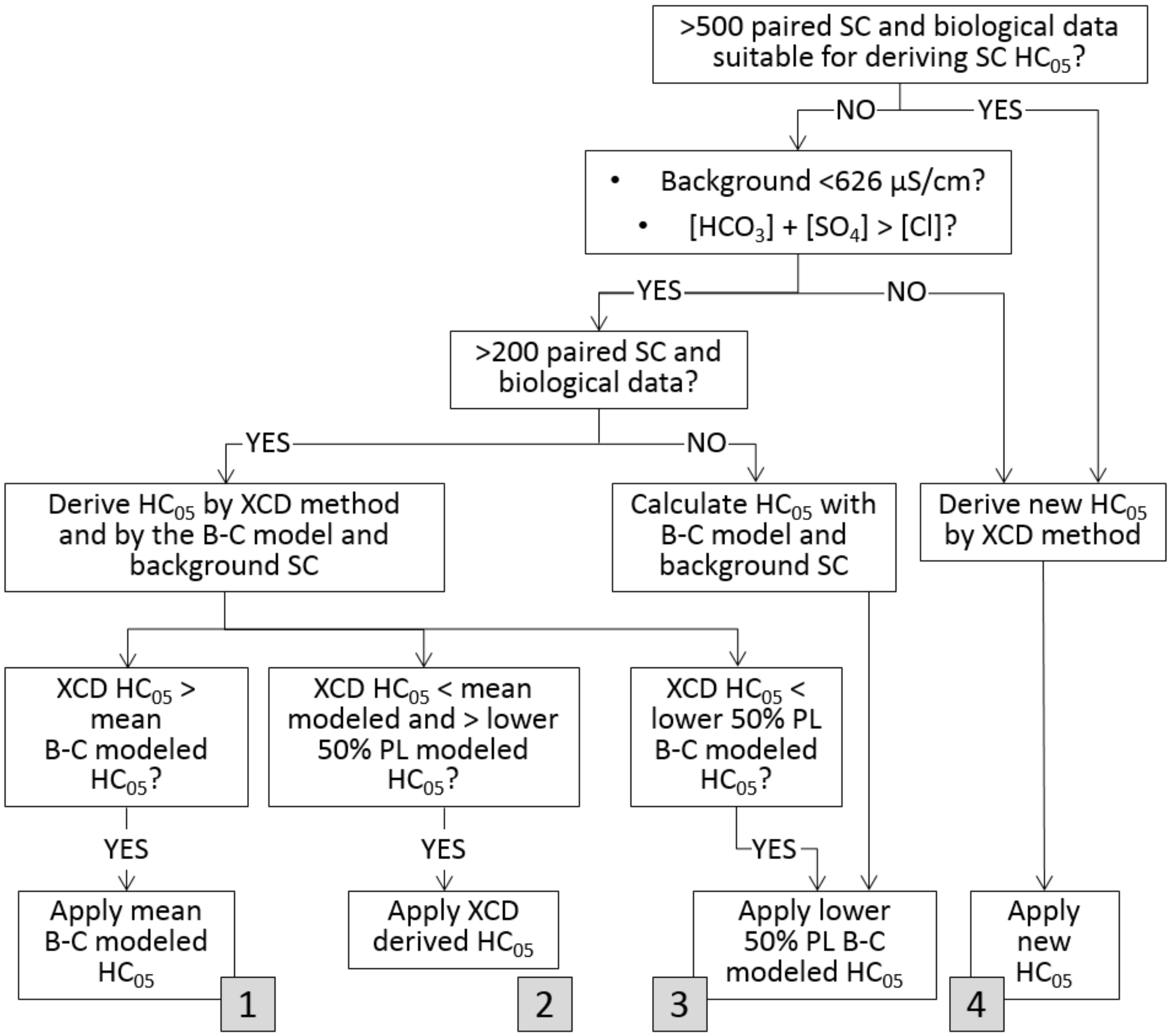

Figure 3-11. **A decision tree for calculating and applying a hazardous concentration of the 5th centile of a hazardous concentration (HC_{05}).** This flow chart may be used when developing a criterion continuous concentration (CCC) for new ecoregion, a new area within an ecoregion, or other defined geographic area using the field extirpation concentration distribution (XCD) method, background-to-criterion (B-C) method and minimally affected or least disturbed background specific conductivity (SC). Numbered product paths are described in the body of the text.

Where the background is less than 626 µS/cm and the waters have a similar ion composition to those used to derive the model, the B-C method can be used (see Figure 3-11). Where there are >200 but <500 sites with paired biological and SC data, HC_{05} values are derived using the XCD method and compared to the mean and lower 50% PL of the B-C model. These values are compared to select the appropriate HC_{05} as follows. (1) If the XCD HC_{05} (see eq 3-1) is greater than the mean B-C modeled HC_{05} (see eq 3-4), then the mean B-C modeled HC_{05} is recommended (see eq 3-4) as a conservative approach to account for uncertainty associated with a smaller data set. (2) If the XCD HC_{05} is between the mean B-C modeled HC_{05} and the lower 50% PL, then the XCD HC_{05} is recommended because the XCD from measured data from the region is more likely to represent the region than the more general B-C model. (3) If the XCD estimate is below the lower 50% PL, then the lower 50% PL is recommended as the HC_{05} (see eq 3-5). This is recommended because the XCD is calculated from a smaller data set. Also, it may be overly protective because it is more uncertain than the modeled results which indicate that 75% of HC_{05} values from areas with a similar background SC are estimated to be greater than a value less than the lower PL. The lower 50% PL is also recommended when there are fewer than 200 paired biological samples because there is no XCD for comparison. For both situations, the SC data and the B-C model is used to estimate the HC_{05}. (4) Where the background SC is greater than 626 μS/cm, the range of the model is exceeded, and it is recommended that data be collected to derive the HC_{05} using the XCD method (see Section 3.1).

3.7.2.2. *Formula for Calculating the Hazardous Concentration of the 5th centile of a Taxonomic Extirpation Concentration Distribution (HC_{05}) from the Background-to-Criterion Model*

The B-C model is described by the following formula:

$$Y = 0.657X + 1.075. \qquad (3\text{-}4)$$

Where:

X is the log10 of the ecoregional background SC

Y is the log10 of the predicted HC_{05}

3.7.2.3. *Formula for Calculating the Lower and Upper 10% Prediction Limits*

The upper and lower PL for a predicted log10 HC_{05} value $\hat{y}$ can be calculated from the regression line using eq 3-5 (Zaiontz, 2014) and log10 transformed SC values (x) as follows:

$$\hat{y} \pm t_{\alpha/2,n-2} S_y \sqrt{1 + \frac{1}{n} + \frac{(x^{\circ} - \bar{x})^2}{SS}} = \text{PL} \qquad (3\text{-}5)$$

Symbol	Explanation	Example from the 23 ecoregion B-C model
$\hat{y}$	Log10 of the mean predicted HC_{05}	Variable differs for each case
n	Number of samples	$n = 24$
α	Alpha error rate for prediction interval (desired confidence level)	50% prediction interval (α = 0.5)
t_{n-2}	Student's *t*-value at specified confidence level (alpha, α) and *n*-2 degrees of freedom	For 50% prediction interval (α = 0.5), $t_{(1-0.5)/2,24-2} = 0.686$
S_y	Residual standard error of prediction (standard deviation)	$S_y = 0.11$
SS	Sum of square of x deviation from their mean, SS = $\sum_{i=1}^{n}(x_i - \bar{x})^2$	$SS = 4.21$
$\bar{x}$	Mean x values used in the model generation	$\bar{x} = 2.15$
x°	A new log10 background *(x)* value for a new prediction interval	SC value differs for each case
PL	Upper and lower prediction limits of mean predicted $\hat{y}$	SC value differs for each case

The estimated backgrounds listed in Tables D-3, D-4, and D-5 of Appendix D for 62 of 85 Level III ecoregions were used to estimate the HC_{05} from the B-C model. HC_{05} values and the lower 50% PLs were estimated using eqs 3-4 and 3-5 and estimated background from probability survey data. Predicted base-flow SC (Olson and Hawkins, 2012) was used to assess whether the 25th centile SC used in the calculation is minimally affected (see Tables D-3 and D-4) or least disturbed background SC (see Table D-5). Although the B-C Model is strongly log-linear within the sampled SC range, the EPA recommends estimation of HC_{05} only for ecoregions with a background <626 µS/cm to avoid extrapolation beyond modeled data. Some regions may have different ionic matrices (e.g., chloride-dominant) for which the derivation of a CCC using this method has not been verified. Those ecoregions are identified in Table D-6. The decision tree depicted in Figure 3-11 was used to select example HC_{05} values that if rounded to two significant figures generates an example CCC.

3.7.2.4. *Criterion Continuous Concentration (CCC) with <200 Paired Biological Data*

When there are insufficient paired data, background SC is used to calculate the lower 50% PL of the mean HC_{05} which is rounded to two significant figures to yield the CCC (see eq 3-5). This result is shown in the Box 3 in Figure 3-11. The estimated CCC at the lower 50% PL for 62 ecoregions can be found in Tables D-3, D-4, and D-5 in Appendix D.

3.7.2.5. *Criterion Continuous Concentration (CCC) with 200 to 500 Paired Biological Data*

If a suitable paired biological and SC data set of 200 to 500 sites is available that meets the requirements outlined in Appendix D.2.1, then the HC_{05} is estimated from that data set using the XCD method (see eq 3-1) and the B-C model (see eq 3-4). The lower of the two estimates is recommended as the HC_{05} unless the XCD estimate is below the lower 50% PL from the B-C model (see Figure 3-11). This result is shown in Boxes 1 or 2 in Figure 3-11. If the XCD estimate is less than the lower 50% PL of the HC_{05} from the B-C model, then the lower PL is used (see eq 3-5). This result is shown in Box 3 in Figure 3-11. In either case, the predicted mean or the lower 50% PL HC_{05} is rounded to two significant figures to yield the CCC. The provisional or comparative values for the CCC based on the mean regression line for 62 ecoregions are shown in Tables D-3, D-4 and D-5.

3.7.2.6. *Calculation of the Criterion Maximum Exposure Concentration (CMEC)*

A CMEC based on water chemistry data can be calculated as described in Section 3.2. If there are insufficient data to calculate a CMEC, the upper 50% PL can be used to approximate a CMEC.

3.7.2.7. *Summary*

Although the B-C regression model is strong, there is scatter in the 24 HC_{05} values, so the lower 50% PL is used. In addition, when there are >200 and <500 paired biological and SC data, the XCD method is applied to check the B-C model results. The B-C model can also be used to evaluate estimates with data sets >500 when they do not meet other requirements for the SC range of exposure, unbiased sampling, seasonal bias, etc. Section 6 provides an example case for deriving an HC_{05} for the Northwestern Great Plains, Ecoregion 43 in Montana, Wyoming, North Dakota, South Dakota, and Nebraska. Section 7 provides an example case for deriving an HC_{05}

for the Level III Cascades, Ecoregion 4, in Washington, Oregon, and California. The estimation of an HC_{05} from background described here is a recommended approach for developing water quality criteria for those ecoregions lacking sufficient data to develop one by the XCD method from a regional data set.

4. CASE STUDY I: EXAMPLE USING EXTIRPATION CONCENTRATION DISTRIBUTION (XCD) METHOD IN A LOW BACKGROUND SPECIFIC CONDUCTIVITY ECOREGION

This section presents a case study for the Central Appalachians (Ecoregion 69) to illustrate how the analytical methods described in Section 3 can be used to derive example SC criteria using the XCD method in an ecoregion with low background SC. Ecoregion 69 results, including estimates of the CCC and CMEC, duration, frequency, and discussion of applicability are included as examples to demonstrate the method. The derivations of the CCC and CMEC analyses and results are based on data from Ecoregion 69 in West Virginia, and SC data from the Ecoregion 69 outside of West Virginia was used to assess applicability of the criteria throughout the ecoregion.

4.1. DATA SET CHARACTERISTICS

The Central Appalachians (Ecoregion 69) stretch from central Pennsylvania through West Virginia and Kentucky to northern Tennessee with small portions in Maryland and Virginia. The primary physiographic feature is a high, rugged plateau composed of sandstone, shale, conglomerate, limestone outcroppings, and coal. Elevation ranges from 366 to 1,402 m, with an average elevation of >790 m. Local relief between valleys and peaks can range from as low as 15 m to as high as 594 m. Rainfall is highly variable due to the topographic diversity, ranging from 96−152 cm/year, with the lowest rainfall in valleys and the highest at the peaks. The region is characterized by distinct summer and winter seasons, with growing seasons in agricultural regions (located within valleys) lasting as long as 165 days. However, pasture and agriculture are limited owing to the rugged terrain, cool climate, and infertile soils. The landcover is mostly forested with oak and northern hardwood forests. The high hills and low mountains are covered by a mixed hardwood forest. Underground and surface bituminous coal mines are common (Woods et al., 1999, 1996). Headwater streams in this ecoregion have some of the freshest (lowest SC) water in the United States. These headwater streams play an important role in diluting downstream waters that are anthropogenically impacted, and serve as refugia for fish and other salt-intolerant organisms.

The data used in this case study are from a large field data set, the West Virginia Department of Environmental Protection (WVDEP's) in-house Watershed Assessment Branch database (WABbase). Chemical and biological samples are from 1996−2011 and 1997−2010, respectively. The WABbase contains data from Level III Ecoregions 66, 67, 69, and 70 in West Virginia (U.S. EPA. 2010; Omernik, 1987; Woods et al., 1996). The WABbase data set provides consistent sampling and analytical methods, high quality, broad spatial coverage and a large number of perennial streams (2,299 distinct locations) in Ecoregion 69.

The WABbase contains data from a mixed sampling design that collects measurements from long-term monitoring stations, targeted sites within watersheds on a rotating basin schedule, randomly selected sample sites (Smithson, 2007), and sites chosen to further define impaired stream segments in support of total maximum daily load (TMDL) development (WVDEP, 2008a). Most sites are sampled once during an annual sampling period, but some sites are sampled monthly for water quality. The data set contains water quality, habitat, watershed characteristics, macroinvertebrate data (both raw data and calculated metrics), and geographic location (WVDEP, 2008a). A wide range of SC levels were sampled, which is useful for modeling the response of organisms to different levels of ionic concentration. The WABbase includes assignation of reference status using a tiered approach. Analyses involving the use of these reference sites were drawn from the Level 1 reference status (WVDEP, 2008b) which selects reference sites that "are thought to represent the characteristics of stream reaches that are minimally affected by human activities and are used to define attainable chemical, biological and habitat conditions for a region" (WVDEP, 2013; Stoddard et al., 2006). Sites are initially selected by a map coordinator based on GIS land use data that indicate minimal human disturbance. Streamside, the appropriateness of the selected site is confirmed based on the level of anthropogenic disturbance, lack of point discharges, habitat quality, pH, dissolved oxygen, and SC (>500 µS/cm) is used to flag a site for further investigation before inclusion as a reference site (WVDEP, 2013).

Macroinvertebrate records in the data set are based on collections from a total of 1 m^2 area of a 100 m reach at each site. Using a 0.5 m wide rectangular kicknet (595 µm mesh), four 0.25 m^2 riffle areas were sampled. In narrow or shallow water, nine areas were sampled with a 0.33 m wide D-frame dipnet of the same mesh size. Composited samples were preserved in 95% denatured ethanol. A random subsample of 200 individuals (±20%) was identified in the laboratory. All contracted analyses for chemistry and macroinvertebrate identification followed WVDEP's internal quality control and quality-assurance protocols (WVDEP, 2008b, 2006).

Quality assurance of the data set was judged by the EPA to be excellent, based on the database itself and supporting documentation.

Several data filters, described in Section 3.1 (see Figure 3-2), were applied prior to finalization of the data set and analyses. A total of 9,806 records from Ecoregion 69 are included in the data set; of these, SC measurements were included in 8,989 samples. Many of these are measurements of water quality without biological sampling. There are 1,911 paired samples with SC measurements and biological samples. Of these, a total of 250 samples were removed from the data set due to low pH ≤6 (237 samples) and high proportion of chloride ions ($[HCO_3^-] + [SO_4^{2-}]) \leq [Cl^-]$ (13 samples). Additional criteria were applied to identify macroinvertebrates for inclusion in the example extirpation concentration distribution: occurrence at reference sites and occurrence in 25 or more samples. Of the 219 macroinvertebrate genera identified in this ecoregion in the WABbase, 193 genera occurred at least once at one of the 64 identified reference sites where invertebrate samples were collected. A total of 142 genera occurred at 25 or more sampling locations. The final example "Criterion-data set" has 1,661 samples belonging to 1,420 sites (stations) (depicted in Figure 4-1). Of these 1,661 samples, 186 (11.2%) were sampled more than once between 1996 and 2010. Summary statistics for the data set used to derive the example CCC is shown in Table 4-1. The statistical package R, Version 2.12.1 (December 2010), was used for all statistical analyses (R Development Core Team, 2011).

SC ranged from 15.4−3,794 µS/cm which allowed the response of organisms to be modeled for a wide range of SC levels. Scatter plots of parameters and SC are depicted in Appendix A.1.

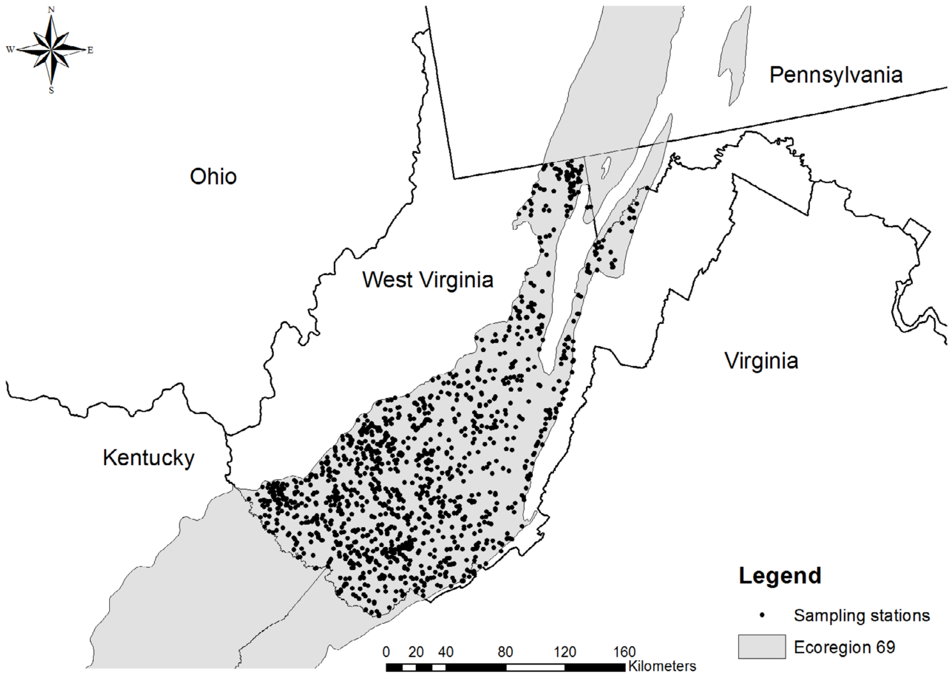

Figure 4-1. **Ecoregion 69 extends from central Pennsylvania to northern Tennessee.** Sampling sites (stations) (N = 1,420) in the example Criterion-data set that were used to derive the criterion continuous concentration (CCC) are indicated as points.

Table 4-1. Summary statistics of the measured water-quality parameters used to derive the example specific conductivity criteria in Ecoregion 69.
The example Criterion-data set has 1,661 samples belonging to 1,420 stations.

Parameter	Units	Min	25th	50th	75th	Max	Geomean	*N*
SC	µS/cm	15.4	94	229	540	3,794	225	1,661
Hardness	mg/L	2.18	28.03	64.31	132.7	1,492	64.43	834
Total alkalinity	mg/L	2	14	41	90	560	37	1,144
SO_4^{2-}	mg/L	1	12	32	126	2,097	39	1,146
Chloride	mg/L	0.5	2	3	8	650	4	930
$SO_4^{2-} + HCO_3^-$	mg/L	8.66	36.3	99.4	252	2,256	99.3	1,142
Ca, total	mg/L	0.67	6.9	16.9	33.5	430	15.8	842
Mg, total	mg/L	0.5	2.4	5.0	12	204	5.6	832
Na, total	mg/L	0.5	1.8	3.5	13	423	5.2	166
K, total	mg/L	0.5	0.7	1.2	2.4	16	1.4	164
TSS	mg/L	1	3	3	5	80	4	1,151
Fe, total	mg/L	0.02	0.09	0.18	0.38	4.9	0.19	1,170
Fe, dissolved	mg/L	0.01	0.02	0.03	0.07	1.1	0.04	995
Al, total	mg/L	0.01	0.06	0.1	0.19	3.3	0.11	1,142
Al, dissolved	mg/L	0.01	0.02	0.03	0.06	0.9	0.04	1,007
Mn, total	mg/L	0.003	0.02	0.03	0.06	4.4	0.03	1,142
Se, total	mg/L	0	0.001	0.001	0.003	1.3	0.002	665
DO	mg/L	2.06	8.47	9.27	10.2	17.1	9.41	1,644
Total phosphorus	mg/L	0.01	0.02	0.02	0.02	1.3	0.02	897
NO_x	mg/L	0.01	0.14	0.28	0.45	11	0.26	910
Fecal	Counts/100 mL	0.5	15	65	300	250,000	71	1,405
pH	SU	6.01	7.00	7.54	7.97	10.48	7.48	1,661
Catchment area	km^2	0.34	4.36	17.6	65.2	17,986	19.3	1,408
Temperature	°C	−0.28	14.2	17.9	20.7	30.2	17.5	1,661
RBP 10Sc	RBP score	53	126	142	156	195	140	1,641
RBP 7Sc	RBP score	30	84	98	110	137	97	1,647
Embeddedness	RBP score	1	11	13	16	20	13	1,649
Percentage fines (sand + silt)	-	0	10	12	20	100	15	1,620

All means are geometric means except pH, DO, Temperature, and Habitat Scores.
RBP = rapid bioassessment protocol (Barbour et al., 1999; RBP 10Sc has 10 parameters while RBP 7 does not include 3 flow-related parameters); TSS = total suspended solids.

4.1.1. Background Specific Conductivity

Background SC was estimated at the 25th centile of the subset of probability-based samples from the example Criterion-data set because its sampling design more closely matched the ecoregional EPA-survey data set. Using this probability-based subsample of the WABbase data set, the estimated background was 80 µS/cm (25th centile, 585 samples from 544 sites; see Figure 4-2). Background was also estimated to be 63 µS/cm based on field data from reference sites from the WABbase data set (75th centile, 112 samples from 82 reference sites; see Figure 4-3). By comparison, the 25th centile was 94 µS/cm for all samples (reference and nonreference sites) from the example Criterion-data set that was used to derive the HC_{05} (1,661 samples from 1,420 sites; see Figure 4-4). The monthly 25th centiles of probability-sampled sites (see Figure 4-2) and all samples in the data set (reference and nonreference sites, see Figure 4-4) were relatively consistent and at or below 100 µS/cm except in July through October (see Figure 4-2). The effects of seasonal variability of SC on the subsequent analyses was further evaluated and are presented in Appendix A. The large size of the data set and the wide range in SC levels in the example Criterion-data set allowed for characterization of the XC_{95}.

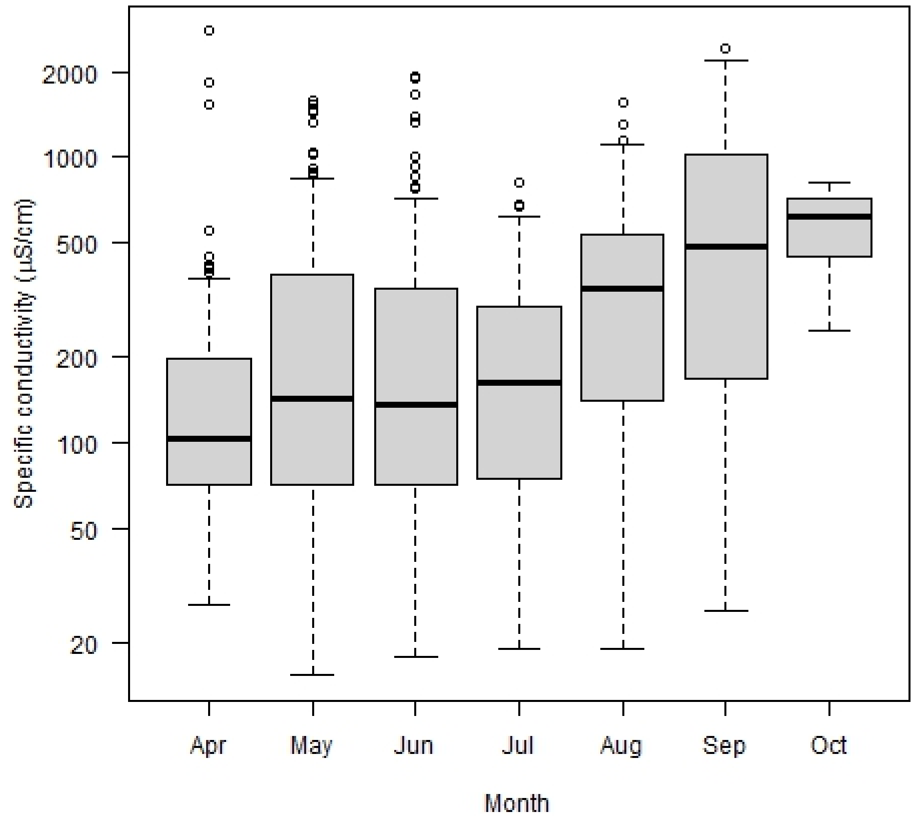

Figure 4-2. Box plot showing seasonal variation of specific conductivity (µS/cm) from probability-sampled sites from Watershed Assessment Branch database (WABbase) 1997−2010. This represents a total of 544 sites with 585 samples from 1997−2010 from Ecoregion 69 with pH >6. Note the difference in scale along the *y*-axis between Figure 4-2 (probability-sampled sites) and Figure 4-3 (reference sites). There are only eight October samples. See Table 4-2 for number of samples per month.

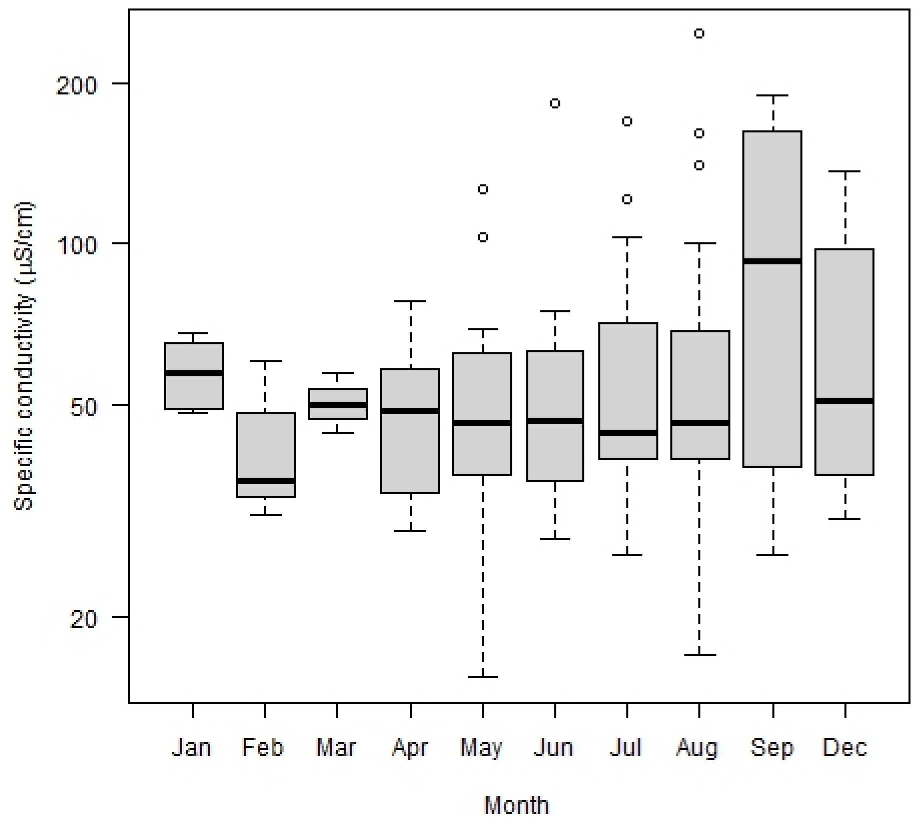

Figure 4-3. Box plot showing seasonal variation of specific conductivity (µS/cm) in the reference streams from Watershed Assessment Branch database (WABbase) 1997−2010.
A total of 112 samples from 82 reference stations were used for this analysis to estimate background specific conductivity. Please note the smaller scale on the *y*-axis compared to Figures 4-2 and 4-4. A total of 112 samples from 82 reference stations were used for this analysis to estimate background specific conductivity. Please note the smaller scale on the *y*-axis compared to Figures 4-2 and 4-4. See Table 4-2 for number of samples per month.

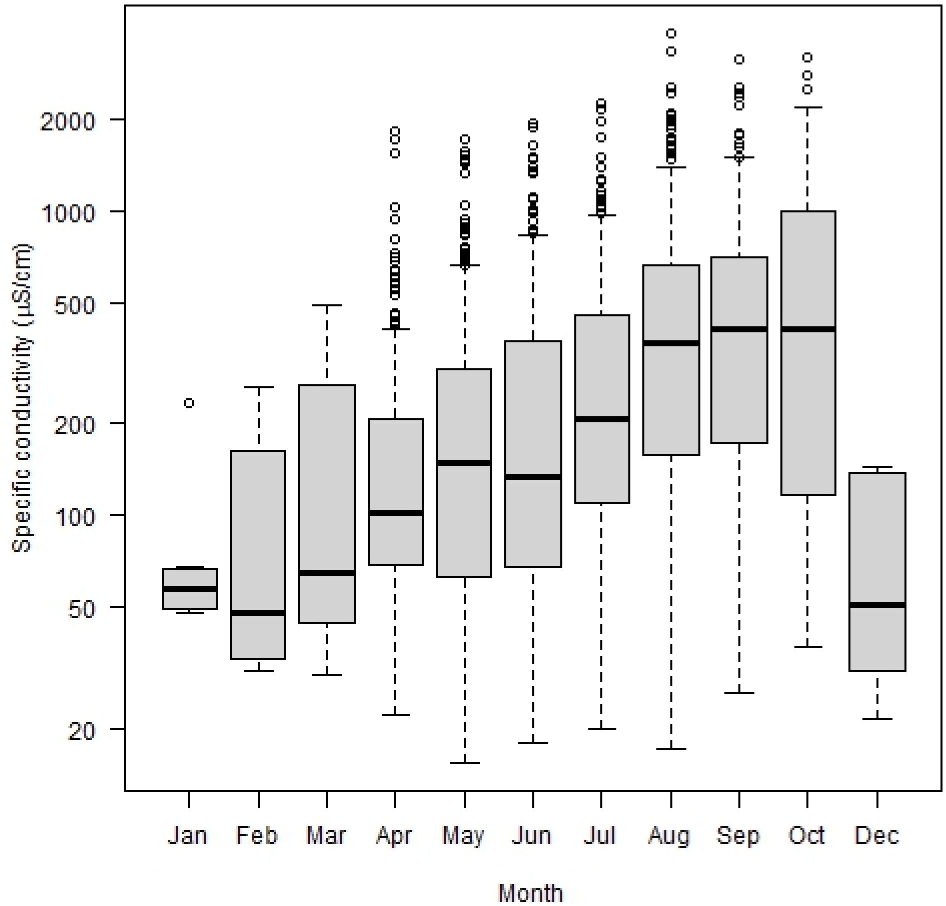

Figure 4-4. Box plot showing seasonal variation of specific conductivity (µS/cm) from all Ecoregion 69 sites from Watershed Assessment Branch database (WABbase) 1997−2010 used to develop the example criteria. This represents a total of 1,661 samples from 1,420 sites from 1997−2010. Note the difference in scale along the *y*-axis between Figure 4-4 (all sites, reference and nonreference) and Figure 4-3 (reference sites). See Table 4-2 for number of samples per month.

4.1.2. Ionic Composition

The ionic composition of the samples in the Ecoregion 69 data set was assessed to ensure that the example criteria were derived for waters dominated by sulfate and bicarbonate anions (see Figure 4-5). Of the 1,674 samples after low pH samples were removed, 56% of samples (938 in total) included measures of calcium, magnesium, sulfate, bicarbonate, and chloride. All but 13 sites (>98%) were dominated by bicarbonate and sulfate anions ($[HCO_3^-] + [SO_4^{2-}]) > [Cl^-]$. The 13 chloride-dominated sites were excluded from the derivation analysis but are shown in Figure 3. Sodium and potassium were less frequently measured, but did not exceed calcium and magnesium where measured for samples in the data set. Sites with no ion measurements were retained in the data set because the data had shown that >98% of samples were dominated by bicarbonate and sulfate anions; thus, it is expected that less than 2% of samples in the Ecoregion 69 Criterion-data set are chloride-dominated.

The analysis may also be defensible for mixtures dominated by sodium, sulfate and bicarbonate ions, e.g., produced water from deep coal mines (Thomas, 2002; Mayhugh and Ziemkiewicz, 2005). This is because the toxicity of these mixtures are more similar to that of calcium, magnesium, sulfate and bicarbonate ions than the toxicity of NaCl (Mount et al., 2016; Kunz et al., 2013; Soucek and Dickinson, 2015).

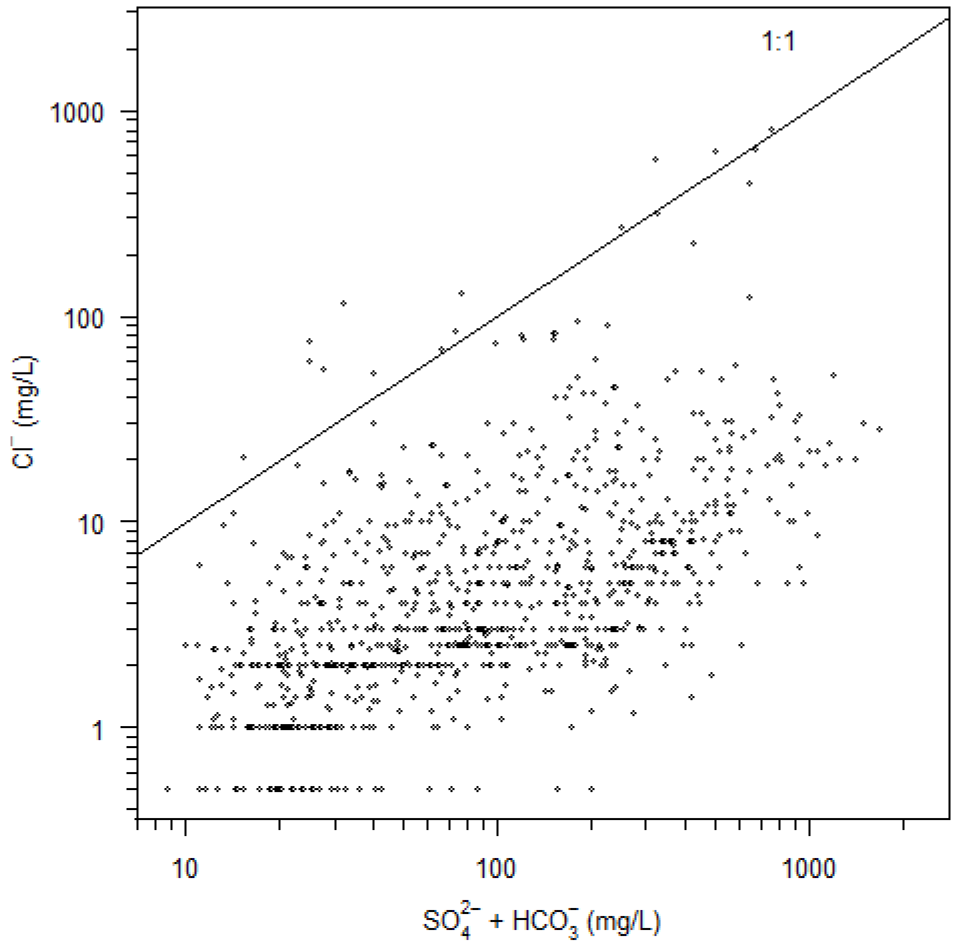

Figure 4-5. Scatter plot of relationship between [Cl⁻] and ([HCO_3^-] + [SO_4^{2-}]) concentrations in streams in Ecoregion 69 data set from 1997−2010 with ionic measurements. Most (98.6%) of the samples (*n* = 938) are below the diagonal line representing the separation between ([HCO_3^-] + [SO_4^{2-}])-dominated and Cl^--dominated mixtures. Sites above the 1:1 line were excluded from the example Criterion derivation data set. Samples depicted here include all sites regardless of pH.

4.1.3. Seasonal Specific Conductivity Regime

For this case study, chemical, physical, and/or biological samples were collected during the sampling years 1997−2010 (January−December). Most (>85%) sites were sampled once during an annual sampling period, but some (e.g., sites being studied to improve stream condition within the TMDL Program) were sampled monthly for water quality parameters (see Table 4-2).

Table 4-2. Number of samples with reported genera and specific conductivity meeting acceptance criteria for the Ecoregion 69 analysis

Number of samples[a]	Month												Total
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
Full data set	8	4	36	159	269	163	197	388	342	89	0	6	1,661
Probability sites	0	0	0	66	190	116	84	68	53	8	0	0	585
Reference sites	6	3	3	10	19	12	27	24	4	0	0	4	82
Percentage of total	0.5	0.2	2.2	9.6	16.2	9.8	11.9	23.4	20.6	5.4	0	0.4	(100)

[a]Number of samples is presented for each month.

Samples collected from the WVDEP-identified reference sites indicate that SC levels are generally low and similar throughout the year, although slightly higher in September (see Figure 4-3). These data show that SC concentrations in flowing waters in the study area can vary somewhat by season, likely depending on stream discharge, rainfall, snowmelt, and other hydrological factors. As described in Section 3.1.4 (and in greater detail in the *EPA Benchmark Report*), the effects of seasonal differences in SC levels and aquatic insect life history were evaluated by comparing HC_{05} values partitioned for season. After careful consideration of the similarity between the spring HC_{05} and the HC_{05} based on the full data set at the low end of the XCD, the example ecoregional criteria were derived using all available data, regardless of the time of year they were collected (see Sections 3.1.4 and Appendix A.2 in this assessment, and U.S. EPA, 2011a).

4.2. RESULTS

4.2.1. Extirpation Concentration (XC_{95}) and Hazardous Concentration (HC_{05}) Values (Example Criterion Continuous Concentration [CCC])

The Ecoregion 69 example Criterion-data set (see Table 4-1) was used to develop XC_{95} values from weighted CDFs. The histogram used to develop weights is depicted in Figure 4-6. The XC_{95} values that were used in the XCDs are listed in the order of least to most salt-tolerant in Appendix A.3. The generalized additive model plots used to designate ~ and > values for those XC_{95} values are depicted in Appendix A.4. The weighted CDFs used to derive the XC_{95}

values are shown in Appendix A.5. The HC_{05} for Ecoregion 69 was calculated at 305.4 μS/cm (see Figures 4-7 and 4-8); the two-tailed 95% confidence bounds were 233−329 μS/cm. Those bounds, derived by bootstrap resampling, indicate that different data sets could yield HC_{05} values within that interval. Rounding to two significant figures, the example CCC for Ecoregion 69 is 310 μS/cm.

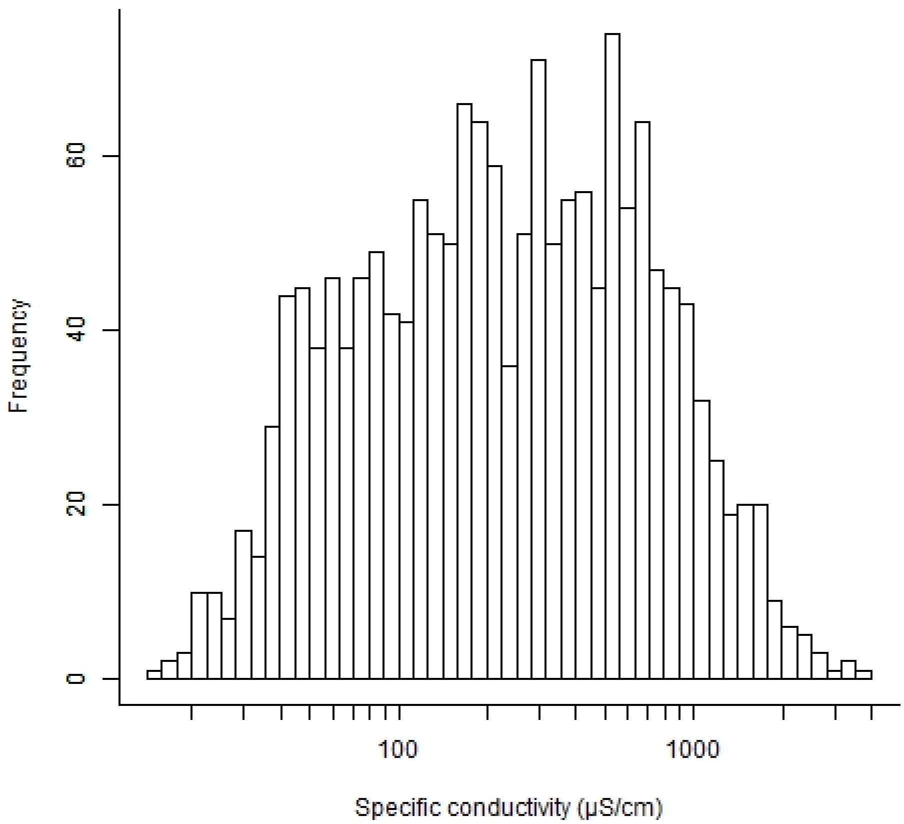

Figure 4-6. Histograms of the frequencies of observed specific conductivity values in samples from Ecoregion 69 sampled between 1997 and 2010. Bins are each 0.017 (1/60) of the range of log10 specific conductivity units wide.

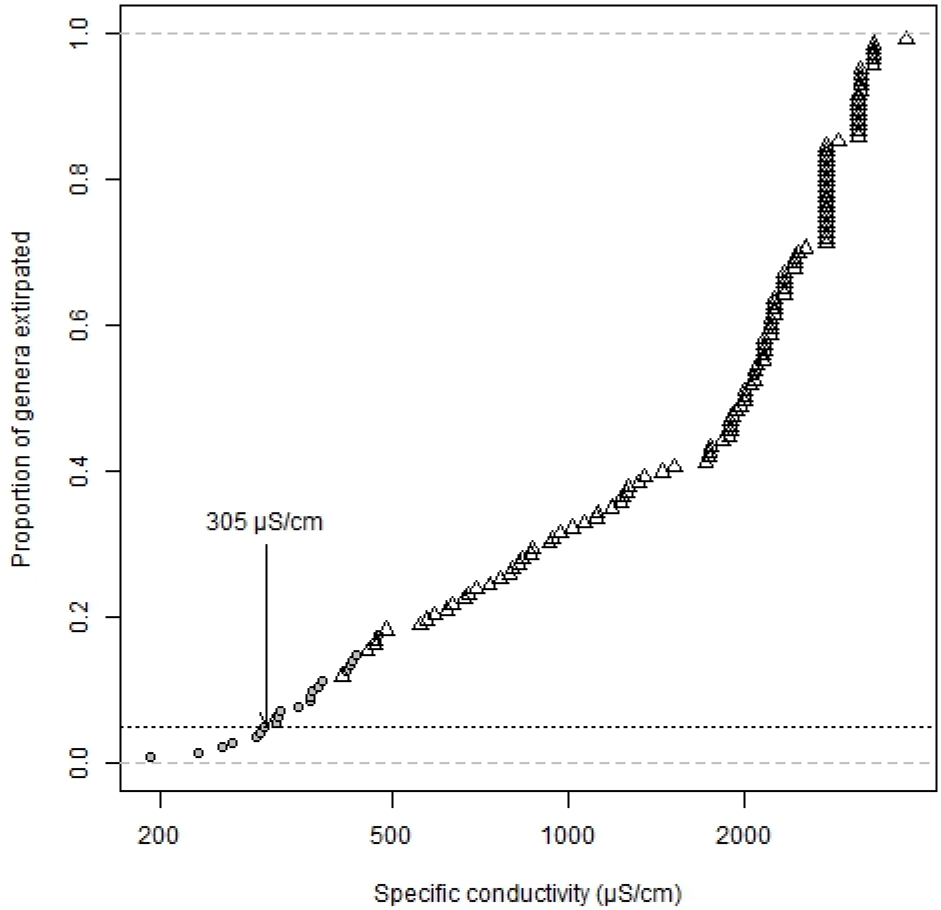

Figure 4-7. Example genus extirpation concentration distribution (XCD) for Ecoregion 69. Each point is an extirpation concentration (XC_{95}) value for a genus. There are 142 genera. The hazardous concentration (HC_{05}) is 305 µS/cm (95% confidence interval is 233−329 µS/cm) and is the specific conductivity at the intersection of the XCD with the horizontal line at the 5th centile. XC_{95} with an approximate or greater than designation are shown as triangles.

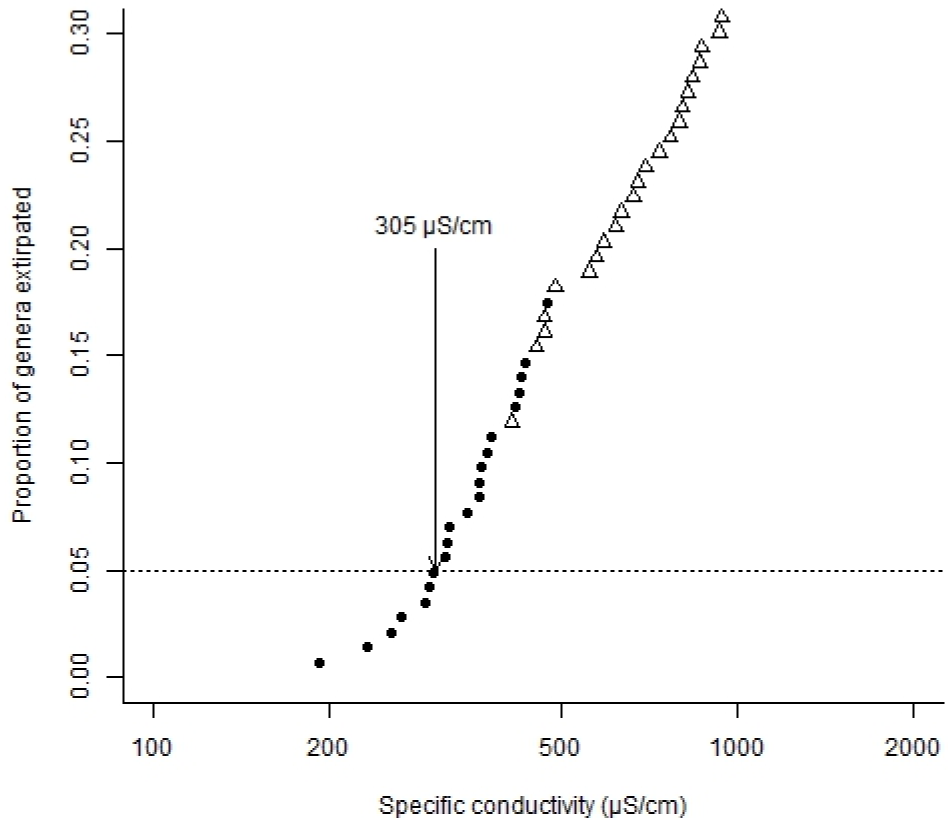

Figure 4-8. The lower end of the example genus extirpation concentration distribution for Ecoregion 69. The dotted horizontal line is the 5th centile. The vertical arrow indicates the hazardous concentration (HC_{05}) of 305 µS/cm (95% confidence intervals 233−329 µS/cm). Only the 50 most salt-intolerant genera are shown to better discriminate the points on the left side of the distribution. The six most salt-intolerant genera (i.e., extirpation concentration [XC_{95}] ≤ 305 µS/cm) are *Leptophlebia, Remenus, Pycnopsyche, Paraleptophlebia, Bezzia,* and *Alloperla*). XC_{95} values with an approximate or greater than designation are shown as triangles.

4.2.2. Example Criterion Maximum Exposure Concentration

At sites meeting the CCC of 310 µS/cm, 90% of the SC observations are estimated to occur below the CMEC (see Section 3.2). The CMEC was derived using the full Ecoregion 69 data set (9,806 samples collected between 1996−2011). Of the 9,806 samples in this ecoregion, there are 5,823 samples in a July to June rotating year representing 564 rotation years, 536 unique stations, with at least 1 sample from July to October and 1 sample from March to

June, and at least 6 samples within a rotation year (see Table 4-3). Note that inclusion of samples is not contingent on biological data. Reference and nonreference sites were included to ensure a range of SC (see Table 4-1).

Table 4-3. Summary data related to the calculation of the example criterion maximum exposure concentration (CMEC) for Ecoregion 69

Number of samples July to June prior to biological sampling	5,811
Number of unique stations/rotation years	536/564
Number of WVDEP reference sites	15
CCC	310 µS/cm
CMEC	630 µS/cm

Of the 564 rotation years (536 unique stations) with multiple SC measurements, the variability of within station SC was found to differ for streams with different mean SC (see Figure 4-9). The locally weighted scatterplot smoothing (LOWESS) lines indicated that the average variability (residual standard deviation for a station) in the middle of the SC gradient is slightly higher than both the lower and higher ends of the gradient. The stations with annual mean SC values between the 25th and 75th centile (which is approximately between 120−520 µS/cm) have relatively similar variances, and therefore, could be used to estimate the standard deviation components of annual mean SC (310 µS/cm). There are 2,855 samples from 278 station years (265 stations) in the selected data sets for Ecoregion 69 with streams having mean SC values between 120 and 520 µS/cm. The grand mean and standard deviation of this data set were determined and the CMEC was calculated. The example CMEC calculation is shown below:

$$\text{CMEC for Ecoregion 69: } 10^{\log10(310)\,+\,1.28*0.243} = 634.1\ \mu\text{S/cm} \qquad (4\text{-}1)$$

The example CMEC (see Table 4-3) rounded to two significant figures yields a CMEC of 630 µS/cm for Ecoregion 69. At this level, where the annual average SC is <310 µS/cm, 90% of the observations are expected to be less than the CMEC.

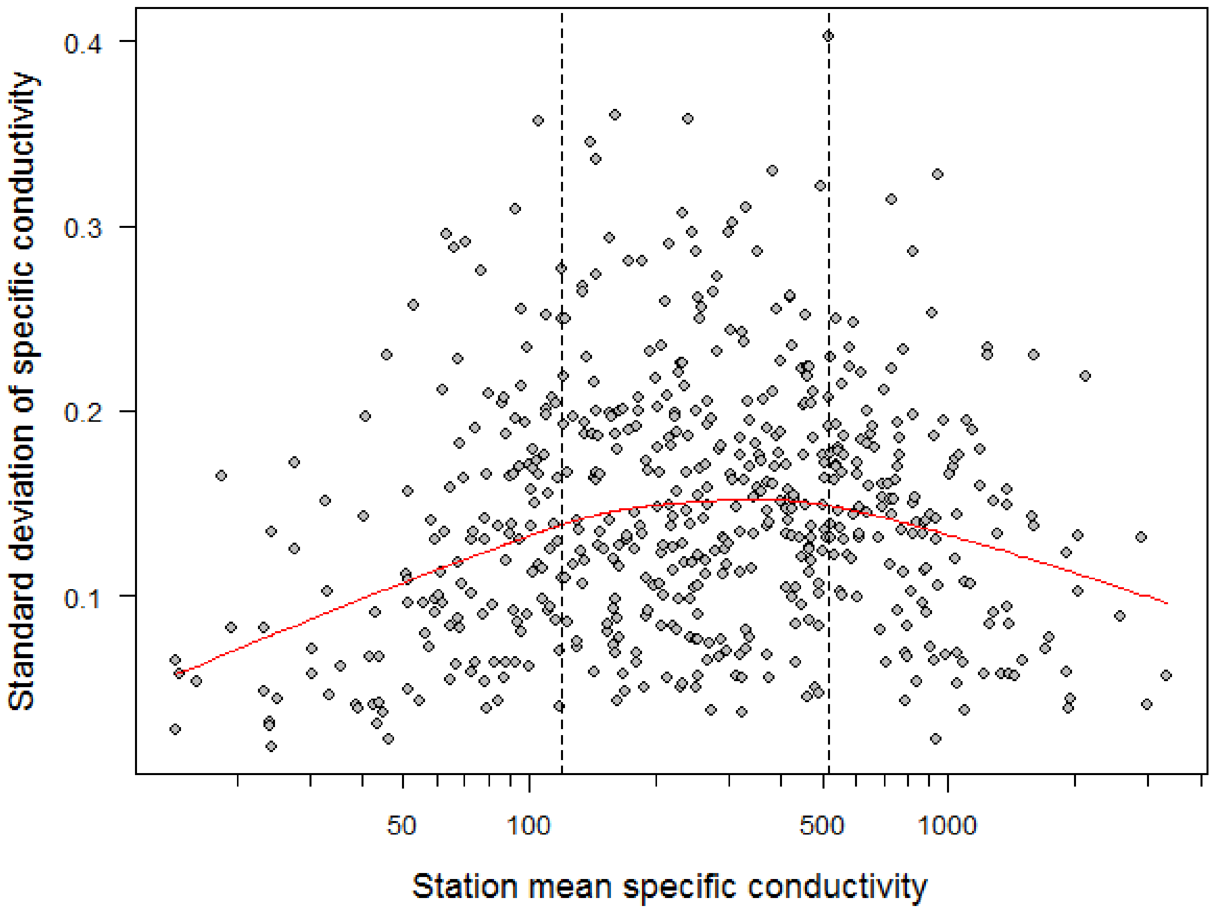

Figure 4-9. Illustration of within site variability (residual standard deviation for each station) along the specific conductivity gradient (station mean) in Ecoregion 69. The *x*-axis is log annual mean specific conductivity. Each dot represents a station. The fitted line is a locally weighted linear scatterplot smoothing (LOWESS, span = 0.75, linear polynomial model), while the two vertical dashed lines represent logarithm mean specific conductivity of 120 and 520 µS/cm, respectively. Within those bounds the standard deviation is fairly constant.

4.3. GEOGRAPHIC APPLICABILITY

The geographical applicability of the criteria throughout Ecoregion 69 was assessed using the background-matching approach (see Section 3.7.1). The background SC of the new area (i.e., the portion of Ecoregion 69 beyond the original data set) was estimated at the 25th centile (see Section 3.7.1.2; and Cormier and Suter, 2013a) and compared with the background SC estimates for the original data set.

Because the example SC criteria presented here have been developed for a dissolved mixture dominated by sulfate and bicarbonate anions, ($[HCO_3^-] + [SO_4^{2-}]) > [Cl^-]$ in mg/L, all chloride-dominated samples, ($[HCO_3^-] + [SO_4^{2-}]) \leq [Cl^-]$ in mg/L, were removed from the data set before estimating background SC. Thereby, the background for the new area is estimated for the same ionic mixture as the example criteria.

4.3.1. Data Sources

Two data sets were used for this example applicability assessment: the original data set used to derive the HC_{05} described in Section 4.1 and an EPA-survey data set (see Table 4-4).

The EPA-survey data set was used to evaluate and characterize ion concentrations and water chemistry in the ecoregion (see Table 4-5). The primary sources of the combined data are from EPA survey programs including the National Rivers and Streams Assessment (NRSA) 2008−2009 surveys (U.S. EPA, 2013b, 2009), Wadeable Streams Assessment (WSA) 2004 survey (U.S. EPA, 2006), Environmental Monitoring and Assessment Program (EMAP) 1993−1998 and Regional-EMAP (R-EMAP) 1999 surveys (U.S. EPA, 2013c), and National Acid Precipitation Assessment Program (NAPAP) 1986 survey (NADP, 2013). Data sets are based on random samples from June through September. Most report SC, alkalinity, hardness, sulfate, chloride, bicarbonate, pH, and other water quality parameters. Ecoregions and sampling sites are shown in Figure 4-10. All samples were collected from first-through fourth-order streams as part of a probability-based design intended to estimate proportions of parameters for various stream classes. The probability-design weights were not used in this characterization. Analysis of water chemistry samples followed EPA procedural and QA/QC protocols from EMAP (U.S. EPA. 2001, 1998b, 1994, 1987), Wadeable Streams Assessment (U.S. EPA, 2004a, b), the NRSA (U.S. EPA, 2009), and NAPAP (Drousé et al., 1986; U.S. EPA, 1987). These data sets were also selected so that methods would be comparable across the data set, and because these studies used probability-based designs (i.e., randomly assigned sampling locations).

Table 4-4. Description of survey data sets combined to form the EPA-survey data set used to assess applicability of the example ecoregional criteria throughout Ecoregion 69

Data set	Sampling period	Total *N*	KY	MD	PA	TN	VA
MAHA EMAP	1993−1995	42	0	3	35	0	4
MAIA EMAP	1997−1998	12	0	0	8	0	4
WSA	2004	9	3	1	0	3	2
NRSA	2008−2009	8	4	0	1	2	1
NAPAP	1986	41	2	6	29	4	0
Region 4 Wadeable Streams R-EMAP	1999−2002	9	7	0	0	2	0
Total		121	16	10	73	11	11

MAHA = Mid-Atlantic Highland Assessment; MAIA = Mid-Atlantic Integrated Assessment.

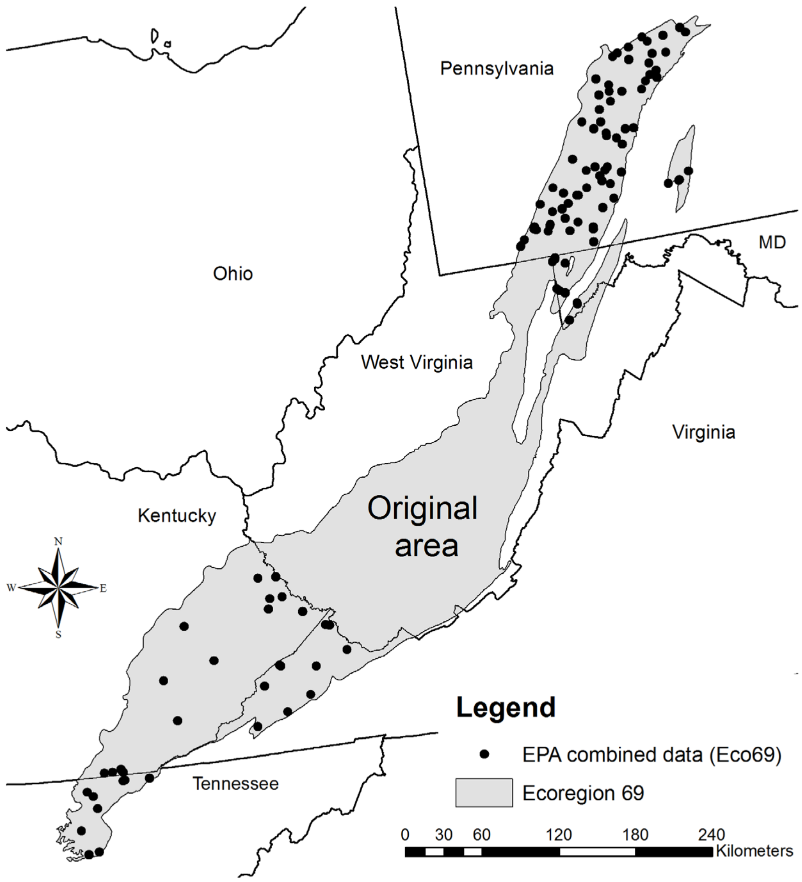

Figure 4-10. **Ecoregion 69 extends from central Pennsylvania to northern Tennessee.** Sampling sites in the EPA-survey data set that were used to estimate background in the "new" area for Ecoregion 69 are indicated as points. The figure depicts 121 samples from 121 stations.

4.3.2. Geographic Applicability Results

A summary of water quality for the EPA-survey data set (see Section 4.3.1) for Ecoregion 69, including major ionic constituents, is provided in Table 4-5. Background SC in the new area was estimated from the full EPA-survey data set because no sample was dominated by chloride ions.

Background SC for bicarbonate and sulfate dominated waters estimated as the 25th centile of the EPA-survey data set for the new area in Ecoregion 69 (outside the area used to develop the example criteria) was 63.5 μS/cm (95% CI 46−89 μS/cm) (see Table 4-6). The 25th centile from the probability sample from the example Criterion-data set was 66 μS/cm (95% CI 60−75) (see Table 4-6). The confidence bounds for background estimated from the example Criterion-data set overlap with the confidence bounds for background estimated for the rest of Ecoregion 69. Therefore, the background SC regime throughout Ecoregion 69 appears to be similar, and thus, the example criteria (CCC = 310 µS/cm, CMEC = 630 µS/cm) are considered geographically applicable throughout the ecoregion. Other estimates of background from the reference sites in the example Criterion-data set (63 µS/cm; 95% CI 60−65 µS/cm) and the example Criterion data set (94 µS/cm; 95% CI 86−101 µS/cm) also overlap with the CI of the background for the rest of Ecoregion 69 (see Table 4-6).

Table 4-5. Summary of water quality parameters for Ecoregion 69 from the EPA-survey data set excluding the sites in West Virginia

Ecoregion	Ion	Min	Centile				Max	Relevant *N*
			10th	25th	50th	75th		
Ecoregion 69	HCO_3^- (mg/L)	0.0	0.1	1.3	12.5	37.3	241.8	102
	SO_4^{2-} (mg/L)	3.2	7.6	10.0	21.4	136.3	1,622.8	112
	Cl^- (mg/L)	0.5	1.1	1.7	3.0	8.6	59.0	112
	Ca^{2+} (mg/L)	1.2	2.2	5.6	13.7	39.1	186.0	112
	Mg^{2+} (mg/L)	0.6	1.0	1.5	4.5	17.3	152.1	112
	Na^+ (mg/L)	0.2	0.6	1.1	2.9	9.3	93.4	112
	K^+ (mg/L)	0.4	0.6	0.8	1.3	2.2	8.0	112
	pH (SU)	3.0	4.7	6.2	7.1	7.7	8.6	121
	[a]$(HCO_3^- + SO_4^{2-})/Cl^-$	1.4	3.0	5.4	13.6	41.4	497.5	102
	SC (µS/cm)	23.7	34.5	63.5	183.5	426.8	2,515	121

[a]Value within category calculated from individual sample ion concentrations. $HCO_3^- + SO_4^{2-}/Cl^-$ in mg/L greater than 1 indicates the mixture is dominated by $HCO_3^- + SO_4^{2-}$.

Table 4-6. Background specific conductivity estimates for Ecoregion 69

Data set	Centile used to estimate background	Background µS/cm	Confidence interval µS/cm	Relevant *N* (sites/samples)
EPA-survey data set from geographic area not represented in the example criterion derivation data set	25^{th}	64	46−89	121/121
WABbase data set, probability sample subset	25^{th}	66	60−75	544/583
WABbase data set, reference sample subset	75^{th}	63	60−65	82/112
Example criterion derivation data set, full data set	25^{th}	94	86−101	1,420/1,661

4.4. SUMMARY OF EXAMPLE CRITERIA FOR ECOREGION 69

The case example for Ecoregion 69 includes an annual geometric mean (i.e., CCC) and a 1-day mean (i.e., CMEC), not to be exceeded more than once in 3 years on average. Both of these distinct expressions of the example SC criteria would need to be met in order to adequately protect aquatic life. These values indicate that freshwater animals in Ecoregion 69 would be protected if the annual geometric mean SC concentration in flowing waters does not exceed 310 µS/cm and the 1-day mean does not exceed 630 µS/cm, more than once every 3 years on average. These example criteria would apply to all flowing freshwaters (ephemeral, intermittent, and perennial streams) in Ecoregion 69 inclusive of portions of Kentucky, West Virginia, Maryland, Virginia, Tennessee, and Pennsylvania. On a site-by-site basis, these example ecoregional criteria apply if the ionic mixture is dominated by anions of bicarbonate and sulfate. For streams crossing into Ecoregion 69, professional judgment may be needed to assess the potential effect of different ionic composition or concentration. Professional judgment is recommended when applying to sites with a catchment area greater than 1,000 km^2 (386 mi^2) owing to lesser representation in the example data set by this class of stream. On a site-by-site basis, alternative specific conductivity criteria may be more appropriate if the natural background of a site is shown to be lower or higher than its regional background specific conductivity.

4.5. EXAMPLE CRITERION CHARACTERIZATION

4.5.1. Factors Potentially Affecting the Extirpation Concentration Distribution (XCD) Model

An assessment of potential confounders and an analysis of the influence of habitat quality and sampling date for Ecoregion 69 can be found in Appendix A.2.

4.5.1.1. *Sensitivity Analyses*

As the minimum number of occurrences of a genus for inclusion in the data set increases, fewer genera are included in the XCD. The HC_{05} increases greatly when a taxon in the lower 5^{th} centile is removed because it does not meet the minimum number of samples and then more slowly alternates between increasing and decreasing as genera either above or below the 5^{th} centile are removed because they do not meet the minimum number of samples (see Figure 4-11). The pattern repeats until all genera above and below the lower 5^{th} centile have the same XC_{95} value (not shown). To maximize the number of genera included in the XCD, a minimum of 25 occurrences was utilized.

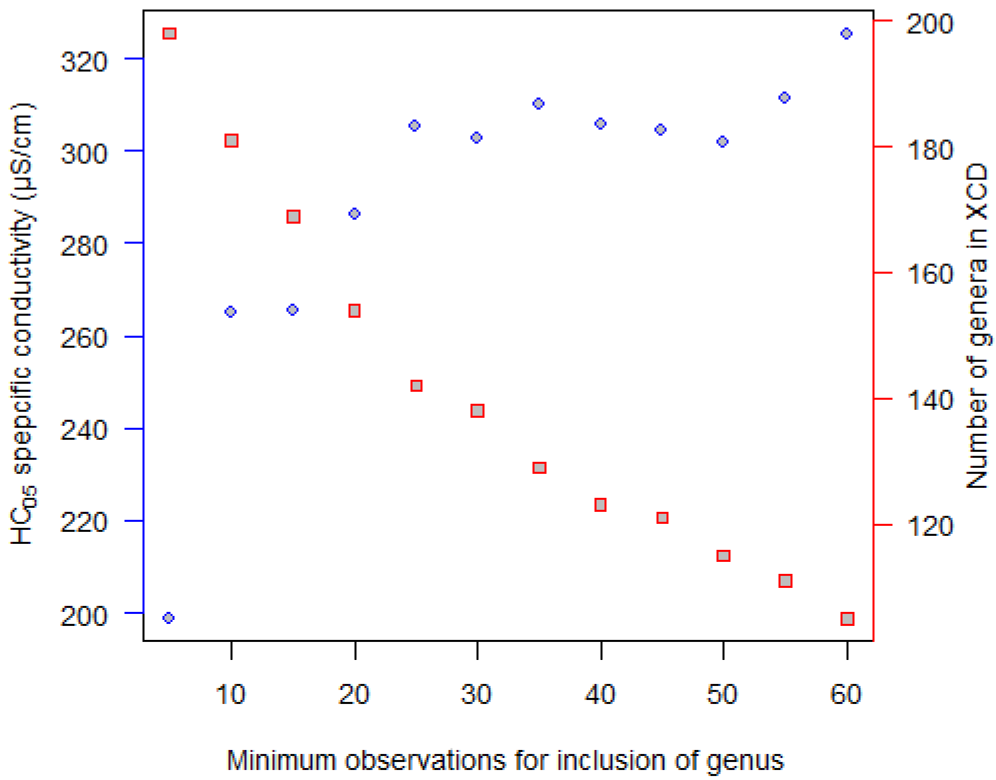

Figure 4-11. Relationship of the number of occurrences of a genus and the hazardous concentration (HC_{05}) based on Ecoregion 69 example Criterion-data set. Estimates of HC_{05} values (blue diamonds, left axis) and the number of taxa in the extirpation concentration distribution (XCD) (red squares, right *y*-axis) based on minimum number of samples (5−60, *x*-axis). As the minimum number of observations of a genus increases, fewer are included in the XCD.

The number of samples in the data set affected the number of genera included in the XCD and the resulting example HC_{05}. The effects of data set size on the HC_{05} estimates and on their confidence bounds were estimated using a bootstrapping technique. The mean of all bootstrapped HC_{05} values, the numbers of genera used for the HC_{05} calculation, and their 95% CI were plotted to show the effect of sample sizes. As shown in Figure 4-12, the HC_{05} for this data set stabilizes, reaching an asymptote at approximately 500−800 sites sampled and 90−120 genera evaluated. Therefore, the original data set was considered adequate for estimating the example.

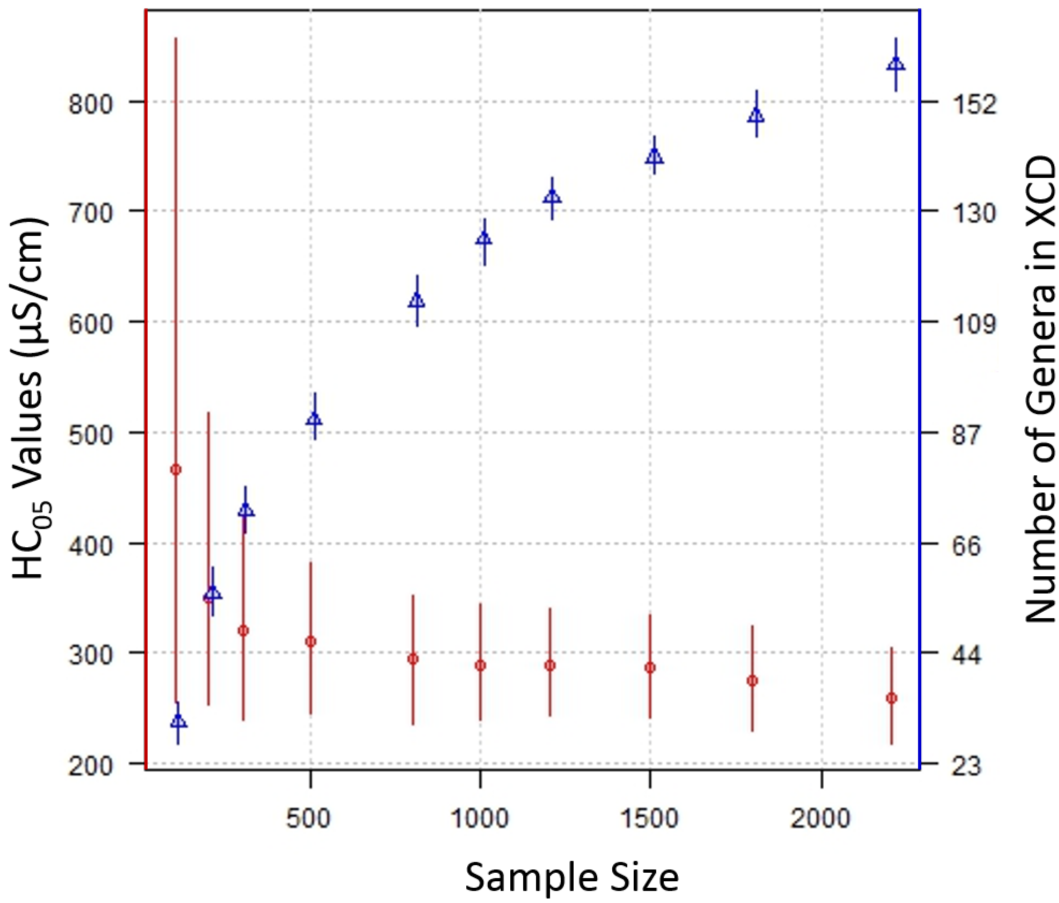

Figure 4-12. **The effect of the size of the data set used to model the hazardous concentration (HC_{05}) based on the Ecoregion 69 example Criterion-data set.** As size of the data set increases, the number of genera included in the extirpation concentration distribution (XCD) increases (triangles). The HC_{05} stabilizes reaching an asymptote at approximately 500−800 sites sampled (circles) and 90−120 evaluated genera.

4.5.2. Validation of the Extirpation Concentration Distribution (XCD) Model

The XCD model was validated and uncertainty around the HC_{05} values was estimated using bootstrapping, as recommended by the EPA SAB in their review of the *EPA Benchmark Report* (U.S. EPA, 2011c). The median HC_{05} estimated from bootstrapping was 281 µS/cm (95% CI 233−329 µS/cm) which is similar to the HC_{05} of 305 µS/cm measured using a 2-point interpolation of the original XCD. The similarity between the two HC_{05} values indicates a similar model would be generated using an independent data set (see Figure 4-13).

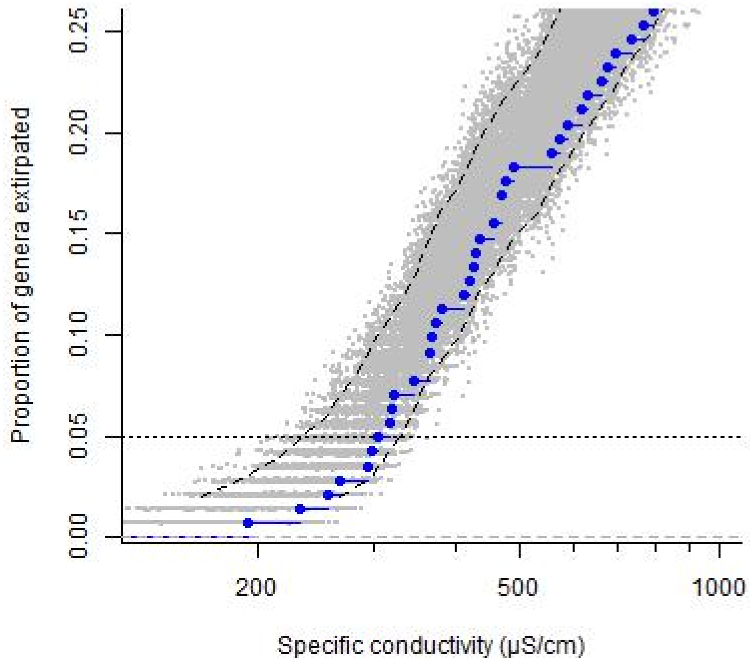

Figure 4-13. Cumulative distribution of the extirpation concentration (XC_{95}) values for the 25% most salt-intolerant genera (blue circles) and 95% confidence intervals (dotted lines) based on 1,000 extirpation concentration distribution (XCD) bootstrapping results. Each tiny gray dot represents an XC_{95} value for a bootstrapping iteration (note that the genera in each percentage varies with each XCD iteration). Each larger blue filled dot represents the calculated XC_{95} of the XCD for the criterion continuous concentration (CCC). The median bootstrapped hazardous concentration (HC_{05}) is 281 µS/cm.

4.5.3. Duration and Frequency

Numeric criteria include magnitude (i.e., how much), duration (i.e., how long), and frequency (i.e., how often) components. Appropriate duration and frequency components of criteria are determined based on consideration of available data and understanding the exposure-response relationship in the context of protecting the aquatic life of a water body. The significant consideration used in setting the duration component of aquatic life criteria is how long the exposure concentration can be above the criteria without affecting the endpoint on which the criteria are based (U.S. EPA, 1991, 1985). Based on the temporal resolution of the available field data set and an analysis of within-site variability of SC levels, EPA developed two different expressions for the example SC criteria in order to provide adequate protection for aquatic life.

In this case, the majority (>85%) of sites used to derive the example CCC for Ecoregion 69 were sampled once during an annual sampling period and thus represent the average stream SC and macroinvertebrate assemblage information over the course of 1 year. As a result, the appropriate duration for the CCC is 1 year. The duration for the CMEC, a level of protection from acutely toxic exposures, is 1 day based on a review of the literature regarding the onset of macroinvertebrate drift in response to elevated SC (see Section 3.3). At sites meeting the CCC, 90% of the SC observations are estimated to occur below the CMEC.

EPA anticipates that an appropriate allowable frequency of exceedance for these example criteria is no more than once in 3 years, based on recovery rates from literature reviews and consideration of the life history of insects able to recolonize a site via drift or aerial dispersal (see Section 3.4). Recovery is expected to occur in 3 years if the following conditions are met: (1) the SC regime returns to a yearly average below the CCC, (2) there are nearby streams with low SC supporting a diverse community, and (3) there is an upstream source of colonizers or the flight or recolonizing distance is within the dispersal range of genetically diverse, reproducing adult colonizers. If any of these conditions are not met, the time necessary for ecosystem recovery (and thus, the allowable frequency of exceedance) would likely be longer than 3 years.

4.6. PROTECTION OF FEDERALLY-LISTED SPECIES

Although the derivation of the example criteria was limited to the macroinvertebrate taxa represented in the data sets, the available evidence indicates that other taxa in the streams would likely be protected as well (see Section 2.6 and Appendix G). Hence, no adjustment was made for unanalyzed taxa. However, on a site-specific basis, the example criterion could be adjusted or recalculated to protect important species, highly valued aquatic communities, or specially protected waters.

5. CASE STUDY II: EXAMPLE USING THE EXTIRPATION CONCENTRATION DISTRIBUTION (XCD) METHOD IN A MODERATE BACKGROUND SPECIFIC CONDUCTIVITY ECOREGION

This section presents a case study for the Western Allegheny Plateau (Ecoregion 70) to illustrate how the analytical methods described in Section 3 can be used to derive example SC criteria using the XCD method in an area with slightly higher background SC than Ecoregion 69 (see Section 4). Ecoregion 70 results, including estimates of the CCC and CMEC, duration, frequency, and discussion of applicability are included as examples to demonstrate the method. The derivations of the CCC and CMEC analyses and results are based on data from Ecoregion 70 in West Virginia, and SC data from the Ecoregion 70 outside of WV was used to assess applicability of the criteria throughout the ecoregion.

5.1. DATA SET CHARACTERISTICS

The Western Allegheny Plateau (Ecoregion 70) extends from the corner of southwestern Pennsylvania and southeastern Ohio into Kentucky and West Virginia. The hilly and wooded terrain of the Western Allegheny Plateau is mostly unglaciated and well dissected, with local relief of 61 to 229 m, and peak elevations of around 610 m. Many of the rivers in this ecoregion are entrenched, as a result of the rugged, hilly terrain, particularly within the Permain Hills (70a) and Monongahela Transition Zones (70b). The ecoregion is predominantly forested, but also consists of a mosaic of urbanized areas, pastures, farms, and coal mines (Woods et al., 1999). Extensive mixed mesophytic forests and mixed oak forests originally grew in the Western Allegheny Plateau and, today, most of its rounded hills remain in forest; dairy, livestock, and general farms, with residential developments concentrated in the valleys. The Western Allegheny Plateau is composed of horizontally bedded sandstone, shale, siltstone, limestone, and coal. The horizontally-bedded sedimentary rock underlying the region has been mined for bituminous coal (Woods et al., 1996).

The data used in this case study are from a large field data set, the WVDEP's in-house WABbase. Chemical and biological samples are from 1996−2011 and 1997−2010, respectively. The WABbase contains data from Level III Ecoregions 66, 67, 69, and 70 in West Virginia (U.S. EPA, 2000a; Omernik, 1987; Woods et al., 1996). The WABbase data set provides consistent sampling and analytical methods, high quality, broad spatial coverage of a large number of perennial streams (2,011 distinct locations) in Ecoregion 70.

The WABbase contains data from a mixed sampling design that collects measurements from long-term monitoring stations, targeted sites within watersheds on a rotating basin schedule, randomly selected sample sites (Smithson, 2007), and sites chosen to further define impaired stream segments in support of TMDL development (WVDEP, 2008a). Most sites are sampled once during an annual sampling period, but most TMDL sites are sampled monthly for water quality. The data set contains water quality, habitat, watershed characteristics, macroinvertebrate data (both raw data and calculated metrics), and geographic location (WVDEP, 2008a). A wide range of SC levels were sampled, which is useful for modeling the response of organisms to different ionic concentrations. Level 1 reference status (WVDEP, 2008b) which selects reference sites that "are thought to represent the characteristics of stream reaches that are minimally affected by human activities and are used to define attainable chemical, biological and habitat conditions for a region" (WVDEP, 2013). Sites are initially selected by a map coordinator based on GIS land use data that indicate minimal human disturbance. Streamside, the appropriateness of the selected site is confirmed based on the level of anthropogenic disturbance, lack of point discharges, habitat quality, pH, dissolved oxygen, and SC (>500 µS/cm) is used to flag a site for further investigation before inclusion as a reference site (WVDEP, 2013).

Macroinvertebrate records in the data set are based on collections from a total of 1 m^2 area from a 100 m reach at each site. Using a 0.5 m wide rectangular kicknet (595 µm mesh), four 0.25 m^2 riffle areas were sampled. In streams narrower than 1 m, nine areas were sampled with a 0.33 m wide D-frame dipnet of the same mesh size. Composited samples were preserved in 95% denatured ethanol. A random subsample of 200 individuals (±20%) was identified in the laboratory. All contracted analyses for chemistry and macroinvertebrate identification followed WVDEP's internal quality control and quality-assurance protocols (WVDEP 2008b, 2006). Quality assurance of the data set was judged by EPA to be excellent, based on the database itself and supporting documentation.

Several data filters, described in Section 3.1 (see Figure 3.2), were applied prior to finalization of the data set and analyses. A total of 12,909 records from Ecoregion 70 are included in the data set; of those, SC measurements were included in 11,600 of these samples.

Many of these are measurements of water quality without biological sampling. Of the 11,600, there are 2,126 paired samples with SC measurements and biological samples identified to genus. Of these, a total of 51 samples were removed from the data set due to low pH ≤6 (48 samples) and high proportion of chloride ions, ($[HCO_3^-] + [SO_4^{2-}]) \leq [Cl^-]$ (3 samples). Additional criteria were used to identify macroinvertebrates for inclusion in the extirpation concentration distribution: occurrence at reference sites and occurrence in 25 or more samples. Of the 217 macroinvertebrate genera identified in this ecoregion of the WABbase, 179 genera occurred at least once at one of the 29 identified reference sites where invertebrate samples were collected and identified to genus. A total of 139 genera occurred at 25 or more sampling locations. The final example Criterion-data set has 2,075 samples belonging to 1,695 stations (as depicted in Figure 5-1). Multiple samples were obtained from 19% of stations. Summary statistics for the data set used to derive the criterion is shown in Table 5-1. The statistical package R, Version 2.12.1 (December 2010), was used for all statistical analyses (R Development Core Team, 2011).

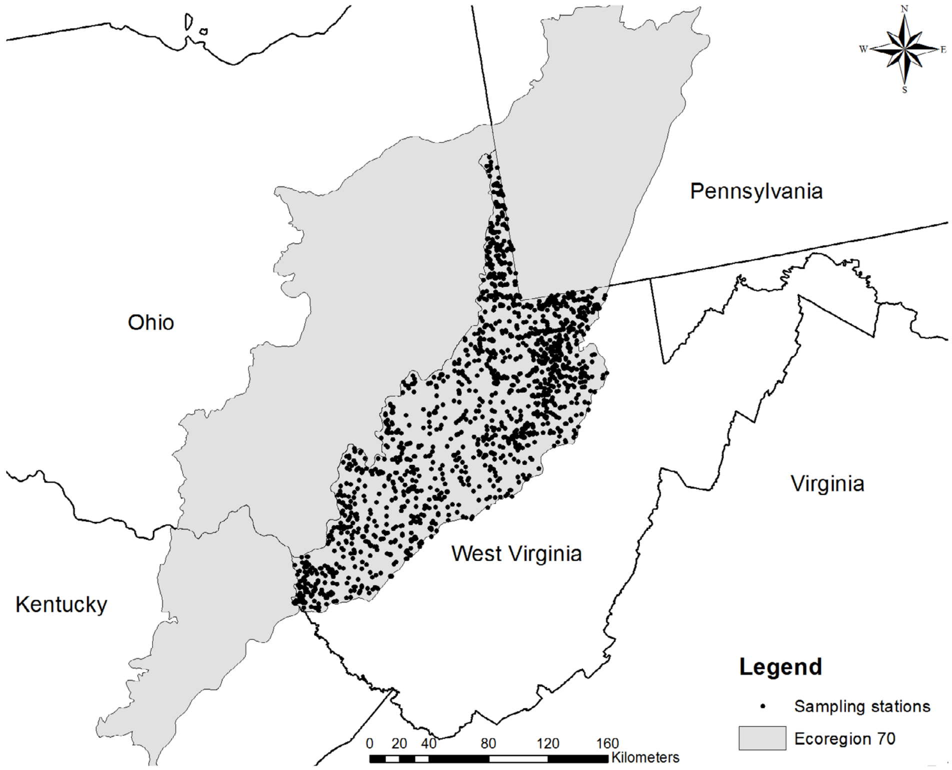

Figure 5-1. Ecoregion 70 extends from central Pennsylvania to northern Tennessee. Sampling sites (stations) ($N = 1{,}695$) in the example Criterion data set that were used to derive the example criterion continuous concentration (CCC) are indicated as points.

Table 5-1. Summary statistics of the measured water-quality parameters used to derive the example specific conductivity criteria in Ecoregion 70. The example Criterion data set has 2,075 samples belonging to 1,695 stations.

Parameter	Units	Min	25th	50th	75th	Max	Mean [a]	*N*
SC	µS/cm	40	169	259	563	11,646	322.8	2,075
Hardness	mg/L	14.21	67.38	106.44	234.4	2,271.3	130.7	1,050
Total alkalinity	mg/L	1	47.35	84.15	135.75	810	77.3	1,378
SO_4^{2-}	mg/L	1	19.4	42	253	6,560	67.7	1,405
Chloride	mg/L	0.5	4	7.4	19	1,153	9.4	1,074
$SO_4^{2-} + HCO_3^-$	mg/L	5.7	87.3	168.2	414.4	6,664.9	192.8	1,375
Ca, total	mg/L	1	19.1	30.8	64	621	36.6	1,052
Mg, total	mg/L	1.1	4.64	7.1	15.9	175	9.2	1,053
Na, total	mg/L	1.4	6.4	18.3	52	2,340	22.2	197
K, total	mg/L	0.6	1.2	2.3	4	25.3	2.3	194
TSS	mg/L	1	3	4	7	506	4.5	1,682
Fe, total	mg/L	0.02	0.16	0.3	0.54	137	0.31	1,673
Fe, dissolved	mg/L	0.02	0.02	0.05	0.07	114	0.048	1,285
Al, total	mg/L	0.01	0.09	0.13	0.26	12	0.15	1,392
Al, dissolved	mg/L	0.02	0.02	0.04	0.05	0.4	0.038	1,304
Mn, total	mg/L	0.003	0.02	0.047	0.118	15.9	0.053	1,269
Se, total	mg/L	0.001	0.001	0.001	0.002	0.033	0.002	524
DO	mg/L	1.02	7.89	9.04	10.33	18.35	9.2	2,038
Total phosphorus	mg/L	0.004	0.02	0.02	0.03	2.36	0.023	971
NO_{2+3}	mg/L	0.01	0.071	0.1	0.3	30	0.133	971
Fecal	Counts/100 mL	0.5	64	200	581.5	180,000	189	1,955
pH	SU	6.07	7.33	7.64	7.96	10.07	7.6	2,075
Catchment area	km^2	0.17	2.88	9.1	38.2	3,912.2	12.2	958
Temperature	°C	0.08	15.9	19.5	22.3	31.9	18.8	2,074
RBP_Sc	RBP score	49	110	123	136	181	122.9	2,055
RBP_7Sc	Seven most relevant parameters	31	72	83	94	129	82.8	2,059
Embeddedness	RBP score	0	10	12	14	19	11.4	2,065
Percentage fines (sand + silt)	Percentage	0	10	20	25	100	20.19	2,033

[a]All means are geometric means except pH, DO, Temperature, and Habitat variables.
RBP = rapid bioassessment protocol (Barbour et al., 1999; RBP 10Sc has 10 parameters while RBP 7 does not include three flow-related parameters).

SC ranged from 40−11,646 µS/cm, which allowed the response of organisms to be modeled for a wide range of SC levels. This maximum is SC is three times higher than the background SC estimated for the data set analyzed in Case Study I (15−3,794 µS/cm). Scatter plots of parameters and SC are depicted in Appendix B.1.

5.1.1. Background Specific Conductivity

Background SC was estimated at the 25th centile from the probability-based samples from the example Criterion-data set because its sampling design more closely matched the ecoregional EPA-survey data set. Using this probability-based subsample of the WABbase data set, the estimated background for Ecoregion 70 was 147 µS/cm (681 samples from 617 sites; see Figure 5-2). Background was also estimated to be 201 µS/cm based on field data from 30 reference sites from the WABbase data set (75th centile; see Figure 5-3). By comparison, the 25th centile for all samples used to derive the example HC_{05} was estimated (166 µS/cm) (see Figure 5-4). The higher estimated background SC based on state-selected reference sites ($n = 30$)[3] reflects the importance of habitat in site selection and the smaller data set. Seasonal patterns of SC are evident in the probability-based samples and example Criterion-data set (see Figures 5-2 and 5-4). The apparent Background SC is <200 µS/cm December through June and >200 µS/cm July through October (no samples were available for November; see Figure 5-4). The effect of seasonal variability of SC on the subsequent analyses was further evaluated and presented in Appendix B. The large size of the data set and the wide range in SC levels in the example Criterion-data set allowed for genus XC_{95} to be calculated.

[3]29 of these sites have biological sampling available as described in Section 5.1.

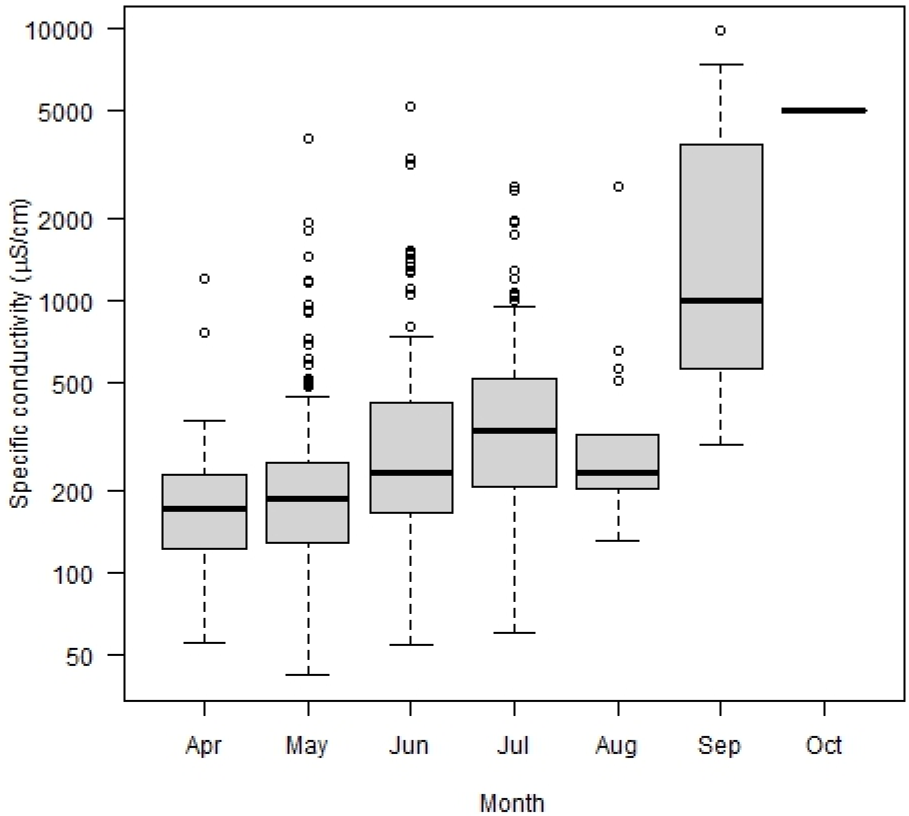

Figure 5-2. Box plot showing seasonal variation of specific conductivity (µS/cm) from probability sites from Watershed Assessment Branch database (WABbase) 1997−2010. This represents a total of 617 sites with 681 samples from 1997−2010 from Ecoregion 70 with pH >6. Note the difference in scale along the *y*-axis between Figure 5-2 (probability sites) compared to Figure 5-3 (reference sites). See Table 5-2 for sample sizes.

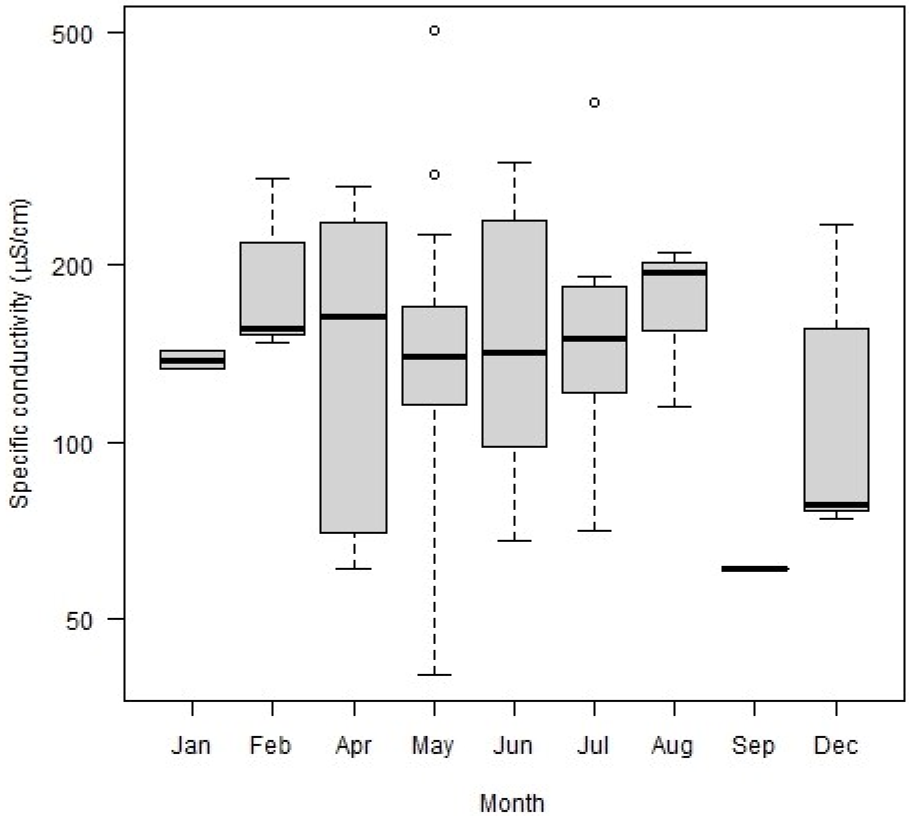

Figure 5-3. Box plot showing seasonal variation of specific conductivity (µS/cm) in the reference streams of Ecoregions 70 from 1997 to 2010. A total of 55 samples from 30 reference stations were used for this analysis. Please note the smaller scale on the *y*-axis compared to Figures 5-2 and 5-4. See Table 5-2 for sample sizes.

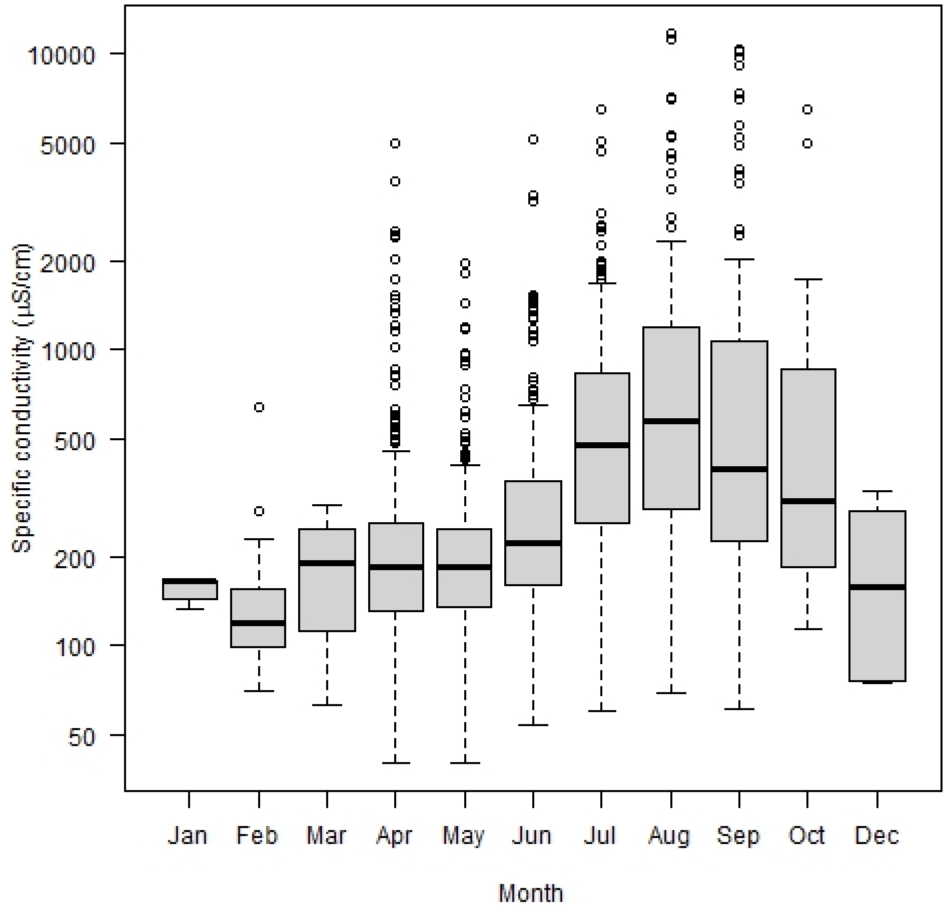

Figure 5-4. Box plot showing seasonal variation of specific conductivity (µS/cm) from all Ecoregion 70 sites from Watershed Assessment Branch database (WABbase) 1997−2010 used to develop the example criteria. The example Criterion-data set has 2,075 samples from 1,695 sites. Note the difference in scale along the *y*-axis between Figure 5-2 (all sites, reference and nonreference) and Figure 5-3 (reference sites). See Table 5-2 for sample sizes.

5.1.2. Ionic Composition

The ionic composition of the samples in the data set for Ecoregion 70 waters was assessed to ensure that the example criteria were derived for waters dominated by sulfate and bicarbonate anions (see Figure 5-5). Of the 2,082 samples after low pH samples were removed, 50.3% of samples (1,048 in total) included measures of calcium, magnesium, sulfate, bicarbonate, and chloride. All but three sites (>99.7%) were dominated by bicarbonate and sulfate anions, ($[HCO_3^-] + [SO_4^{2-}]) > [Cl^-]$. The three chloride-dominated sites were excluded from the derivation analysis but are shown in Figure 3. Sodium and potassium were less frequently measured, but did not exceed calcium and magnesium where measured. Sites with no ion measurements were retained in the data set because the data had shown that >99.7% of samples were dominated by bicarbonate and sulfate anions; thus, it is expected that less than 1% of samples in the Ecoregion 70 Criterion-data set are chloride-dominated. However, the analysis may also be defensible for ionic mixtures dominated by sodium, sulfate and bicarbonate ions, e.g., produced water from deep coal mines (Thomas, 2002; Mayhugh and Ziemkiewicz, 2005). This is because the toxicity of these mixtures are more similar to that of calcium, magnesium, sulfate and bicarbonate ions than the toxicity of NaCl (Mount et al., 2016; Kunz et al., 2013; Soucek and Dickinson, 2015).

5.1.3. Seasonal Specific Conductivity Regime

Chemical, physical, and/or biological samples were collected during the sampling years 1997−2010 (January−December). Most sites were sampled once during an annual sampling period, but some (e.g., sites being studied to improve stream condition within the TMDL Program) were sampled monthly for water quality parameters (see Table 5-2).

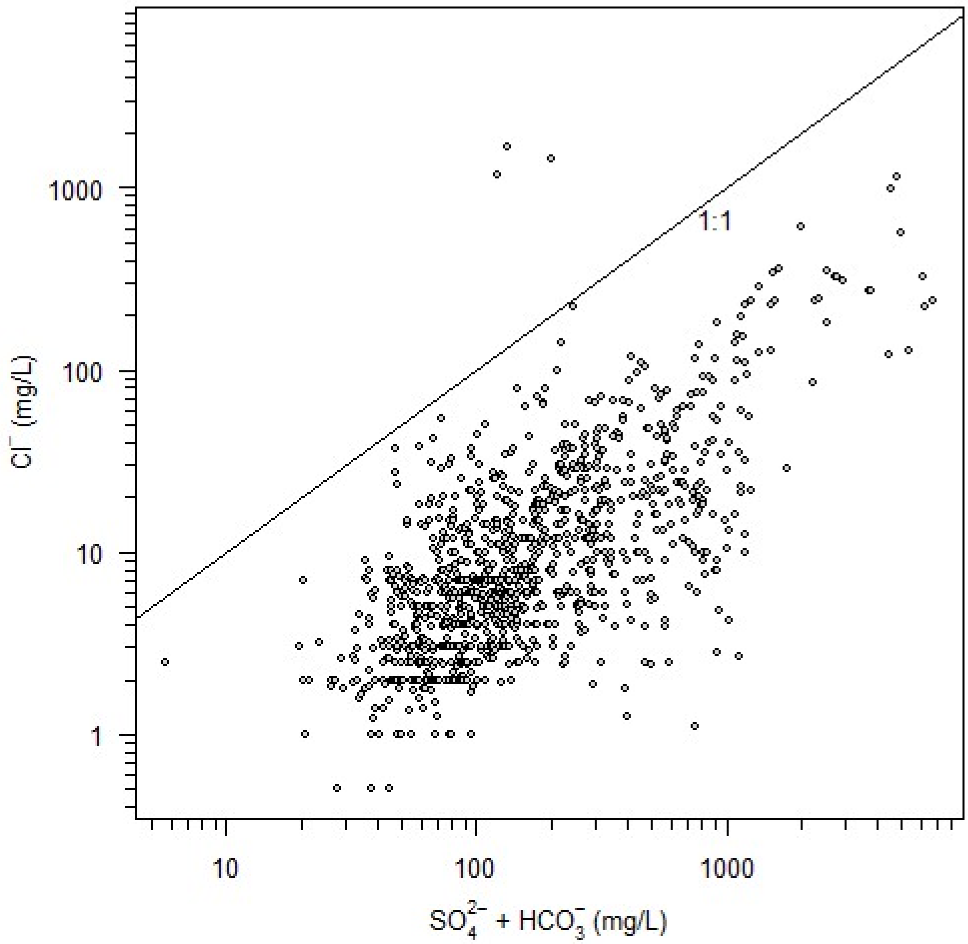

Figure 5-5. Scatter plot of relationship between [Cl^-] and ([HCO_3^-] + [SO_4^{2-}]) concentrations in streams of Ecoregion 70 data set. Most (99.7%) samples (*n* = 1,045) are below the diagonal line representing the separation between (HCO_3^- + SO_4^{2-})-dominated and Cl^--dominated mixtures. Sites above the 1:1 line were excluded from the example criterion derivation data set. The Ecoregion 70 data set includes all samples with (HCO_3^- + SO_4^{2-}), and Cl^- measurements. Samples depicted here include all sites regardless of pH.

Table 5-2. Number of samples with reported genera and specific conductivity meeting data-inclusion acceptance criteria for the Ecoregion 70 analysis

Number of samples[a]	Month												Total
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
Full data set	5	33	11	362	381	290	333	439	193	24	0	4	2,075
Probability sites	0	0	0	151	262	157	70	17	18	1	0	0	676
Reference sites	2	3	0	6	15	8	7	3	1	0	0	3	48
Percentage of total	0.2	1.6	0.5	17.4	18.4	14	16	21.1	9.3	1.2	0	0.2	100

[a]Number of samples is presented for each month.

Samples collected from the WABbase-identified reference sites indicate that SC levels are generally low and similar throughout the year, although slightly higher in summer/fall (see Figures 5-2 and 5-3). These data show that SC concentrations in flowing waters in the study area can vary somewhat by season, likely depending on stream discharge, rainfall, snowmelt, and other hydrological factors. As described in Section 3.1.4 (and in greater detail in U.S. EPA, 2011a), the effects of seasonal differences in SC levels and aquatic insect life history were evaluated by comparing HC_{05} values partitioned for season. After consideration of the similarity between the spring HC_{05} and the HC_{05} based on the full data set at the low end of the genus XCD, the example ecoregional criteria were derived using all available data, regardless of the time of year they were collected (see Section 3.1.4 and Appendix B.2 in this assessment and U.S. EPA, 2011a).

5.2. RESULTS

5.2.1. Extirpation Concentration (XC_{95}) and Hazardous Concentration (HC_{05}) Values (Example Criterion Continuous Concentration)

The Ecoregion 70 example Criterion-data set (see Table 5-1) was used to develop XC_{95} values from weighted CDFs. The histogram used to develop weights is depicted in Figure 5-6. The XC_{95} values that were used in the XCDs are listed in the order of least to most salt-tolerant in Appendix B.3. The GAM plots used to designate ~ and > values for those XC_{95} values are depicted in Appendix B.4, and the weighted CDFs used to derive the XC_{95} values used to assign the XC_{95} values are shown in Appendix B.5. The example HC_{05} was calculated at 338 µS/cm (see Figures 5-7 and 5-8); the two-tailed 95% confidence bounds were 272−365 µS/cm. Those bounds, derived by bootstrap resampling, indicate that different data sets could yield HC_{05} values within that interval. Rounding to two significant figures, the example CCC is 340 µS/cm.

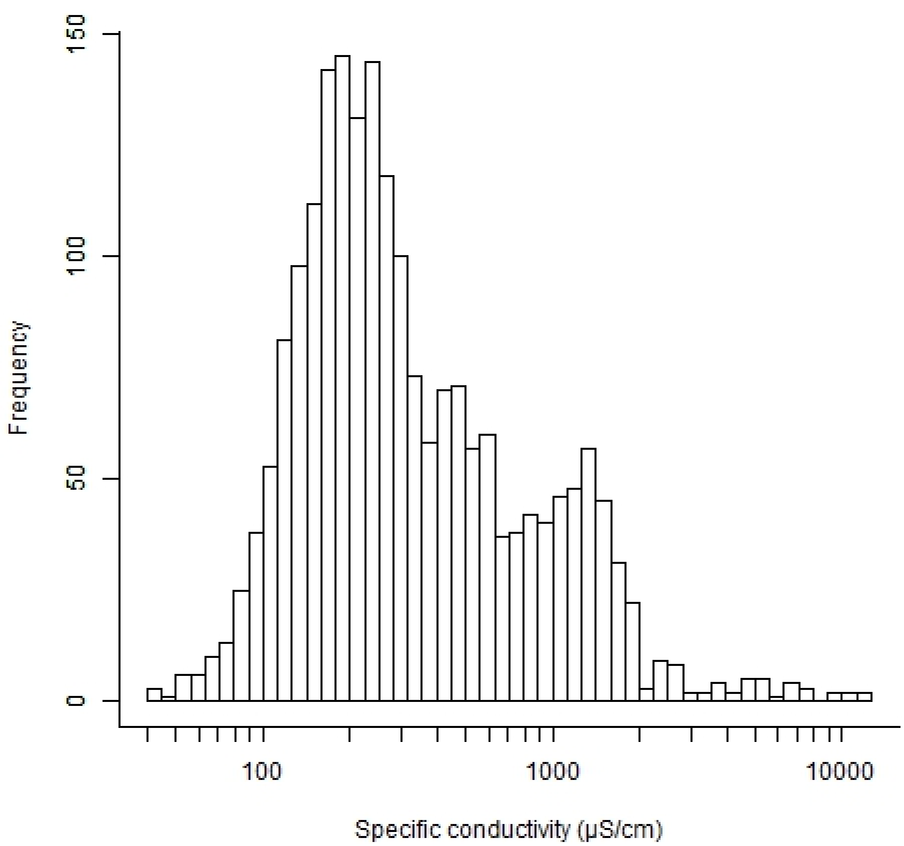

Figure 5-6. Histograms of the frequencies of observed specific conductivity values in samples from Ecoregion 70 sampled between 1997 and 2010. Bins are each 0.017 (1/60) of the range of log10 specific conductivity units wide.

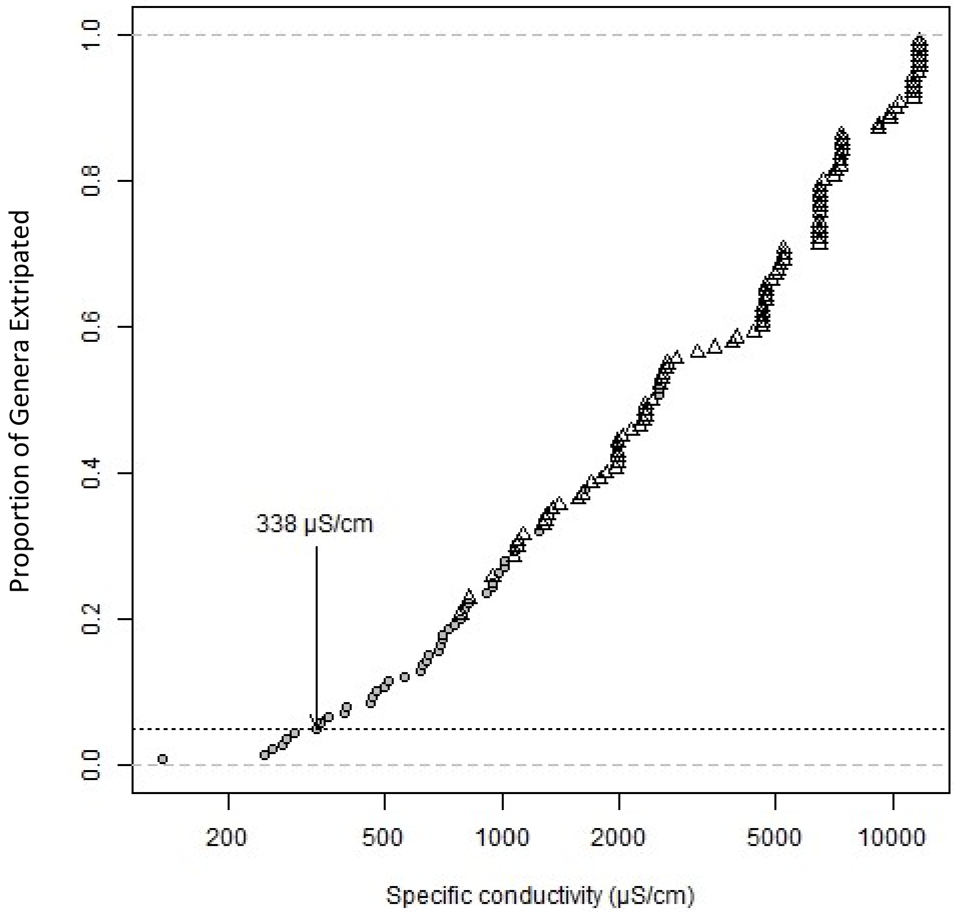

Figure 5-7. Example genus extirpation concentration distribution (XCD) for Ecoregion 70. Each point is an extirpation concentration (XC_{95}) value for a genus (*n* = 139 genera). The hazardous concentration (HC_{05}) is 338 µS/cm (95% confidence interval 272−365 µS/cm) and is the specific conductivity at the intersection of the genus XCD with the horizontal line at the 5th centile. XC_{95} values with an approximate or greater than designation are shown as triangles.

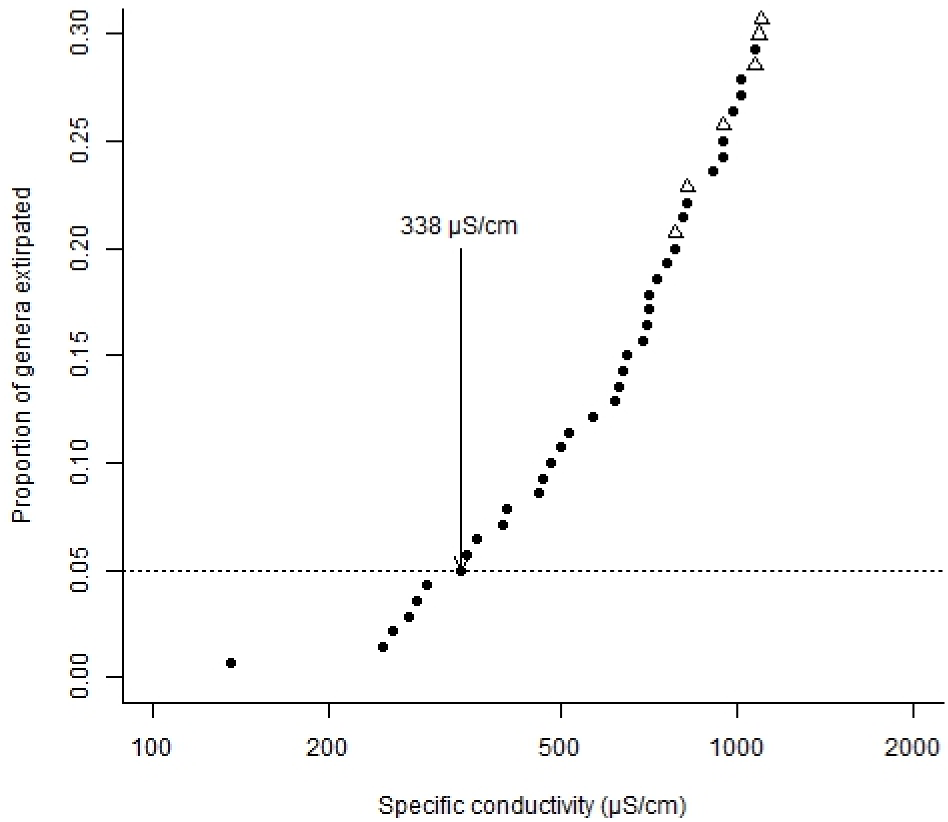

Figure 5-8. The lower end of the example genus extirpation concentration distribution for Ecoregion 70. The dotted horizontal line is the 5th centile. The vertical arrow indicates the hazardous concentration (HC_{05}) of 338 µS/cm (95% confidence interval 272−365 µS/cm). Only the 50 most salt-intolerant genera are shown to better discriminate the points on the left side of the distribution. The six most salt-intolerant genera (i.e., extirpation concentration [XC_{95}] ≤ 338 µS/cm) are *Drunella, Utaperla, Cinygmula, Alloperla, Ephemerella* and *Heptagenia.* XC_{95} values with an approximate or greater than designations are shown as triangles.

5.2.2. Example Criterion Maximum Exposure Concentration

At sites meeting the CCC of 340 µS/cm, 90% of the SC observations are estimated to occur below the CMEC (see Section 3.2). The CMEC was derived using the Ecoregion 70 data set. Out of the 12,909 samples collected between 1996−2011, 8,302 samples had a July-to-June rotating year representing 819 rotation years and 805 unique stations, with at least 1 sample from July to October and one from March to June, and at least 6 samples within a rotation year with

SC measurements (see Table 5-3). Note that inclusion of samples is not contingent on biological data. Reference and nonreference sites were included to ensure a range of SC (see Table 5-1).

Table 5-3. Summary data related to the calculation of the example criterion maximum exposure concentration (CMEC) for Ecoregion 70

Number of samples June to July prior to biological sampling	8,302
Number of rotation years (# unique stations)	819 (805)
Number of WVDEP reference sites	12
CCC	340 µS/cm
CMEC	680 µS/cm

Of the 819 rotation years (805 unique stations) with multiple SC measurements, the variability of within station SC was found to differ among streams (see Figure 5-9); however, the LOWESS line indicated that the average variability (residual standard deviation for a station) is not very different across the entire gradient in Ecoregion 70. The stations with annual mean SC between the 25^{th} and 75^{th} centile (120 and 520 µS/cm) were used to estimate the variance components of annual mean SC (at 340 µS/cm). The selected data sets with mean SC values between 120 and 520 µS/cm in respective data sets have a sample size of 518 rotation years (513 stations) and 5,272 observations. The grand mean and standard deviation of this data set were determined and the CMEC was calculated. The CMEC calculation is shown below:

$$\text{CMEC for Ecoregion 70: } 10^{\log 10(340) + 1.28 * 0.237} = 684\ \mu\text{S/cm} \qquad (5\text{-}1)$$

The example field-based calculated CMEC rounded to two significant figures yields a CMEC of 680 µS/cm for Ecoregion 70. At this level, where the annual average SC <340 µS/cm, 90% of the observations are expected to be less than the CMEC.

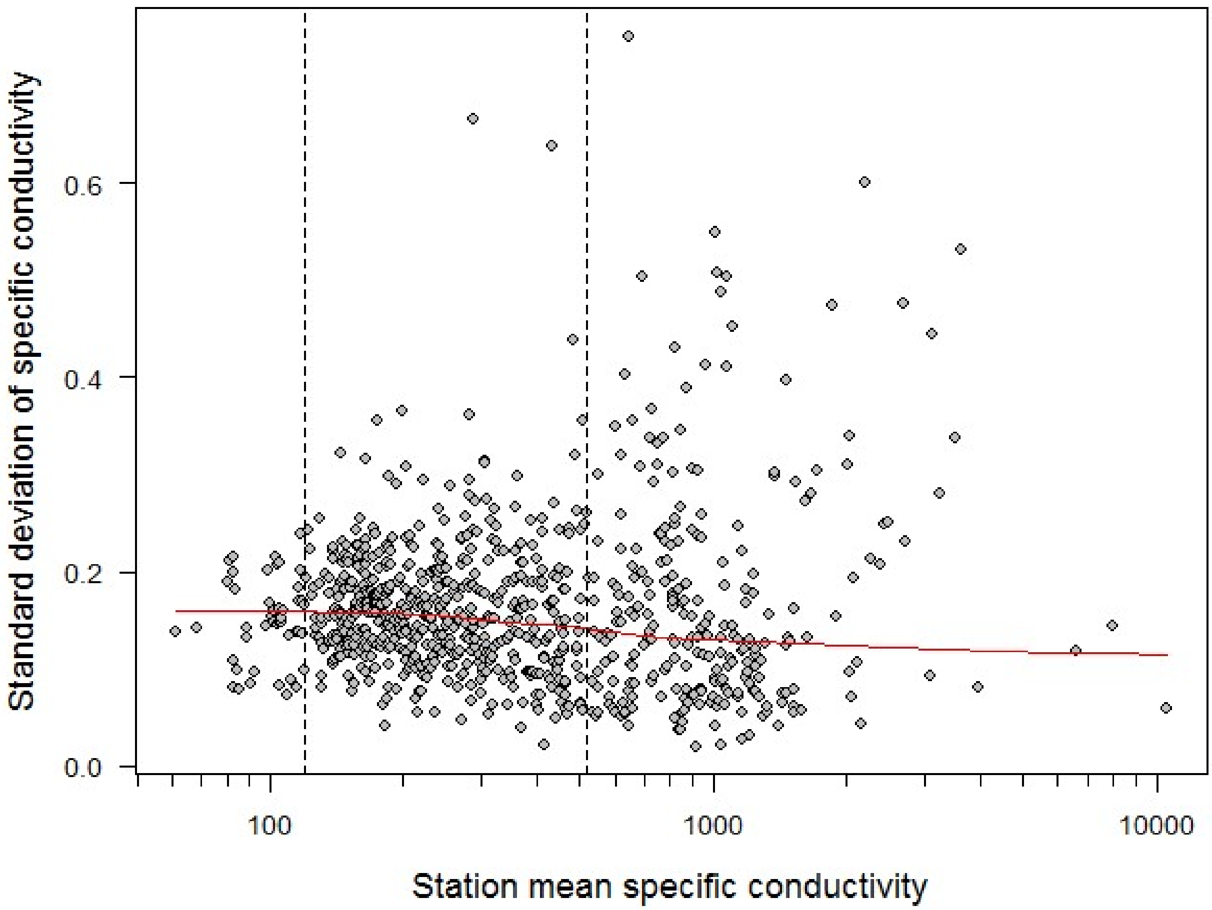

Figure 5-9. Illustration of within site variability (residual standard deviation for each station) along the specific conductivity gradient (station mean) in Ecoregion 70. The *x*-axis is log annual mean specific conductivity. Each dot represents a station. The fitted line is the locally weighted scatterplot smoothing (LOWESS, span = 0.75, linear polynomial model), while the two vertical dashed lines represent logarithm mean specific conductivity of 120 and 520 µS/cm respectively. Within those bounds, the standard deviation is fairly constant.

5.3. GEOGRAPHIC APPLICABILITY

The geographical applicability of the example criteria throughout Ecoregion 70 was assessed using the background-matching approach (see Section 3.7.1). The background SC of the new area (i.e., Ecoregion 70 beyond West Virginia) was estimated at the 25th centile (see Section 3.7.1.2; and Cormier and Suter, 2013a) and compared with the background estimates for Ecoregion 70 within West Virginia.

Because the example SC criteria presented here have been developed for a dissolved mixture dominated by sulfate and bicarbonate anions ($[HCO_3^-] + [SO_4^{2-}]) > [Cl^-]$ in mg/L), all chloride-dominated samples ($([HCO_3^-] + [SO_4^{2-}]) \leq [Cl^-]$ in mg/L) were removed from the data set before estimating background SC. Thereby, the background for the new area is estimated for the same ionic mixture as the example criteria.

5.3.1. Data Sources

Two data sets were used for this example applicability assessment: the original data set used to derive the HC_{05} described in Section 5.1 and an EPA-survey data set.

An EPA-survey data set was used to evaluate and characterize ion concentrations and water chemistry in the ecoregion. The primary sources of the combined data are from EPA survey programs: the NRSA 2008−2009 data set (U.S. EPA, 2013b), WSA 2004 data set (U.S. EPA, 2006), EMAP 1993−1998 data sets and R-EMAP 1999 data set (U.S. EPA, 2013c), and NAPAP data set collected in 1986 (NADP, 2013) (see Table 5-4). Data sets are based on single random samples from June through September. Most report SC, alkalinity, hardness, sulfate, chloride, bicarbonate, pH and other water quality parameters. Ecoregions and sampling sites are shown in Figure 5-10. All samples were collected from first-through fourth-order streams as part of a probability-based design intended to estimate proportions of parameters for various stream classes. The probability-design weights were not used in this characterization. Analysis of water chemistry samples followed EPA procedural and quality assurance/quality control protocols from EMAP (U.S. EPA, 2001, 1998b, 1994, 1987), Wadeable Streams Assessment (U.S. EPA, 2004a, b), NRSA (U.S. EPA, 2009), and NAPAP (Drousé et al., 1986; U.S. EPA, 1987). These data sets were also selected so that methods would be comparable across the data set and because these studies used probability-based designs (i.e., randomly assigned sampling locations).

Table 5-4. Description of survey data sets combined to form the EPA-survey data set used to assess applicability of example ecoregional criteria throughout Ecoregion 70

Data set	Sampling period	Total *N*	KY	OH	PA
MAHA EMAP	1993−1995	14	0	0	14
MAIA EMAP	1997−1998	10	0	0	10
WSA	2004	16	5	6	5
NRSA	2008−2009	14	4	6	4
NAPAP	1986	5	0	0	5
Region 4 Wadeable Streams R-EMAP	1999−2002	2	2	0	0
Total		**61**	**11**	**12**	**38**

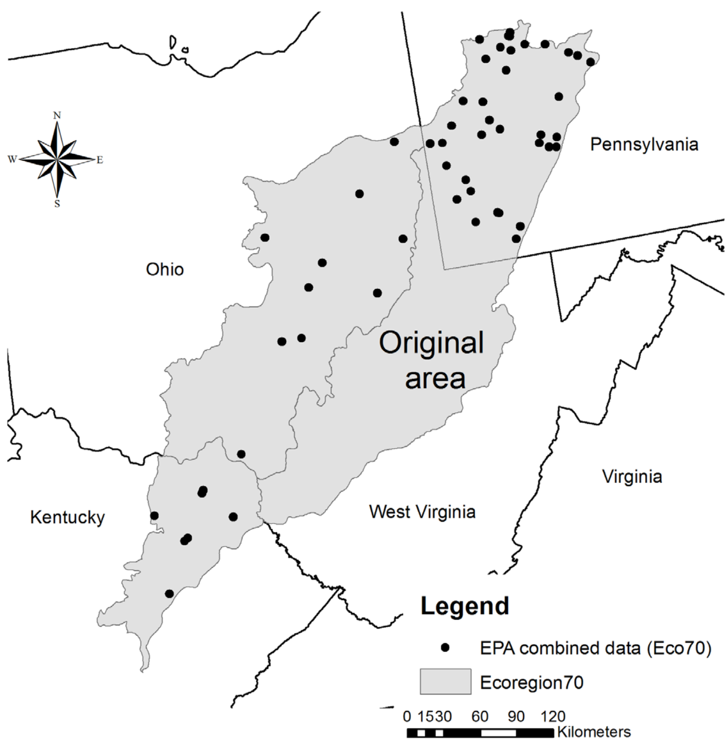

Figure 5-10. **Ecoregion 70 extends from southwestern Pennsylvania and southeastern Ohio into Kentucky.** Sampling sites in the EPA-survey data set that were used to estimate background in the "new" area for Ecoregion 70 are indicated as points. There are 61 samples from 61 stations.

5.3.2. Geographic Applicability Results

A summary of water quality for the ecoregion, including major ionic constituents, for the EPA-survey data set is listed in Table 5-5. Sites with $HCO_3^- + SO_4^{2-}$ concentrations on a mass basis greater than or equal to Cl^- were used to estimate background SC. This mixture is common in the ecoregion, and only one site was dominated by chloride anions in the EPA-survey data set and none in the example Criterion-data set. Therefore, this one site was excluded so the natural background was estimated from the altered EPA-survey data set.

Background SC for bicarbonate and sulfate dominated waters estimated as the 25th centile of the EPA-survey data set for the area in Ecoregion 70 outside the area used to develop the example criteria was 197 µS/cm (95% CI 145−272 µS/cm) (see Table 5-6). The 25th centile from the probability sample from the example Criterion-data set was 147 µS/cm (95% CI 136−159) (see Table 5-6). The confidence bounds for background estimated from the example Criterion-data set overlap with the confidence bounds for background estimated for the rest of the ecoregion. Therefore, the background SC regime throughout Ecoregion 70 appears to be similar, and the example criteria (CCC = 340 µS/cm, CMEC = 680 µS/cm) are considered geographically applicable throughout the ecoregion. Other estimates of background from the reference sites in the example Criterion-data set (201 µS/cm; 95% CI 164−210 µS/cm) and the example Criterion data set (169 µS/cm; 95% CI 161−171 µS/cm) also overlap with the CI of the background for the rest of the ecoregion. As a validation of background specifically for the portion of Ecoregion 70 in Ohio, a weight of evidence was performed (see Appendix C).

Table 5-5. Summary of water quality parameters for Ecoregion 70 EPA-survey data set

Ion or specific conductivity	Min	Centile				Max	[a]*N*
		10th	25th	50th	75th		
HCO_3^- (mg/L)	0.0	1.8	18.1	52.2	121.0	241.8	42
SO_4^{2-} (mg/L)	11.5	17.2	24.1	52.6	144.7	955.8	58
Cl^- (mg/L)	1.0	3.8	6.0	9.6	26.3	204.5	58
Ca^{2+} (mg/L)	4.9	10.5	19.0	47.8	69.3	240.8	58
Mg^{2+} (mg/L)	1.8	3.5	6.2	12.5	22.7	87.7	58
Na^+ (mg/L)	1.0	3.1	4.3	9.1	22.8	161.2	58
K^+ (mg/L)	1.0	1.6	1.9	2.4	3.0	9.6	58
pH (SU)	4.0	6.3	7.3	7.7	8.1	8.6	60
[b]$(HCO_3^- + SO_4^{2-})/Cl^-$	1.7	3.7	8.0	10.6	22.8	103.8	42
SC (µS/cm)	66.7	108	197	398	631	1,860	60

[a]Relevant *N* indicates the number of samples from the large data set relevant to each water quality parameter.
[b]Value within category calculated from individual sample ion concentrations. $HCO_3^- + SO_4^{2-}/Cl^-$ in mg/L greater than 1 indicates the mixture is dominated by $HCO_3^- + SO_4^{2-}$. One site dominated by Cl^- was removed from the data set.

Table 5-6. Background specific conductivity estimates for Ecoregion 70

Data set	Centile used to estimate background	Estimated background µS/cm	Confidence interval µS/cm	Relevant *N* (stations/ samples)
EPA-survey data set from geographic area in Ecoregion 70 not represented in the example criterion derivation data set	25th	197	135−240	60/61
WABbase data set, probability sample subset	25th	147	136−159	617/681
WABbase data set, reference sample subset	75th	201	164−210	30/55
Ecoregion 70 example criterion derivation data set, full data set	25th	169	161−171	1,695/2,075

5.4. SUMMARY OF EXAMPLE CRITERIA FOR ECOREGION 70

The case example for Ecoregion 70 includes an annual geometric mean (i.e., CCC) and a 1-day mean (i.e., CMEC), not to be exceeded more than once in 3 years on average. Both of these distinct expressions of the example SC criteria would need to be met in order to adequately protect aquatic life. These values indicate that freshwater animals would be protected if the annual geometric mean SC concentration does not exceed 340 µS/cm and the 1-day mean does not exceed 680 µS/cm, more than once every 3 years on average. These example criteria would apply to all flowing freshwaters (ephemeral, intermittent, and perennial streams) in Ecoregion 70 inclusive of portions of Kentucky, West Virginia, Pennsylvania, and Ohio. On a site-by-site basis, these example ecoregional criteria apply if the ionic mixture is dominated by anions of bicarbonate and sulfate. For streams crossing into Ecoregion 70, professional judgment may be needed to assess the potential effect of different ionic composition or concentration. Professional judgment is recommended when applying to sites with a catchment area greater than 1,000 km^2 (386 mi^2) owing to lesser representation in the example data set by this class of stream. On a site-by-site basis, alternative SC criteria may be more appropriate if the natural background of a site is shown to be lower or higher than its regional background specific conductivity.

5.5. EXAMPLE CRITERION CHARACTERIZATION

5.5.1. Factors Potentially Affecting the Extirpation Concentration Distribution (XCD) Model

An assessment of potential confounders and an analysis of the influence of habitat quality and sampling date for Ecoregion 70 can be found in Appendix B.2.

5.5.1.1. *Sensitivity Analyses*

As the minimum number of occurrences of a genus for inclusion in the data set increases, fewer genera are included in the XCD. The HC_{05} increases greatly when a taxon in the lower 5^{th} centile is removed because it does not meet the minimum number of samples and then more slowly alternates between increasing and decreasing as genera either above or below the 5^{th} centile are removed because they do not meet the minimum number of samples (see Figure 5-11). The pattern repeats until all genera above and below the lower 5^{th} centile have the same XC_{95} value (not shown). To maximize the number of genera included in the XCD, a minimum of 25 occurrences was utilized.

The number of samples in the data set affected the number of genera included in the XCD and the resulting example HC_{05}. The effects of data set size on the HC_{05} estimates and on their confidence bounds were estimated using a bootstrapping technique. The mean of all bootstrapped HC_{05} values, the numbers of genera used for the HC_{05} calculation, and their 95% CI were plotted to show the effect of sample sizes. As shown in Figure 5-12, the HC_{05} for this data set stabilizes, reaching an asymptote at approximately 500−800 sites sampled and 90−100 genera evaluated. Therefore, the original data set was considered adequate for estimating the example CCC.

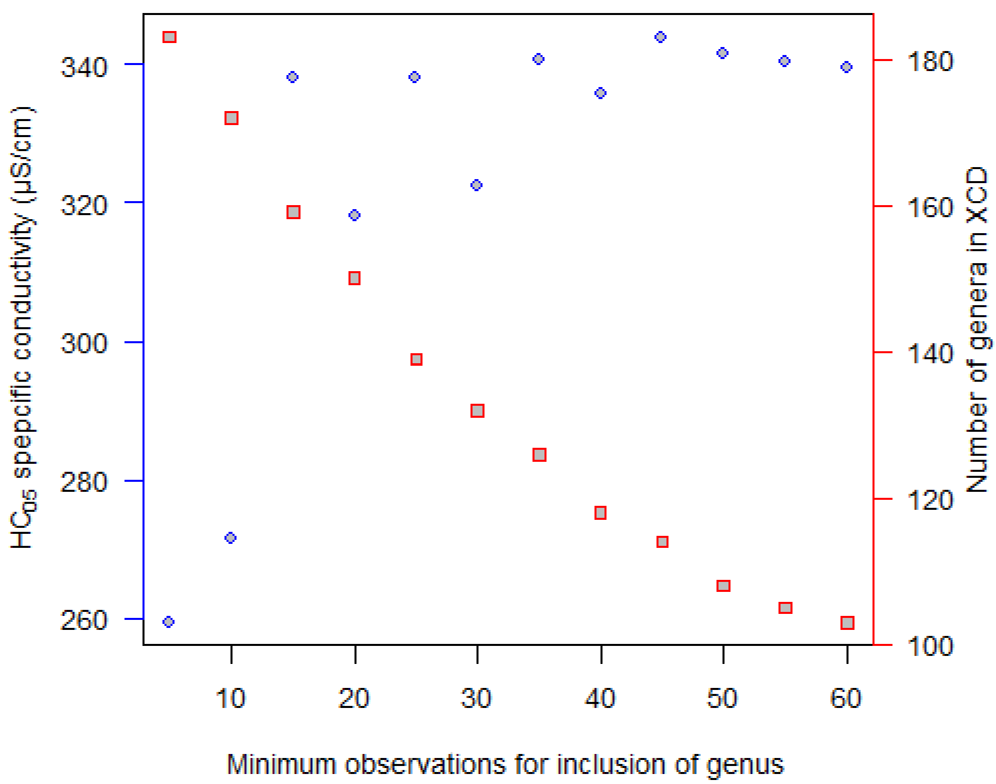

Figure 5-11. Relationship of the number of occurrences of a genus on the hazardous concentration (HC_{05}) based on Ecoregion 70 example Criterion-data set. Estimates of HC_{05} values (blue diamonds, left axis) and the number of taxa in the extirpation concentration distribution (XCD) (red squares, right *y*-axis) based on minimum number of samples (5−60, *x*-axis). As the minimum number of occurrences of a genus increases, fewer are included in the XCD.

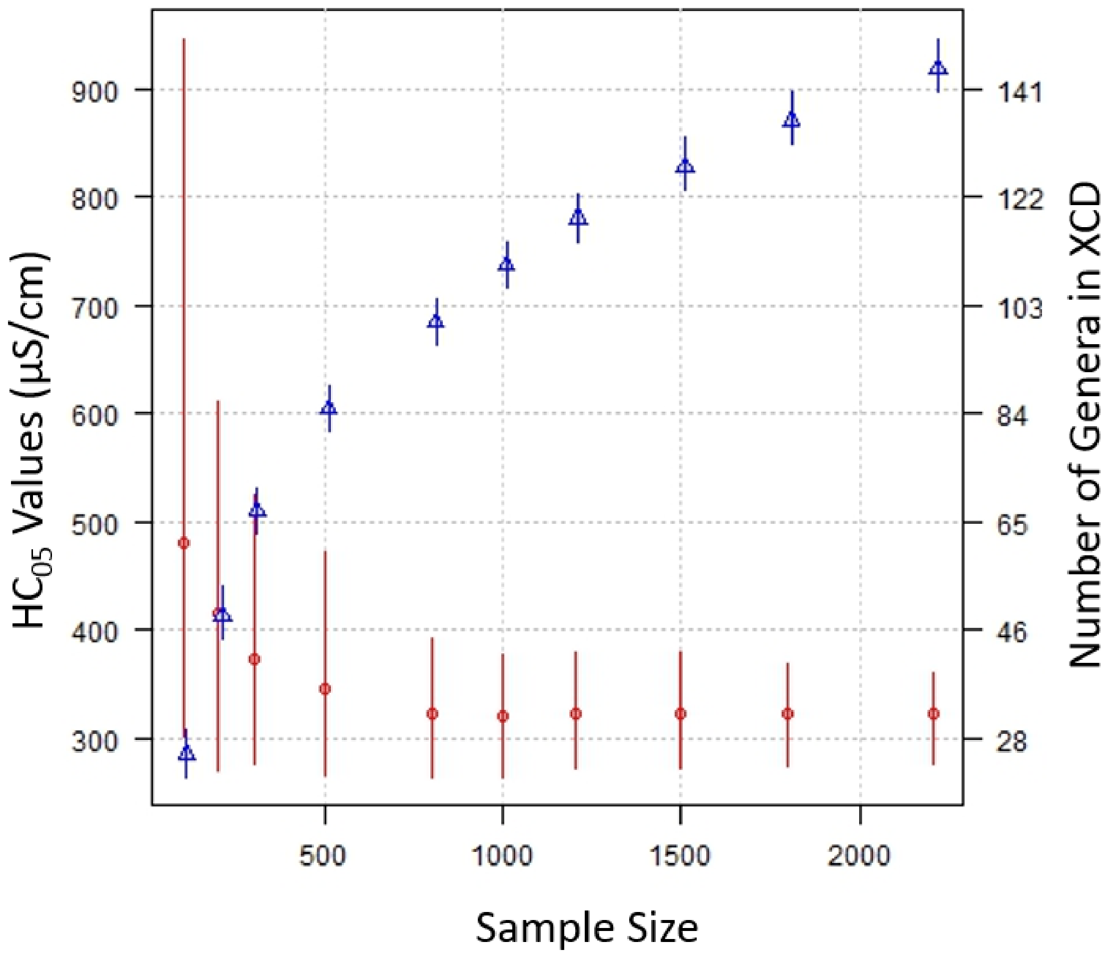

Figure 5-12. **Adequacy of the size of the data set used to model the hazardous concentration (HC_{05}) based on the Ecoregion 70 example Criterion-data set.** As size of the data set increases, the number of genera included in the genus extirpation concentration distribution (XCD) increases (triangles). The HC_{05} stabilizes, reaching an asymptote at approximately 500−800 sites sampled (circles) and 90−100 evaluated genera.

5.5.2. Validation of the Model

As recommended by the SAB (U.S. EPA, 2011c), the XCD model was validated and uncertainty around the HC_{05} values was estimated using bootstrapping. The median HC_{05} estimated from bootstrapping was 323 µS/cm (95% CI 272−365 µS/cm) which is similar to the HC_{05} of 338 µS/cm measured using a two-point interpolation from the original XCD. The similarity between the two HC_{05} values indicates a similar model would be generated using an independent data set (see Figure 5-13).

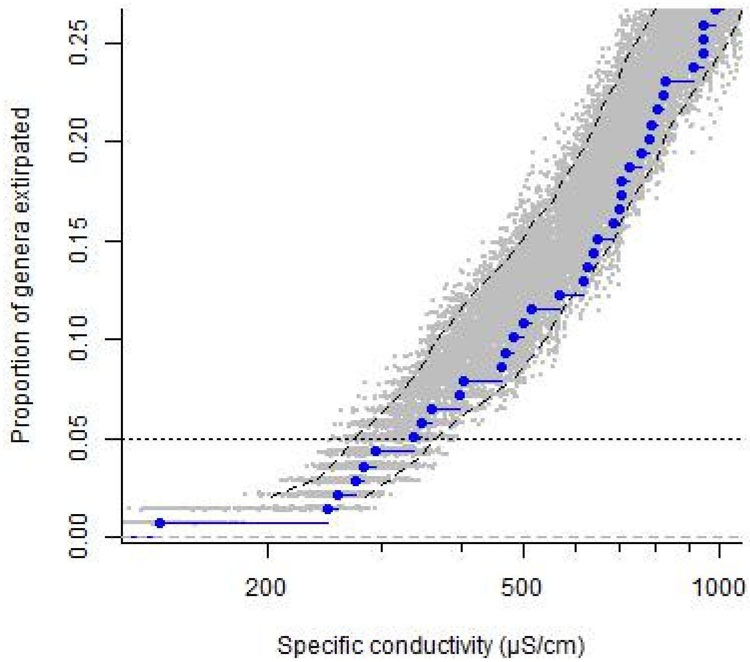

Figure 5-13. 95% confidence intervals (hatched oblique lines) for the lower portion of the Ecoregion 70 genus extirpation concentration distribution (XCD). Each tiny gray dot represents an extirpation concentration (XC_{95}) value from one of 1,000 XCD bootstrapping iterations (note that the genera and their order varies with each XCD-iteration). Each of the 36 blue filled dots represents the calculated XC_{95} of the XCD for the example criterion continuous concentration (CCC). Hazardous concentration (HC_{05}) based on the bootstrap medians is 323 µS/cm.

5.5.3. Duration and Frequency

Numeric criteria include magnitude (i.e., how much), duration (i.e., how long), and frequency (i.e., how often) components. Appropriate duration and frequency components of criteria are determined based on consideration of available data and understanding the exposure-response relationship in the context of protecting the aquatic life of a water body. The significant consideration used in setting the duration component of aquatic life criteria is how long the exposure concentration can be above the criteria without affecting the endpoint on which the criteria are based (U.S. EPA, 1985, 1991). Based on the temporal resolution of the available field data set and an analysis of within-site variability of SC levels, EPA developed two different expressions for the example SC criteria in order to provide adequate protection for aquatic life.

In this case, the majority (>81%) of sites used to derive the example CCC for Ecoregion 70 were sampled once during an annual sampling period and thus represent the average stream chemistry (SC) and macroinvertebrate assemblage information over the course of 1 year. As a result, the appropriate duration for the CCC is 1 year. The duration for the CMEC, a level of protection from acutely toxic exposures, is 1 day based on a review of the literature on the onset of macroinvertebrate drift in response to elevated SC (see Section 3.3). At sites meeting the CCC, 90% of the SC observations are estimated to occur below the CMEC.

EPA anticipates that an appropriate allowable frequency of exceedance for these example criteria is no more than once in 3 years, based on recovery rates from literature reviews and consideration of the life history of insects able to recolonize a site via drift or aerial dispersal (see Section 3.4). Recovery is expected to occur in 3 years if the following conditions are met: (1) the SC regime returns to a yearly average below the CCC, (2) there are nearby streams with low SC supporting a diverse community, and (3) there is an upstream source of colonizers or the flight or recolonizing distance is within the dispersal range of genetically diverse, reproducing adult colonizers. If any of these conditions are not met, the time necessary for ecosystem recovery (and thus, the allowable frequency of exceedance) would likely be longer than 3 years.

5.6. PROTECTION OF FEDERALLY-LISTED SPECIES

Although the derivation of the example criteria was limited to the macroinvertebrate taxa represented in the data sets, the available evidence indicates that other taxa in the streams would likely be protected as well (see Section 2.6 and Appendix G). Hence, no adjustment was made for unanalyzed taxa. However, on a site-specific basis, the example criterion could be adjusted or recalculated to protect important species, highly valued aquatic communities, or specially protected waters.

6. CASE STUDY III: EXAMPLE USING THE BACKGROUND TO CRITERION (B-C) REGRESSION METHOD

This section presents an example calculation of an ecoregional CCC using the B-C method (see Section 3.7.2 and Appendix D). In this example, a CCC for the Northwestern Great Plains, Level III Ecoregion 43, was calculated using SC data from the ecoregion and the B-C method because there were insufficient paired SC and biological data to use the XCD method in this ecoregion.

First, the water chemistry data set was screened for ionic composition to ensure samples were dominated by sulfate and bicarbonate anions, and sampled sites were mapped to determine whether the geographic distribution of sites was representative of (dispersed throughout) the ecoregion. Minimally affected background SC of the new ecoregion was estimated at the 25^{th} centile of probability samples (see Section 3.7.1.2; Cormier and Suter, 2013a). Least disturbed background SC was estimated at the 25^{th} centile of a combined data set of targeted and probability samples. The CCC was calculated using the least disturbed 25^{th} centile background SC as the independent variable (x) in the B-C regression model to yield an HC_{05} (y). Depending on available data and analytical results (see Figure 3-11), an HC_{05} may take the form of (1) the y-value at the mean of the regression line from the B-C mode, (2) the y-value at the lower 50% PL of the regression line, or (3) an HC_{05} derived from a data set based on ≥200 paired SC and biological samples. In this example case for Ecoregion 43, there were <200 paired SC and biological samples, so the lower 50% PL was used to develop the example CCC.

6.1. DATA SET CHARACTERISTICS

The Northwest Great Plains is mostly an unglaciated, semiarid, rolling plain with rolling hills and occasional buttes and badlands (Woods et al., 2002). Elevation ranges from 458 to 1,200 meters (McNab and Avers, 1994). The area covers approximately 347,000 km^2 and extends from southeastern Montana and northeastern Wyoming into western parts of North Dakota and South Dakota. Ecoregion 43 is bordered by the Northwestern Glaciated Plains to the north and east, the Middle Rockies and Wyoming Basin to the west, the Eastern High Plains and Nebraska Sand Hills to the south. An outcropping of the Middle Rockies occurs in the south of the ecoregion. The shallow soil is underlain with shale, siltstone, and sandstone. Where there is sandstone, aquifers can produce groundwater. Otherwise, there are few perennial rivers, and the rainfall is erratic with approximately 250−510 ml/year. The low precipitation and high evapotranspiration lead to less groundwater recharge and baseflow contributing to streams; therefore, many small streams are intermittent or ephemeral. Grazing and ranching is a common land use with some dryland and irrigated agriculture. Surface coal mining and oil and gas production also occur. The often alkali-rich soils in the steppes are dominated by sagebrush; whereas, the buttes are more moist and can support forests.

Only existing data were used for this example assessment (see Table 6-1). An EPA-survey data set and a U.S. Geological Survey (USGS) data set were combined to characterize ion concentrations and water chemistry and then used to calculate a provisional CCC. The USGS data set was also used to calculate the CMEC. The statistical package R, Version 2.12.1 (December 2010), was used for all statistical analyses (R Development Core Team, 2011).

Table 6-1. EPA and U.S. Geological Survey (USGS) chemistry data sets included in this study.
Years indicate the period during which the samples were collected. Western Environmental Monitoring and Assessment Program (EMAP) survey sites are included in the count of sites from the National Wadeable Streams Assessment (NWSA).

Survey	Years	# of sites	# of samples
EPA probability samples			
EMAP and Regional EMAP	1993−2003	12	12
NWSA	2000−2004	53	53
NRSA	2008−2009	53	53
USGS mixed sampling			
USGS: full data set	1946−2008	281	45,489
USGS: subset ≥ six samples per rotation year, July−June	1946−2008	148	41,648

This B-C regression model was developed using biological data paired with SC data from 24 data sets with waters having ionic mixtures dominated by calcium, magnesium, sulfate and bicarbonate ions and where background SC did not exceed 626 µS/cm. Therefore, the model is most appropriate for waters with similar ionic characteristics. The model has not been thoroughly tested with waters dominated by other mixtures, i.e., ($[SO_4^{2-}] + [HCO_3^-]) < [Cl^-]$, and ($[Ca^{2+}] + [Mg^{2+}]) < ([Na^+] + [K^+])$ in mg/L. In particular, the B-C model is not appropriate for waters dominated by NaCl (Haluszczak et al., 2013, Entrekin et al., 2011; Gregory et al., 2011; Veil et al., 2004) or road salt (Forman and Alexander, 1998; Kelly et al., 2008; Environment Canada and Health Canada, 2001; Evans and Frick, 2001; Kaushal et al., 2005). However, the model and this analysis may be defensible for ionic mixtures dominated by sodium, sulfate and bicarbonate ions (Brinck et al., 2008; Dahm et al., 2011; Jackson and Reddy, 2007; National Research Council, 2010; Clark et al., 2001; Veil et al., 2004). This is because the toxicity of these mixtures are more similar to that of calcium, magnesium, sulfate and bicarbonate ions than the toxicity of NaCl (Mount et al., 2016; Kunz et al., 2013; Soucek and Dickinson, 2015).

In this example case study, more than half of the sampled sites were dominated by sulfate, bicarbonate, sodium, and potassium ions, ($[SO_4^{2-}] + [HCO_3^-]) > [Cl^-]$, and ($[Ca^{2+}] + [Mg^{2+}]) < ([Na^+] + [K^+])$ in mg/L. No samples were excluded based on cations. All samples in the EPA-survey data set were used because no samples were dominated by chloride ions. A USGS data set of 281 sites was used to verify ionic composition. Of 7,461 samples, 7,456 (>99.9%) were dominated by sulfate plus bicarbonate. The five samples not dominated by sulfate and bicarbonate occurred at sites sampled multiple times that more often than not were dominated by sulfate and bicarbonate, so these sites were retained. All sites in the combined EPA-USGS data set had pH data, but none were <6 nor >9.8, so no sites were removed from the data set.

6.1.1. EPA-Survey Data Set

Data sources, sampling period, and number of samples used to estimate background SC in the new area (Ecoregion 43) are provided in Table 6-1. The NRSA 2008−2009 data set (U.S. EPA, 2013b), WSA 2004 data set (U.S. EPA, 2006), EMAP 1993−1998 data sets and R-EMAP 1999 data sets (U.S. EPA, 2013c), are based on single random (i.e., probability-based design) samples from June through September.

EPA-survey data sampling sites within the ecoregion are shown in Figure 6-1. Water quality parameters collected in Ecoregion 43 are included in Table 6-2. Most of the samples have reported SC, alkalinity, hardness, sulfate, chloride, bicarbonate, and pH, as well as other water quality parameters. When necessary, ionic concentrations in milliequivalents (meq/L) were converted to mg/L [(meq/L) × (ion MW)/(ionic charge)] (Hem, 1985).

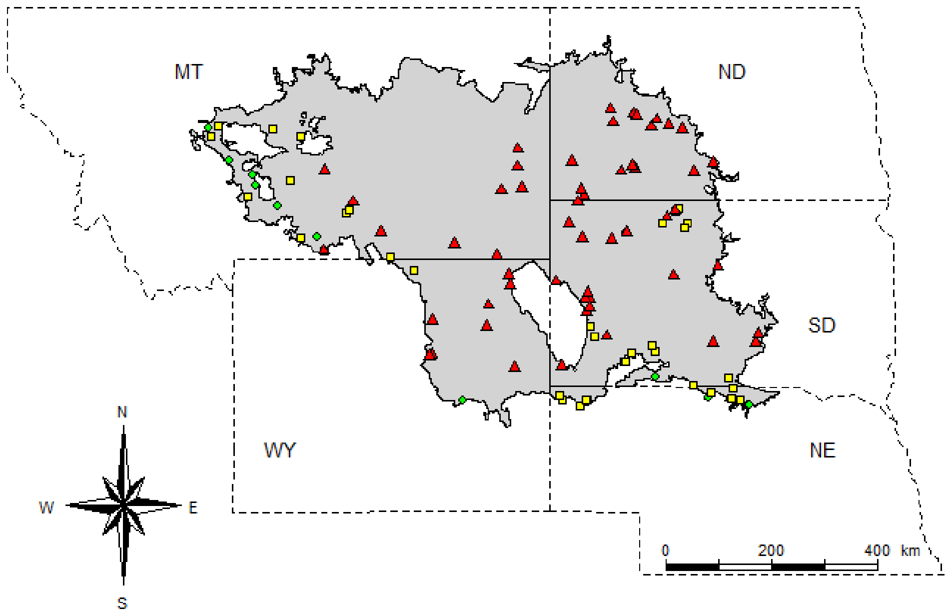

Figure 6-1. Sampling sites in the EPA-survey data set that were used to estimate minimally affected background in Ecoregion 43. For the purpose of demonstrating the background-to-criterion approach, Level III Ecoregion 43 which encompasses portions of Montana, North and South Dakota, Wyoming, and Nebraska is shaded gray. A total of 115 sampling sites is depicted. Sampling locations are color-coded by site-specific conductivity range: green diamonds <300 µS/cm, yellow squares 300−1,000 µS/cm, and red triangles ≥1,000 µS/cm. Geodetic reference system = North American Datum (NAD83).

Table 6-2. Summary of data for the example case for Northwestern Great Plains, Level III Ecoregion 43 from EPA-survey samples.
Geometric means were calculated except for pH and ion ratio.

Parameter	*N*	Minimum	25th	50th	Mean	75th	Maximum
SC (µS/cm)	118	57	483	1,257	1,041	2,406	5,769
pH (SU)	118	6.70	8.06	8.27	8.30	8.47	9.88
Ca^{2+} (mg/L)	111	3.62	37.6	53.5	62.6	139	511
Mg^{2+}(mg/L)	111	0.65	9.34	33.0	26.8	82.1	240
Na^{+} (mg/L)	106	1.23	23.7	162.97	99.2	390	1,059
K^{+} (mg/L)	106	0.44	4.16	7.95	6.73	12.6	80.1
HCO_3^{-} (mg/L)	53	45.8	205	277	286	429	987
SO_4^{2-} (mg/L)	106	3.33	52.4	367	214	1,074	2,750
Cl^{-} (mg/L)	106	0.20	3.80	8.50	8.63	18.9	520
[a] $HCO_3^{-} + SO_4^{2-}/Cl^{-}$	53	3.26	35.2	73.3	99.4	129	464

[a]Ratio of mg/L $HCO_3^{-} + SO_4^{2-}/Cl^{-}$ greater than 1 indicates the mixture is dominated by $HCO_3^{-} + SO_4^{2-}$.

All samples were collected from first-through fourth-order streams as part of a probability-based design intended to estimate proportions of parameters for various stream classes. The probability-design sampling weights for stream order were not used in the characterization. Analysis of water chemistry samples followed procedural and QA/QC protocols of EPA and EMAP (U.S. EPA, 2001, 1998b, 1994, 1987), Wadeable Streams Assessment (U.S. EPA 2006, 2004a, b), NRSA (U.S. EPA, 2009), and NAPAP (Drousé et al., 1986; U.S. EPA, 1987).

6.1.2. U.S. Geological Survey (USGS) Data Set

The USGS survey data set used in this example is composed of 45,489 water quality samples, from 281 stations within Ecoregion 43 (see Table 6-1; Figure 6-2). Some stations were sampled only once while others were sampled as many as 5,445 times. The data were collected between 1946 and 2015 during all seasons. Water quality parameters collected in Ecoregion 43 are included in Table 6-3. Most of the samples have reported SC, alkalinity, hardness, sulfate, chloride, bicarbonate, and pH, as well as other water quality parameters. Analysis of water chemistry samples followed procedural and QA/QC protocols for USGS data sets (Mueller et al. 1997).

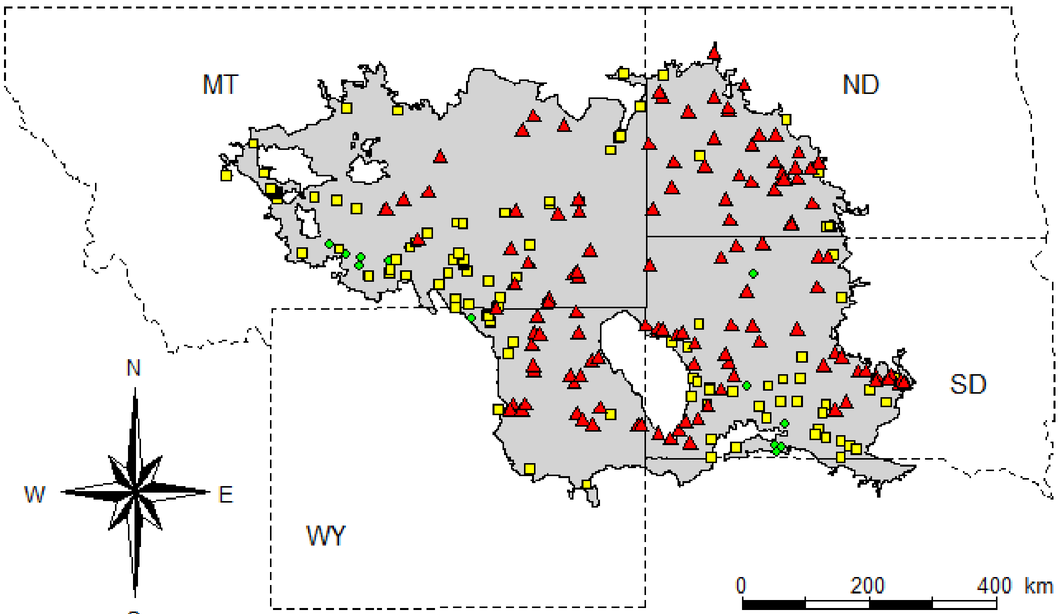

Figure 6-2. Sampling sites in the U.S. Geological Survey (USGS) data set in Ecoregion 43. Ecoregion 43 is shaded gray. Geometric mean specific conductivity at sampling locations is color-coded: green diamonds <300 µS/cm, yellow squares 300−1,000 µS/cm, and red triangles ≥1,000 µS/cm. Geodetic reference system = North American Datum (NAD83).

Table 6-3. Summary of data for the example case for Northwestern Great, Level III Ecoregion 43 from U.S. Geological Survey (USGS).
Geometric means were calculated for all variables except for pH and ion ratio.

Parameter	*N*	Minimum	25th	50th	Mean	75th	Maximum
SC (µS/cm)	281	85	564	1,045	986	1,816	7,330
pH (SU)	170	7.30	8.1	8.2	8.2	8.3	9.0
Ca^{2+} (mg/L)	118	21.87	50.9	69.0	76.4	114.9	464
Mg^{2+} (mg/L)	118	5.81	28.4	46.6	45.0	75.5	654
Hardness (mg/L)	52	121.1	290	479.7	432.4	608.1	1,040
Na^{+} (mg/L)	118	1.55	59.4	180.3	132.5	325	1,186
K^{+} (mg/L)	110	0.74	5.1	9.2	8.1	13.3	25.1
HCO_3^{-} (mg/L)	92	2.00	224.4	278.5	204.2	405	765
SO_4^{2-}(mg/L)	120	4.56	189.5	464.2	362.4	808.3	2,283
Cl^{-} (mg/L)	117	0.97	6.9	10.7	14.7	27.6	938
[a]$HCO_3^{-} + SO_4^{2-}/Cl^{-}$	86	2.35	22.9	77.6	82.6	116	363

[a]ratio of mg/L $HCO_3^{-} + SO_4^{2-}/Cl^{-}$ greater than 1 indicates the mixture is dominated by $HCO_3^{-} + SO_4^{2-}$.

All samples were collected from first-through fourth-order streams for various research purposes and thus the targeted sampling design may emphasize areas with increased anthropogenic disturbance or some geologic formations. In this respect, the data may skew the background SC estimates. Background SC was not estimated from this data set because sampling stations were not randomly assigned. However, after weighing the potential bias that could be introduced with the benefits of having greater coverage across the ecoregion, the EPA-survey and USGS data sets were combined and used to estimate background SC.

Because the USGS data set contained multiple measurements in an annual rotation from sampling locations, the data set was used to estimate a CMEC and to explore the variability of SC patterns within the region. Therefore, a second data set was selected by excluding stations with fewer than six SC measurements throughout the year. A minimum of one sample during the first 6 months and one in the last 6 months of the previous year were also required so that at least one low and high SC sample was included in the data set. The second USGS data set that included 41,648 samples from 168 stations was used to calculate the variance near the CCC for the CMEC calculation.

6.1.3. Modeled Mean Baseflow Background Specific Conductivity (SC)

Predicted mean natural base-flow SC in catchments of Northwestern Great Plains, Ecoregion 43, was also considered for comparison purposes (Olson and Hawkins, 2012). The stream length weighted, mean natural SC (each SC was multiplied by the proportion of stream segment length) at base flow for each ecoregion (see Appendix D). Figure 6-3 shows the predicted SC at 300m resolution in order to emphasize the general trends across Ecoregion 43.

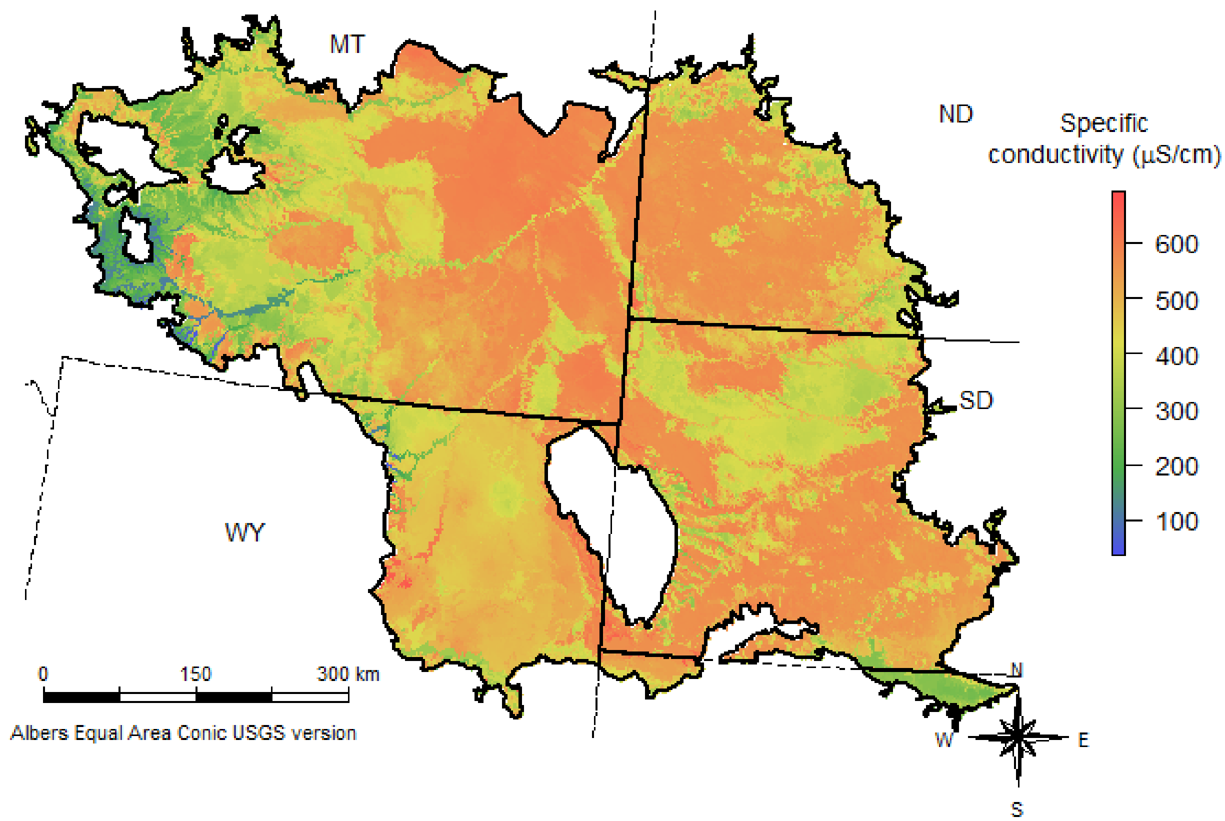

Figure 6-3. Predicted mean natural base-flow specific conductivity in catchments of Northwestern Great Plains, Ecoregion 43, using the Olson-Hawkins model. Albers projection used for mapping.

6.1.4. Characterization of Ionic Matrices

6.1.4.1. *EPA-Survey Data Set Ionic Characteristics*

A summary of water quality ionic constituents including major ionic constituents for Ecoregion 43 is provided in Table 6-2. Centiles were calculated using each sample observation because most measurements were single grab samples. There were no chloride-dominated sites where ($[Cl^-] \geq [HCO_3^-]$) + $[SO_4^{2-}]$ in the EPA-survey data ($N = 118$); therefore, no sites were excluded from the data sets. Sodium was the dominant cation at more than half the sampled sites. This mixture was judged as acceptable for use with the B-C model, but with less confidence than a calcium and magnesium dominated mixture. Ionic characteristics for Ecoregion 43 are shown for the EPA-survey in Table 6-2 and the USGS data set in Table 6-3.

6.1.4.2. *U.S. Geological Survey (USGS) Data Set Ionic Characteristics*

Table 6-3 summarizes the water quality parameters including major ionic constituents for Ecoregion 43 in the USGS data set. Unlike the EPA-survey data set, the USGS data set contains targeted sites of interest rather than probability samples. Also, in some cases, there are multiple measurements from the same site, and other sites are autocorrelated with downstream sampling locations. Therefore, the distribution of sites in this data set is not necessarily representative of Ecoregion 43 in its entirety; however, the data set can be used to define the overall pattern of SC for the ecoregion because it contains samples in areas not represented in the EPA-survey data set. Centiles were calculated using the geometric mean of site measurements (except pH and the ionic ratio) and were qualitatively compared to the probability-based EPA-survey data set.

Two samples with pH <6 and a few observations (less than 10) with some ion concentrations recorded as 0 were removed from the USGS data set. Only 5 out of 45,489 samples collected in this data set were dominated on some days by chloride, $[Cl^-] \geq ([HCO_3^-] + [SO_4^{2-}])$ in mg/L; however, on average, these 5 sites were not dominated by chloride ions, so those samples were not removed. Sodium was the dominant cation in more than half of the samples. This mixture was judged as acceptable for use with the B-C model, but with less confidence than a calcium and magnesium dominated mixture.

6.1.5. Comparison of Background Specific Conductivity (SC) Estimates

In this case example, the stream length weighted average predicted mean base flow SC from the Olson-Hawkin's model in Ecoregion 43 is 489 µS/cm. Raw values for predicted mean flow for stream segments are shown as box plot in Figure 6-4. The 25th centile of the EPA-survey data set is 483 µS/cm. The USGS SC data set has a slightly narrower overall range and mid-range of values resulting in a slightly higher quartile SC than the EPA survey data set

(564 µS/cm) (see Figure 6-4 and Table 6-3). When the USGS data set is combined with the EPA-survey data set, the 25th centile (542 µS/cm) is also greater than the predicted mean baseflow of 489 µS/cm (see Table 6-4, Figure 6-4). Therefore, this background SC estimated from the combined EPA-USGS data set is least disturbed.

The 25th centile of the combined EPA-survey and USGS data set was used to calculate the HC_{05} following the decision tree described Section 3.7.2 and Figure 3-11. Because the lower quartile ranges from 85 to 564 µS/cm, additional analysis is recommended for streams known to have low SC regimes.

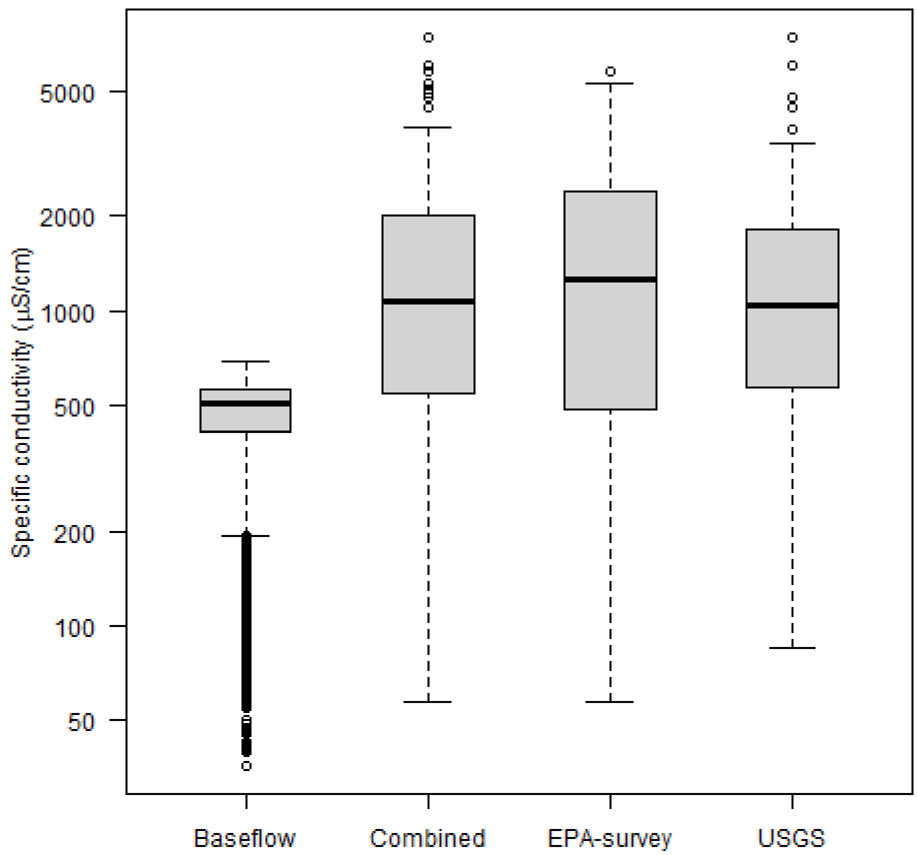

Figure 6-4. Box plots of specific conductivity (SC) distributions for EPA-survey, U.S. Geological Survey (USGS), combined EPA-survey and USGS data sets and predicted mean base-flow. The USGS data set captures a slightly narrower midrange of values possibly owing to the targeted sampling and the mean values rather than the single measurements in the EPA sample. The 25th centile of the combined and USGS data set is greater than the mean predicted base-flow. The mean baseflow model predicts many outliers for the region <200 µS/cm.

Table 6-4. Summary of data for the example case for Northwestern Great Plains, Level III Ecoregion 43 from the combined EPA-survey and U.S. Geological Survey (USGS) data set.
Geometric means for sampled sites were calculated for all variables except for pH and ion ratio.

Parameter	*N*	Minimum	25th	50th	Mean	75th	Maximum
SC (µS/cm)	399	57	542	1,074	1,002	2,006	7,330
pH (SU)	288	6.7	8.1	8.2	8.3	8.4	9.9
Ca^{2+} (mg/L)	229	3.6	46.2	63.0	69.4	122.5	511.2
Mg^{2+} (mg/L)	229	0.65	20.0	43.7	35.0	77.6	654.4
Hardness (mg/L)	163	11.7	184.5	377.8	325.0	642.5	1,828.1
Na^{+} (mg/L)	224	1.2	49.1	173.7	115.5	337.7	1,185.6
K^{+} (mg/L)	216	0.44	4.5	8.7	7.4	12.9	80.1
HCO_3^{-} (mg/L)	145	2.0	212.9	277.8	231.0	410.8	986.7
SO_4^{2-}(mg/L)	226	3.33	129.5	452.9	283.0	940.8	2,750.7
Cl^{-} (mg/L)	223	0.20	5.3	9.9	11.4	20.6	937.8
[a]$HCO_3^{-} + SO_4^{2-}/Cl^{-}$	139	2.35[b]	33.6[b]	76.25[b]	89[b]	123.0[b]	464[b]

[a]Ratio of mg/L $HCO_3^{-} + SO_4^{2-}/Cl^{-}$ greater than 1 indicates the mixture is dominated by $HCO_3^{-} + SO_4^{2-}$.

6.1.6. Calculation of Ecoregion 43 Criterion Continuous Concentration (CCC) from Background

Because available paired SC and biological data represented <200 sites, the HC_{05} was estimated at the lower 50% PL using the B-C model (see Figure 6-5). The 25th centile of SC from the EPA-survey data set of Ecoregion 43 was used to identify the lower 50% PL using eqs 3-4 and 3-5. The B-C model development is described in Appendix D. The *x*-variable is the background SC in Ecoregion 43 which was log10 transformed. The calculated *y*-value is the predicted mean log10 HC_{05}. In this example case, the least disturbed background is 542 µS/cm (see Table 6-4). It was estimated at the 25th centile from the combined EPA-survey and USGS data set to improve representation of samples from the entire ecoregion. The calculation of the predicted mean log10 of HC_{05} (*y*) is shown in eqs 6-1 and 6-2 and that value is used to estimate the lower 50% PL using eq 6-3.

The B-C model is described by the following formula:

$$Y = 0.657X + 1.075 \quad (6\text{-}1)$$

Where:

X is the log10 of the ecoregional background SC

Y is the log10 of the predicted HC_{05}

The background for Ecoregion 43 (542 μS/cm) is converted to log10, replacing X in the formula with that value and Y is computed (see eq 6.2). The predicted value Y is converted from log10 to a number that is the modeled HC_{05} for that region. In Ecoregion 43 the mean modeled HC_{05} is 740 μS/cm after rounding to two significant figures.

$$\text{Log Predicted } HC_{05} = (0.657 \times 2.73) + 1.075 = 2.87\ \mu S/cm \quad (6\text{-}2)$$

Then

$$\text{Predicted } HC_{05} = 10^{2.87}\ \mu S/cm = 743\ \mu S/cm$$

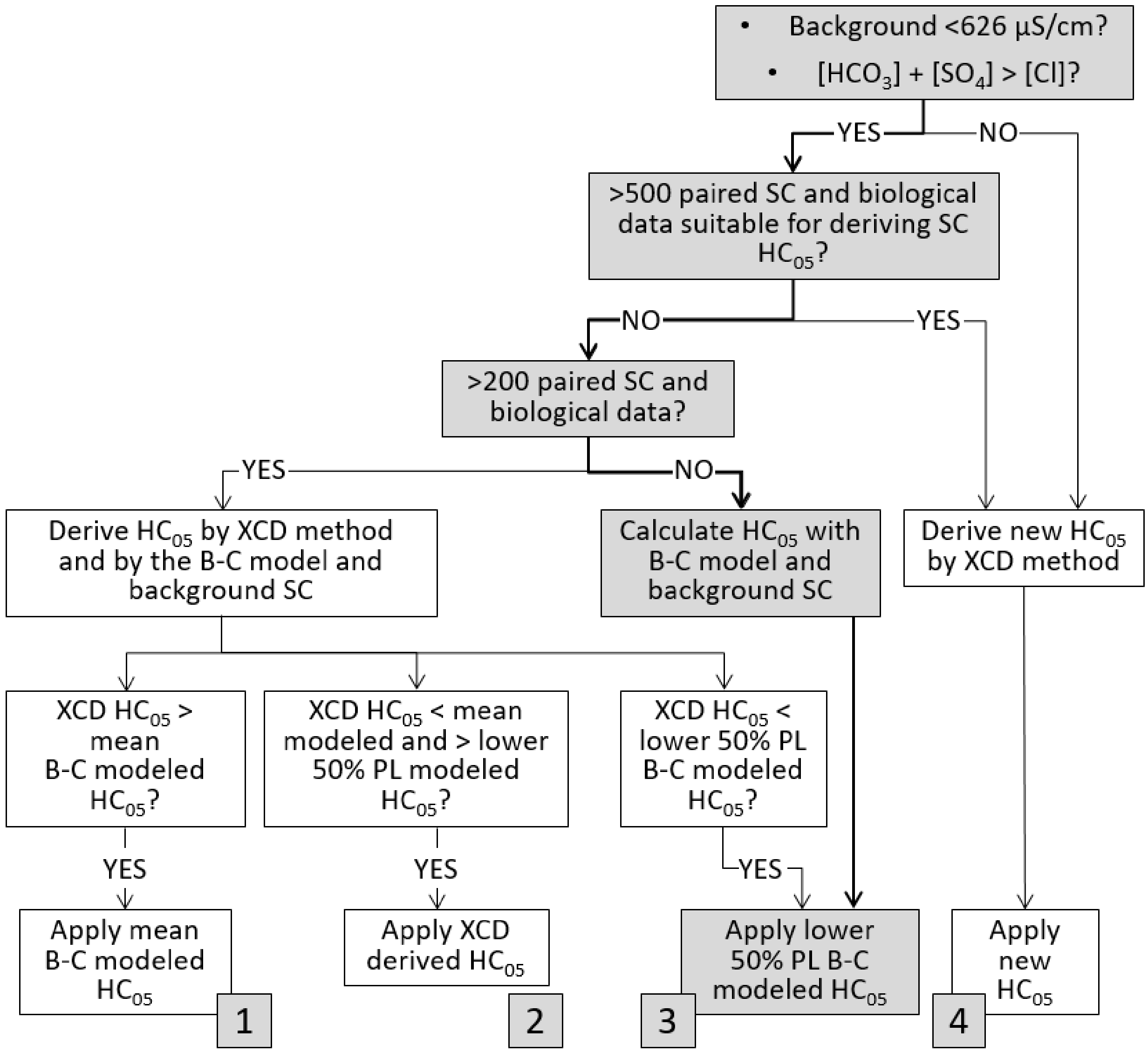

Figure 6-5. Process and decision path case example for Ecoregion 43. Decision path is highlighted in gray and connected by bold lines. Because there was no previously derived hazardous concentration at the 5th centile (HC_{05}) of a taxonomic extirpation concentration distribution (XCD) for Ecoregion 43, and the background was <626 µS/cm, and there were <200 paired specific conductivity (SC) measurements, the HC_{05} was calculated with the background-to-criterion (B-C) model at the lower 50% prediction limit (PL).

6.1.7. Formula for Calculating the Lower 50% Prediction Limit

Because the available paired SC and biological data constitute <200 sites, the HC_{05} was estimated at the lower 50% PL (see Figure 6-5). The 25th centile of background SC and the predicted mean HC_{05} of a region and variance of the B-C model is used to calculate the lower

50% PL (see eq 6-3). In this case example for Ecoregion 43, the background is 542 µS/cm and the mean modeled HC_{05} is 743 µS/cm. Both values are converted to log10 (x, y) as shown in eq 6-4. The prediction interval from the regression line for a mean predicted value $\hat{\bar{y}}$ can be calculated as follows or more conveniently using statistical software thus avoiding rounding errors:

$$\hat{\bar{y}} \pm t_{\alpha/2,n-2} S_y \sqrt{1 + \frac{1}{n} + \frac{(x^{\circ} - \bar{x})^2}{SS}} = \log 10 \text{ PL} \tag{6-3}$$

Symbol	Explanation	Example from the B-C model
$\hat{\bar{y}}$	Log10 of mean predicted HC_{05}	2.87 µS/cm, log10 of 743 µS/cm
n	Number of samples in the model	$n = 24$
α	Alpha error rate for prediction interval (desired confidence level)	50% prediction interval (α = 0.5)
t_{n-2}	*t*-value at specific level (alpha, α) and degrees of freedom ($n - 2$) of interval	For 50% prediction interval (α = 0.5), $t_{(1-0.5)/2,24-2} = 0.686$
S_y	Residual standard error of prediction (standard deviation)	$S_y = 0.11$
SS	Sum of square of x deviation from their mean, SS = $\sum_{i=1}^{n}(x_i - \bar{x})^2$	$SS = 4.21$
$\bar{x}$	Mean x values used in the model generation	$\bar{x} = 2.15$
x°	x value for a new prediction interval	Log10 542 µS/cm = 2.73
PL	Upper and lower prediction limits of mean predicted $\hat{\bar{y}}$	calculated in eq 6-4

Using x° = log10 (542) µS/cm and the mean predicted HC_{05} for Ecoregion 43 value, ($\hat{\bar{y}}$ = 2.87, the log10 of 743 µS/cm) the lower 50% PL is calculated as follows in eq 6-4. Note, the upper 50% PL is not calculated but is included in the formula because it may be used to estimate a CMEC where there are insufficient data to calculate a CMEC using the method described in Section 3.2.

$$log10\ (743)\ \pm 0.686 \times\ 0.11\sqrt{1+\frac{1}{24}+\frac{(log_{10}(542)-2.15)^2}{4.21}} \quad (6\text{-}4)$$

So:

$$2.87 - 0.686\ \times 0.11\sqrt{\frac{25}{24}+\frac{(2.73-2.15)^2}{4.21}}$$

$$2.87 - 0.686\ \times 0.11 \times 1.06$$

$$10^{2.79} = 617\ \mu S/cm$$

The log of the lower calculated 50% PL is 2.79 which equals 617 µS/cm after back transformation. The lower 50% PL rounded to two significant figures yields a CCC of 620 µS/cm.

6.1.8. Example Criterion Maximum Exposure Concentration

At sites meeting the CCC of 620 µS/cm, 90% of the SC observations are estimated to occur below the CMEC (see Section 3.2). The CMEC was derived using the USGS data set (45,489 samples collected between 1946−2015). This data set was used because it contained multiple measures of SC within a year whereas the EPA survey data set consisted of single measurements at each site. Of the 45,489 samples in this ecoregion, there are 41,648 samples in a July-to-June rotating year representing 1,241 rotation years, 148 unique stations, with at least 1 sample from July to October and one from March to June and at least 6 samples within a rotation year. Note that inclusion of samples is not contingent on biological data.

Of the 1,241 rotation years (148 unique stations) with multiple SC measurements, the variability of within station SC slightly differed for streams with different mean SC (see Figure 6-6). However, the LOWESS indicated that the average variability (residual standard deviation for a station) was relatively stable (see Figure 6-6); therefore, the entire data set was used to estimate the standard deviation components of annual mean SC (620 µS/cm). The grand mean and standard deviation of this data set was determined, and the CMEC was calculated. The example calculation of the CMEC for Ecoregion 43 is shown below using eq 3-2 from Section 3.2:

$$10^{\log10(620) + 1.28*0.333} = 1{,}656\ \mu S/cm \quad (6\text{-}5)$$

The example CMEC (see Table 6-5) rounded to two significant figures yields a CMEC of 1,700 µS/cm for Ecoregion 43. If this level is not exceeded, where the annual geometric mean SC <620 µS/cm, 90% of the observations are expected to be less than the CMEC.

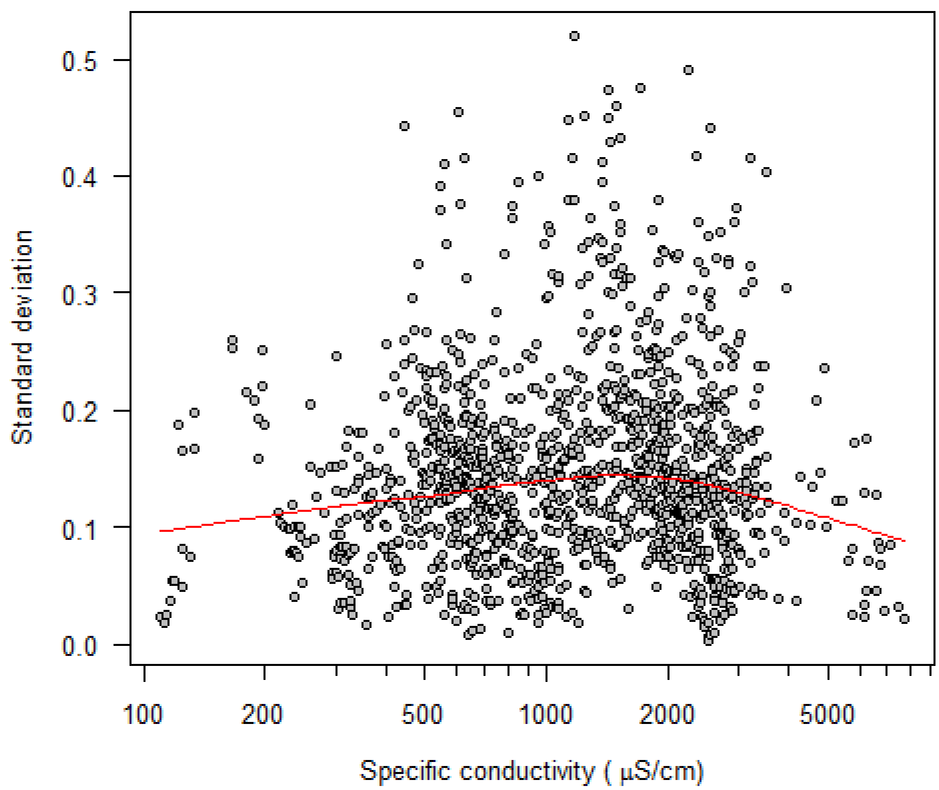

Figure 6-6. Illustration of within site variability (residual standard deviation for each station) along the specific conductivity gradient (station mean) in Ecoregion 43. The *x*-axis is annual mean specific conductivity. Each dot represents a station. The fitted line is a locally weighted scatterplot smoothing (LOWESS, span = 0.75, linear polynomial model).

Table 6-5. Summary data related to the calculation of the example criterion maximum exposure concentration (CMEC) for Ecoregion 43

Number of samples July to June prior to biological sampling	41,648
Number of unique stations/rotation years	148/1,241
CCC	620 µS/cm
CMEC	1,700 µS/cm

6.2. EXAMPLE CRITERION CHARACTERIZATION FOR ECOREGION 43 BASED ON A BACKGROUND-TO-CRITERION MODEL

The case example for Ecoregion 43 includes an annual geometric mean (i.e., CCC) and a 1-day mean (i.e., CMEC), not to be exceeded more than once in 3 years on average. Both of these distinct expressions of the example SC criteria would need to be met in order to adequately protect aquatic life. These values indicate that freshwater animals are protected if the annual geometric mean SC concentration in flowing waters does not exceed 620 µS/cm and the 1-day mean does not exceed 1,700 µS/cm more than once every 3 years on average. These example criteria would apply to all flowing freshwaters (ephemeral, intermittent, and perennial streams) in Ecoregion 43 inclusive of portions of Montana, Wyoming, South Dakota, North Dakota, and Nebraska. On a site-by-site basis, these example ecoregional criteria apply if the ionic mixture is dominated by anions of bicarbonate and sulfate and either sodium or calcium cations. For streams crossing into Ecoregion 43 from ecoregions with either lower or higher background SC, professional judgment may be needed to assess the potential effect of different ionic composition or concentration. Professional judgment is recommended when applying to sites with a catchment area greater than 1,000 km^2 (386 mi^2) owing to lesser representation in the data set by this class of stream in the development of the B-C model. On a site by site basis, alternative SC criteria may be more appropriate if the natural background of a site is shown to be lower or higher than its regional background SC.

In particular, some streams in Ecoregion 43 may have consistently low SC throughout the year (Keya Paha Tablelands [43i], Niobara River Breaks [43r], Noncalcareous Foothill Grasslands [43s], Shield-Smith Valleys [43t], Limy Foothill Grassland [43u], and Pryor-Big Horn Foothills [43v]). Because all or most of the sampled sites in these Level IV ecoregions were measured at less than 500 µS/cm, a finer resolution (subecoregional) analysis is recommended to adequately protect these areas of Ecoregion 43. Specifically, because the more western parts of the ecoregion provide sources of freshwater and dilution to the rest of the ecoregion and ecoregions to the east, subdividing the ecoregion according to background SC and developing different SC criteria may help to protect regional water quality where geophysical and climatic conditions lead to higher natural SC regimes.

The weight-of-evidence method described in Appendix C could be used to evaluate subregions or stream classes that may have different background SC in this large ecoregion where natural background SC may range from <100 µS/cm to >1,000 µS/cm. For example, higher criteria may be appropriate for areas such as the Little Missouri Badlands (43b).

6.3. PROTECTION OF FEDERALLY-LISTED SPECIES AND OTHER HIGHLY VALUED TAXA

Although the example criteria were derived using the macroinvertebrate taxa represented in the data sets, the available evidence indicates that other taxa in the streams would likely be protected as well (see Section 2.6 and Appendix G). Hence, no adjustment was made for unanalyzed taxa. However, on a site-specific basis, the example criterion could be adjusted or recalculated to protect important species, highly valued aquatic communities, or specially protected waters.

7. CASE STUDY IV: EXAMPLE USING THE BACKGROUND TO CRITERION (B-C) REGRESSION METHOD FOR A REGION WITH LOW CONDUCTIVITY

This section presents an example calculation of an ecoregional CCC using the B-C method (see Section 3.7.2 and Appendix D). In this example, a CCC for the Cascades Level III Ecoregion 4 was calculated using SC data from the ecoregion and the B-C method because there were insufficient paired SC and biological data to accurately estimate the XC_{95} values for the XCD method in this ecoregion.

First, the water chemistry data set was screened for ionic composition to ensure samples were dominated by sulfate and bicarbonate anions and calcium and magnesium cations. Sampled sites were mapped to determine whether the geographic distribution of sites was representative of (dispersed throughout) the ecoregion. Minimally affected background SC of the new ecoregion was estimated at the 25th centile of probability samples (see Section 3.7.1.2; Cormier and Suter, 2013a). Least disturbed background SC was estimated at the 25th centile of a combined data set of targeted and probability samples. The CCC was calculated using the 25th centile least disturbed background SC as the independent variable (x) in the B-C regression model to yield an HC_{05} (y). Depending on available data and analytical results (see Figure 3-11), an HC_{05} may take the form of (1) the y-value at the mean of the regression line from the B-C model, (2) the y-value at the lower 50% PL of the regression line from the B-C model, or (3) an HC_{05} derived from a data set based on ≥200 paired SC and biological samples. In this example case for Ecoregion 4, the range of SC conditions was narrow with few sites exceeding 200 µS/cm and only two SC measurements exceeding 1,000 µS/cm, so any HC_{05} would be uncertain using paired SC and biological measurements. Therefore, an HC_{05} was not calculated using paired SC and biological measurements and the lower 50% PL was used to develop the example CCC.

7.1. DATA SET CHARACTERISTICS

7.1.1. Ecoregion Description

The Cascades (Ecoregion 4) is a mountainous ecoregion extending from the central portion of western Washington into Oregon and, after a separation by the Klamath River, another separate mountainous area in northern California (U.S. EPA, 2013d). The mountain ranges of the Cascades continue northerly into the North Cascades (Ecoregion 77), and south into the Sierra Nevada (Ecoregion 5). To the east lies the Eastern Cascades Slopes and Foothills (Ecoregion 9). To the west in Washington state is the Puget Lowlands (Ecoregion 2) and in Oregon the fertile Willamette Valley (Ecoregion 3). To the west in southern Oregon and northern California, the Cascades are bounded by the Klamath Mountains/California High North Coast Range (Ecoregion 78).

Some peaks in Ecoregion 4 are snow-capped or glaciated year round. Both active and dormant volcanoes are located on the high plateau in the eastern part of the ecoregion. The highest strato-volcano is Mount Ranier with an elevation of 4,392 m (USGS, 2016). The western Cascades in Oregon and Washington are dissected by numerous, steep-sided stream valleys (U.S. EPA, 2013d).

This geologic area is underlain by Cenozoic volcanics that have been affected by alpine glaciation (U.S. EPA, 2013d). Soils are characterized by frigid temperature regimes and at lower elevations in the south. Some soils are mesic. Common soils include andisols, formed in volcanic ash containing high proportions of glass and amorphous colloidal materials, and inceptisols, nearly like the parent material and having little or no clay, iron, aluminum or organic matter. The mean annual precipitation ranges from 180 cm and ranges from 115 in the Southern Cascades to 360 cm on some of the highest peaks of the Cascades Subalpine/Alpine subecoregion (OWEB 2001, Wilkins et al. 2011). Two of the larger rivers include the Columbia and Klamath Rivers.

The Cascades have a moist, temperate climate that supports an extensive and highly productive coniferous forest that is intensively managed for logging. Conifers dominate except at the highest elevations where there are alpine meadows and rocky alpine zones.

7.1.2. General Data Set Description

Only preexisting water chemistry data were used for this example assessment (see Table 7-1). EPA-survey, State, and USGS data sets were combined to characterize ion concentrations and water chemistry and then calculate a provisional CCC. The USGS data set was also used to calculate the CMEC because this data set had many within-year samples at each site. The statistical package R, Version 2.12.1 (December 2010), was used for all statistical analyses (R Development Core Team, 2011).

Table 7-1. EPA and U.S. Geological Survey (USGS) chemistry data sets included in this study.
Years indicate the period during which the samples were collected. Western Environmental Monitoring and Assessment Program (EMAP) survey sites are included in the count of sites from the National Wadeable Streams Assessment (NWSA). Specific conductivity was not measured at all sites.

Survey	Years	# of sites	# of samples
EPA probability samples (sample size in parenthesis)			
NWSA, NRSA, Region 10 R-EMAP	1995−2009	152	152
Total		152	152
State data from EPA Region 10			
Oregon	1990−2014	418	2,511
Washington	1990−2015	121	562
Total (only 90 with SC)		539	3,073
State: subset ≥ six samples per year, January−December	1990−2015	19	1,111
USGS mixed sampling			
USGS: full data set	1958−2016	290	6,258
USGS: subset ≥ six samples per year, January−December	1959−2014	50	5,019

The B-C regression model was developed using biological data paired with SC data from 24 data sets with waters having ionic mixtures dominated by calcium, magnesium, sulfate and bicarbonate ions and where background SC did not exceed 626 μS/cm. Therefore, the model is most appropriate for waters with similar ionic characteristics.

Because the B-C regression model was developed with an ionic mixture dominated by bicarbonate and sulfate (i.e., $([HCO_3^-] + [SO_4^{2-}]) > [Cl^-]$ in mg/L), samples dominated by chloride (i.e., $([HCO_3^-] + [SO_4^{2-}]) \leq [Cl^-]$ in mg/L) should be removed from the data set prior to estimating background SC. In this case, no samples were dominated by chloride ions and so all samples in the EPA-survey data set were used. The State data set of 539 sites was used to verify ionic composition. Of 359 samples of the State data set with ionic measurements, all samples were dominated by sulfate plus bicarbonate. Therefore, none was removed from the final analysis. All sites in the combined EPA-USGS data set had pH data. Because none were <6 or >8.7, no sites were removed from the data set.

7.1.3. EPA-Survey Data Set

Data sources, sampling period, and number of samples used to estimate background SC in the new area (Ecoregion 4) are provided in Table 7-1. The NRSA 2008−2009 data set (U.S. EPA, 2013b), NWSA 2004 data set (U.S. EPA, 2006), R-EMAP 1999 data sets (U.S. EPA, 2013c), are based on single random (i.e., probability-based design) samples from June through September.

EPA-survey data sampling sites within the ecoregion are shown in Figure 7-1. Water quality parameters collected in Ecoregion 4 are included in Table 7-2. Most of the samples have reported SC, alkalinity, hardness, sulfate, chloride, bicarbonate, and pH, as well as other water quality parameters. When necessary, ionic concentrations in milliequivalents (meq/L) were converted to mg/L [(meq/L) × (ion MW)/(ionic charge)] (Hem, 1985).

Table 7-2. Summary of data for the example case for Cascades, Level III Ecoregion 4, from EPA-survey samples.
Calculated means are geometric except for pH values and ion ratios.

Parameter	*N*	Minimum	25th	50th	Mean	75th	Maximum
SC (µS/cm)	152	1.56	33.9	44.9	44.8	61.8	205.0
pH (SU)	144	6.17	7.3	7.5	7.5	7.7	9.0
Ca^{2+} (mg/L)	70	0.04	3.1	4.8	4.7	8.1	22.7
Mg^{2+}(mg/L)	70	0.03	0.7	1.3	1.2	1.9	8.8
Na^{+} (mg/L)	70	0.08	2.1	2.8	2.7	4.0	10.4
K^{+} (mg/L)	70	0.01	0.2	0.5	0.5	0.8	3.2
HCO_3^{-} (mg/L)	46	0.46	18.3	26.4	25.4	40.0	132.2
SO_4^{2-} (mg/L)	121	0.07	0.4	1.0	1.0	2.3	34.3
Cl^{-} (mg/L)	136	0.14	0.6	0.9	0.8	1.1	12.2
[a] $HCO_3^{-} + SO_4^{2-}/Cl^{-}$	46	3.88	24.5	32.3	50.4	58.4	211.1

[a]Ratio of mg/L $HCO_3^{-} + SO_4^{2-}/Cl^{-}$ greater than 1 indicates the mixture is dominated by $HCO_3^{-} + SO_4^{2-}$.

All samples were collected from first-through fourth-order streams as part of a probability-based design intended to estimate proportions of parameters for various stream classes. The probability-design sampling weights for stream order were not used in the characterization. Analysis of water chemistry samples followed procedural and QA/QC protocols of EPA and EMAP (U.S. EPA, 2001, 1998b, 1994, 1987), Wadeable Streams Assessment (U.S. EPA 2006, 2004a, b), NRSA (U.S. EPA, 2009), and NAPAP (Drousé et al., 1986; U.S. EPA, 1987).

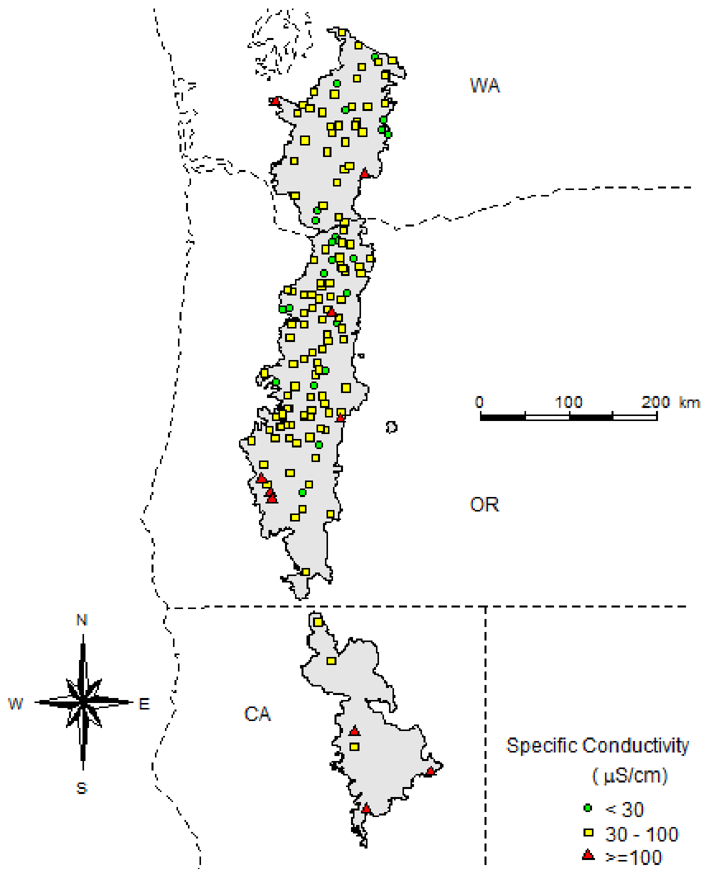

Figure 7-1. Sampling sites in the EPA survey data set that were used to estimate minimally affected background in Ecoregion 4.
For the purpose of demonstrating the background-to-criterion approach, Level III Ecoregion 4, is shaded gray. A total of 152 sites with specific conductivity measurements are depicted. Sampling locations are color coded by site-specific conductivity range: green diamonds <30 µS/cm, yellow squares 30−100 µS/cm, and red triangles ≥100 µS/cm. Geodetic reference system = North American Datum (NAD83).

7.1.4. State Data Set (EPA Region 10)

The State data set includes data from the Oregon DEQ 1990−2014 data set (Oregon DEQ, 2009) and the Washington Department of Ecology (WDE) 1990−2015 data set (WDE, 2014). Monthly sampling was performed throughout the year in many sites.

State sampling sites within the ecoregion are shown in Figure 7-2. Water quality parameters collected in Ecoregion 4 are included in Table 7-3. Most of the samples have reported SC, sulfate, chloride, and pH.

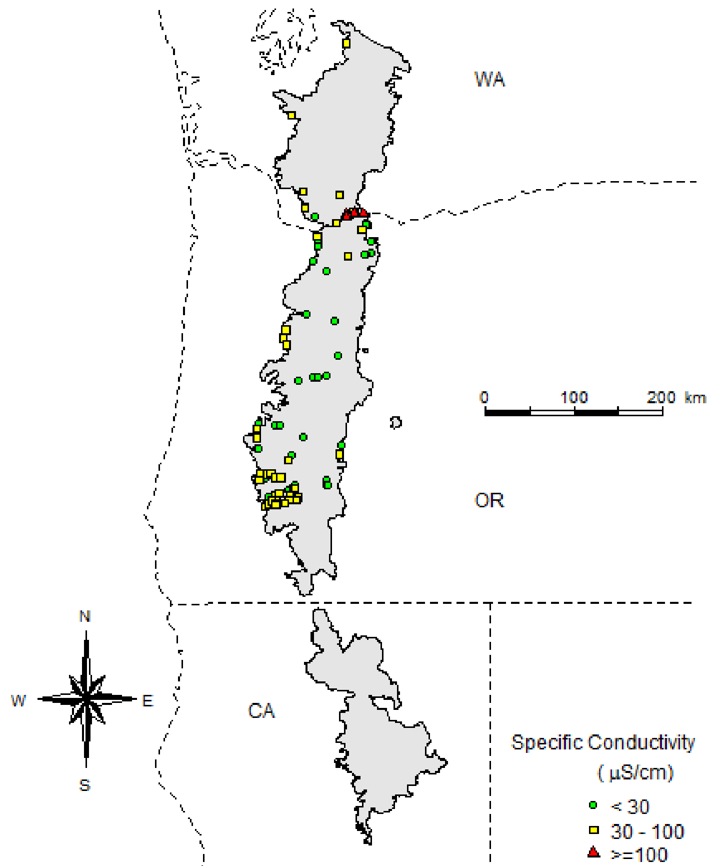

Figure 7-2. Sampling sites in State data set that were used to estimate minimally affected background in Ecoregion 4.
For the purpose of demonstrating the background-to-criterion approach, Level III Ecoregion 4 is shaded gray. A total of 539 sampling sites are in the state data set, but only 95 sites with conductivity measurements are shown here. Sampling locations are color coded by site-specific conductivity range: green diamonds <30 µS/cm, yellow squares 30−100 µS/cm, and red triangles ≥100 µS/cm. Geodetic reference system = North American Datum (NAD83).

Table 7-3. Summary of data for the example case for Cascades, Level III Ecoregion 4 from State Data from Oregon and Washington.
Calculated means are geometric except for pHs and ion ratios.

Parameter	*N*	Minimum	25th	50th	Mean	75th	Maximum
SC (μS/cm)	95	6.8	24.0	32.4	31.9	39.4	126
Ca^{2+} (mg/L)	78	1.0	2.3	3.0	3.7	4.3	1,100
HCO_3^- (mg/L)	300	7.7	19.9	25.8	27.5	38.0	3,966
SO_4^{2-} (mg/L)	252	0.1	0.3	0.6	0.8	1.7	36.2
Cl^- (mg/L)	315	0.3	0.7	1.0	1.1	1.5	250.0
[a]$HCO_3^- + SO_4^{2-}/Cl^-$	203	3.3	18.0	28.2	37.6	46.0	225.7

[a]ratio of mg/L $HCO_3^- + SO_4^{2-}/Cl^-$ greater than 1 indicates the mixture is dominated by $HCO_3^- + SO_4^{2-}$.

7.1.5. U.S. Geological Survey (USGS) Data Set

The USGS survey data set used in this example is composed of 6,258 water quality samples, from 290 stations within Ecoregion 4 (see Table 7-1; Figure 7-3). Some stations were sampled only once while others were sampled as many as 641 times. The data were collected between 1958 and 2016 during all seasons. Water quality parameters collected in Ecoregion 4 are included in Table 7-4. Most of the samples have reported SC, alkalinity, hardness, chloride, bicarbonate, and pH, as well as other water quality parameters. Analysis of water chemistry samples followed procedural and QA/QC protocols for USGS data sets (Mueller et al. 1997).

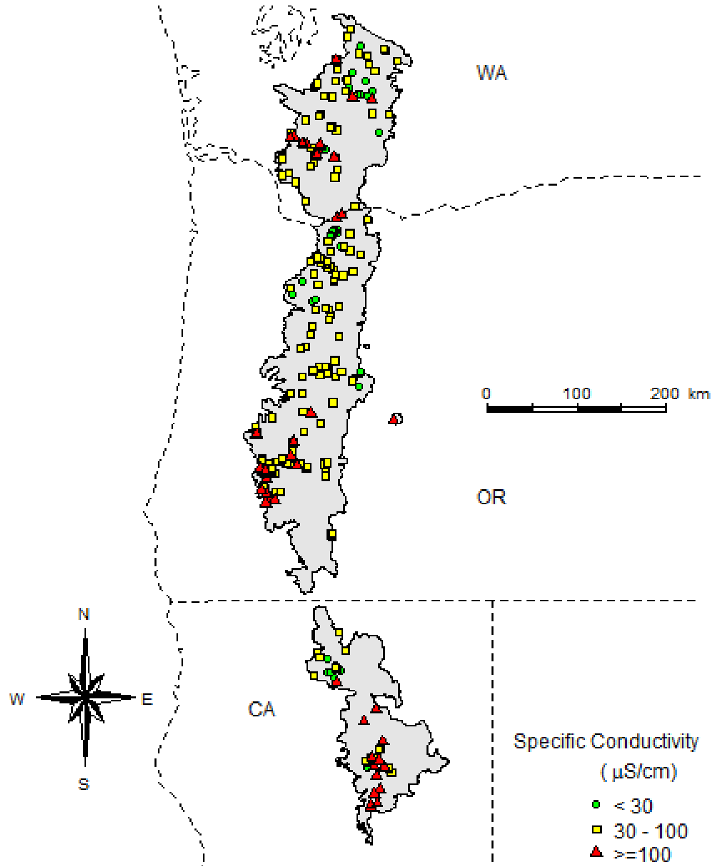

Figure 7-3. Sampling sites in the U.S. Geological Survey (USGS) data set in Ecoregion 4.
Ecoregion 4 is shaded gray. Geometric mean specific conductivity at 290 sampling locations is color-coded: green diamonds <30 µS/cm, yellow squares 30−100 µS/cm, and red triangles ≥100 µS/cm. Geodetic reference system = North American Datum (NAD83).

Table 7-4. Summary of data for the example case for Cascades, Level III Ecoregion 4 from the U.S. Geological Survey (USGS).
Calculated means are geometric except for pH.

Parameter	*N*	Minimum	25th	50th	Mean	75th	Maximum
SC (µS/cm)	282	3.50	38	53.9	53.6	78.3	370
pH (SU)	274	6.2	7.2	7.5	7.4	7.7	8.7
Ca^{2+} (mg/L)	5	1.6	1.7	2.1	2.9	5.6	15.7
Mg^{2+} (mg/L)	158	0.10	0.78	1.39	1.31	2.43	9.08
Na^{+} (mg/L)	5	1.2	1.2	1.3	1.7	2.5	5.6
K^{+} (mg/L)	5	0.1	0.2	0.2	0.3	0.5	1.3
HCO_3^- (mg/L)	57	3.0	29.8	35.0	37.0	51.3	122.0

All samples were collected from first-through fourth-order streams for various research purposes, and those targeted sampling designs may emphasize areas with increased anthropogenic disturbance. In this respect, the data may skew the background estimates. Background SC was not estimated from this data set alone because sampling stations were not randomly assigned.

Because the USGS and State data sets contained multiple measurements in a year from sampling locations, the data sets were used to estimate a CMEC and explore the variability of SC patterns within the region. Therefore, a second data set was selected by excluding stations with fewer than 6 SC measurements throughout the year. A minimum of one sample during the spring (March to June) and one in the summer (July to October) were also required so that at least one lower and one higher SC sample were included in the data set. The second USGS data set included 5,019 samples from 50 stations, while the second State data set include 1,111 samples from 19 stations. The USGS and State data sets were combined was used to calculate the variance near the CCC for the CMEC calculation.

7.1.6. Modeled Mean Base Flow Background Specific Conductivity (SC)

Predicted mean natural base-flow specific conductivity in catchments of Cascades, was also considered for comparison purposes (Olson and Hawkins, 2012). The stream length weighted, mean natural SC from the modeled base flow were calculated for the SC at base flow for each ecoregion (see Appendix D). Figure 7-4 shows the predicted SC with 300 m resolution in order to emphasize the general trends across Ecoregion 4.

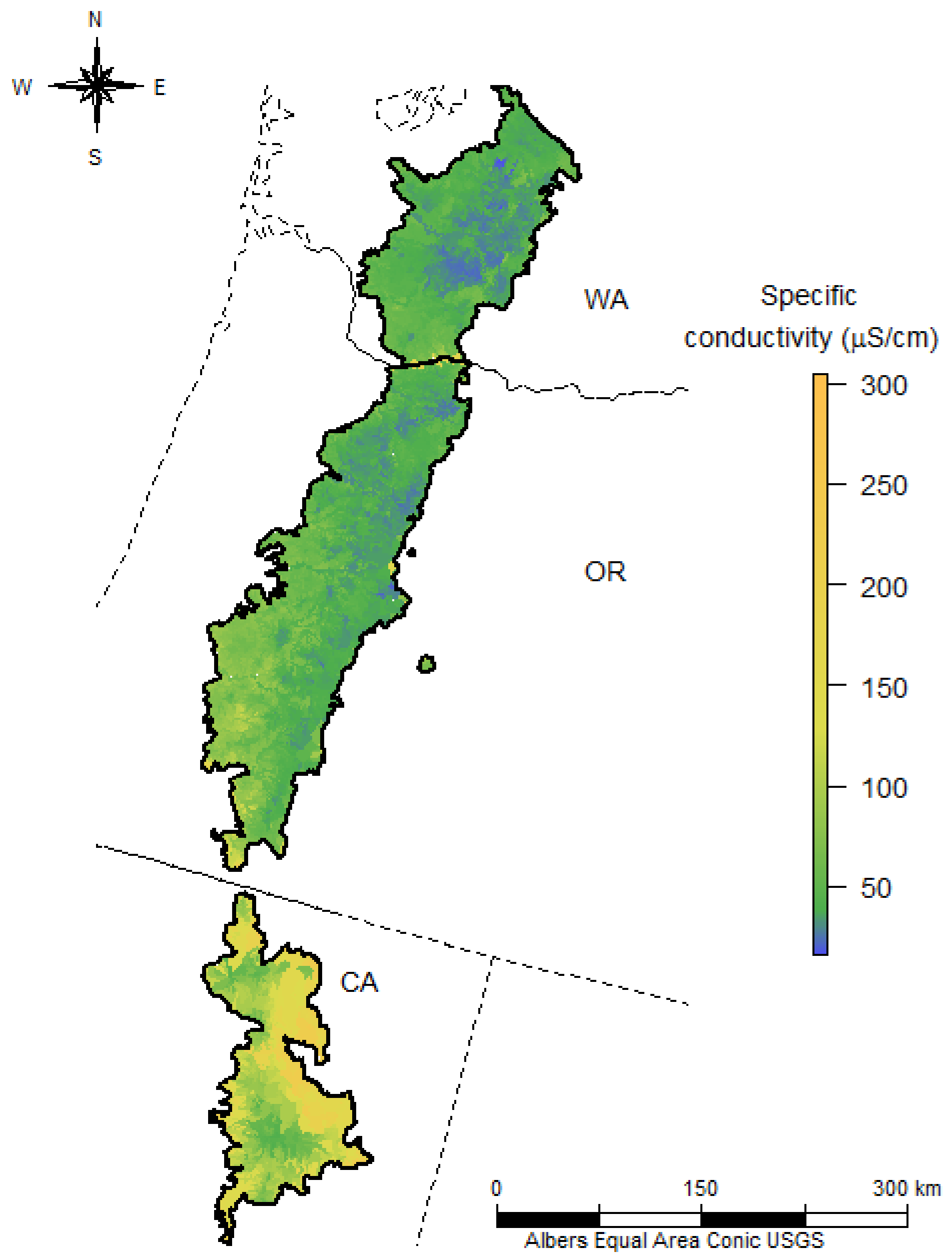

Figure 7-4. Predicted mean natural base-flow specific conductivity in catchments of the Cascades, Ecoregion 4, using the Olson-Hawkins model. Albers projection used for mapping.

7.1.7. Characterization of Ionic Matrices

7.1.7.1. *EPA-Survey Data Set Ionic Characteristics*

A summary of water quality ionic constituents including major ionic constituents from EPA-survey data for Ecoregion 4 is provided in Table 7-2. Centiles were calculated using each sample observation because most measurements were single grab samples. There were no chloride-dominated sites where $[Cl^-] \geq ([HCO_3^-] + [SO_4^{2-}])$ in mg/L in the EPA-survey data ($N = 152$); therefore, no sites were excluded from the data sets. Calcium plus magnesium were the dominant cations $([Ca^{2+}] + [Mg^{2+}]) > ([Na^+] + [K^+])$.

7.1.7.2. *State Data Set Ionic Characteristics*

A summary of water quality ionic constituents including major ionic constituents from State data for Ecoregion 4 is provided in Table 7-3. Centiles were calculated using each sample observation because most measurements were single grab samples. There were no chloride-dominated sites (where $[Cl^-] \geq ([HCO_3^-] + [SO_4^{2-}])$ in the state data ($N = 539$); therefore, no sites were excluded from the data sets. Likewise, calcium plus magnesium were the dominant cations, $([Ca^{2+}] + [Mg^{2+}]) > ([Na^+] + [K^+])$.

7.1.7.3. *U.S. Geological Survey (USGS) Data Set Ionic Characteristics*

Table 7-4 summarizes the water quality parameters including major ionic constituents for Ecoregion 4 in the USGS data set. Unlike the EPA-survey data set, the USGS data set are targeted sites of interest rather than probability samples. Also, in some cases, there are multiple measurements from the same site, and other sites are auto-correlated with downstream sampling locations. Therefore, the distribution of sites in this data set is not necessarily representative of Ecoregion 4 in its entirety; however, the data set can be used to define the overall pattern of SC for the ecoregion because it contains samples in areas not represented in the EPA-survey data set. Centiles were calculated using the geometric mean of site measurements (except pH and the ionic ratio) and were qualitatively compared to the probability-based EPA-survey data set. No sulfate measurements were found so $([HCO_3^-] + [SO_4^{2-}])/[Cl^-]$ ratios were not determined for this data set. Calcium plus magnesium were the dominant cations $([Ca^{2+}] + [Mg^{2+}]) > ([Na^+] + [K^+])$.

Two samples from the USGS data set were in the 1,000 µS/cm range and were associated with salt springs at Lake Paulina and Longmire Meadow mineral springs and were removed from the data set (Ingebritsen et al., 2014). Six sites (421−1,030 µS/cm) were sampled on the flanks of Mount St. Helen after the 1980 eruption; and, these were removed. These eight sites represent a small proportion of the data set, and the example criterion would not apply to these or similar areas with naturally higher background SC. The background SC was 39.5 µS/cm with these eight sites included and 38.2 µS/cm with them removed.

7.1.8. Comparison of Background Specific Conductivity (SC) Estimates

SC data from the three data sets, the combined data, and the Olson-Hawkins base flow model are summarized in Figure 7-5. The stream length weighted average predicted mean base flow SC from the Olson-Hawkins model in Ecoregion 4 is 65.7 µS/cm. The 25th centile of the EPA-survey data set is 33.9 µS/cm, of the State data set is 24 µS/cm, and of the USGS SC data set is 39.5 µS/cm. All three are less than the mean base flow SC (65.7 µS/cm), so these background SC are characterized as minimally affected (see Figure 7-5 and Tables 7-2, 7-3, and 7-4). When data are combined, the 25th centile (≈33 µS/cm) is still lower than the predicted mean base flow of 65.7 µS/cm (see Table 7-5, Figure 7-5). Therefore, this background SC estimated from the combined State-EPA-USGS data set also represents minimally affected background SC.

The 25th centile of the combined data set was used to calculate the HC_{05} following the decision tree described Section 3.7.2 and Figure 3-11.

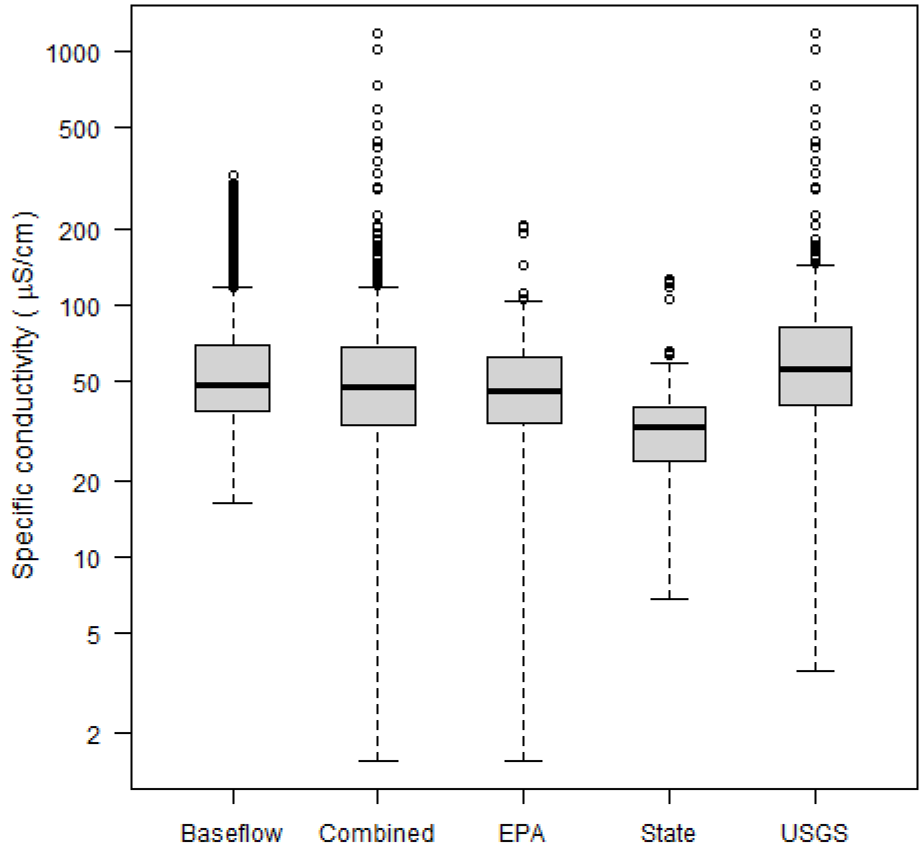

Figure 7-5. Box plots of specific conductivity (SC) distributions for EPA-survey, State, U.S. Geological Survey (USGS), and combined data sets, and the predicted base flow SC for all stream segments.
The State data set captures a slightly narrower range of values possibly owing to the targeted sampling. The USGS samples greater than 400 µS/cm include some samples from mineral springs and Mt. St. Helen ash flows which have been removed from the combined data set. The 25th centiles of the observed data sets are fairly similar to the 25th centile of predicted base-flow.

Table 7-5. Summary of data for the example case for Cascades, Level III Ecoregion 4 from the combined data set.
Calculated means for sampled sites are geometric for all variables except for pH and ion ratio.

Parameter	*N*	Minimum	25th	50th	Mean	75th	Maximum
SC (µS/cm)	529	1.56	32.7	46.4	46.4	66	370
pH (SU)	418	6.2	7.2	7.5	7.4	7.7	9.0
Ca^{2+} (mg/L)	153	0.04	2.51	3.59	4.06	6.36	1,100
Mg^{2+} (mg/L)	228	0.03	0.77	1.34	1.29	2.20	9.08
Na^{+} (mg/L)	75	0.08	2.03	2.76	2.59	3.96	10.45
K^{+} (mg/L)	75	0.01	0.22	0.49	0.44	0.77	3.17
HCO_3^{-} (mg/L)	403	0.46	20.4	28.0	28.4	40.2	3,966
SO_4^{2-} (mg/L)	373	0.07	0.34	0.78	0.85	2.17	36.2
Cl^{-} (mg/L)	451	0.14	0.63	1.00	1.00	1.40	250
$HCO_3^{-} + SO_4^{2-}/Cl^{-}$	249	3.29	18.3	28.6	40	48.5	226

[a]ratio in mg/L $HCO_3^{-} + SO_4^{2-}/Cl^{-}$ greater than 1 indicates the mixture is dominated by $HCO_3^{-} + SO_4^{2-}$.

7.2. CALCULATION OF THE CRITERION CONTINUOUS CONCENTRATION (CCC)

7.2.1. Calculation of the Ecoregion 4 mean Hazardous Concentration (HC_{05}) from Background

Because paired SC and biological data are available for <200 sites, the HC_{05} was estimated at the lower 50% PL of the B-C model (see Figure 7-6). The 25th centile of SC from the combined data set of Ecoregion 4 was used to identify the lower 50% PL using eqs 3-4 and 3-5. The B-C model development is described in Appendix D. The *x*-variable is the background SC in Ecoregion 4 which was log10 transformed. The calculated *y*-value is the predicted mean log10 HC_{05}. In this example case, the minimally affected background is 33 µS/cm (see Table 7-5). It was estimated at the 25th centile from the combined data set. The calculation of the predicted mean log10 of HC_{05} (*y*) is shown in eqs 7-1 and 7-2 and that value is used to estimate the lower 50% PL using eq 7-3.

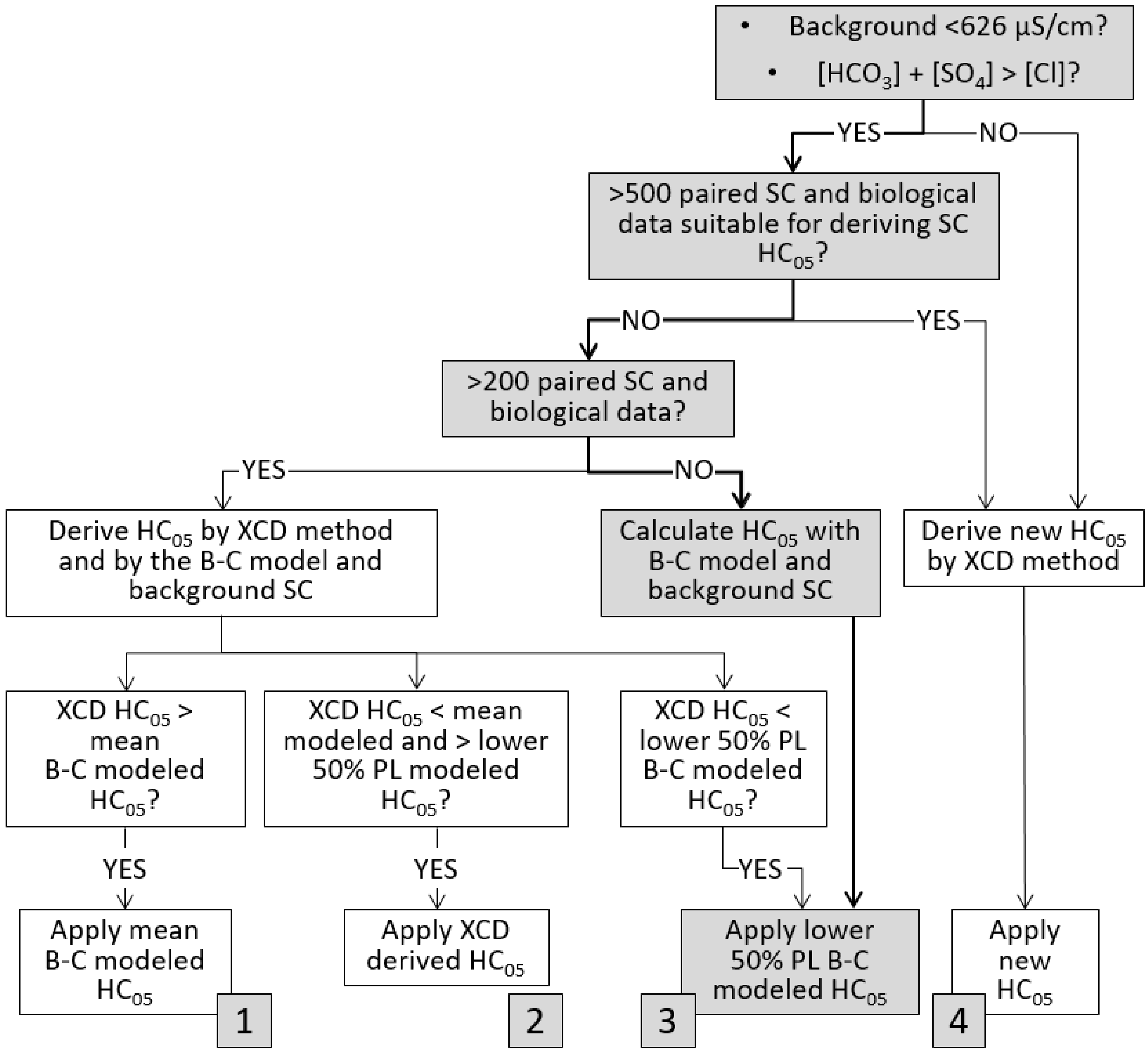

Figure 7-6. Process and decision path case example for Ecoregion 4. Decision path is highlighted in gray and connected by bold lines. Because there was no previously derived hazardous concentration at the 5th centile (HC_{05}) of a taxonomic extirpation concentration distribution (XCD) for Ecoregion 4, and the background was <33 µS/cm, and there were <200 paired specific conductivity (SC) measurements, the HC_{05} was calculated with the background-to-criterion (B-C) model at the lower 50% prediction limit (PL).

The B-C model is described by the following formula:

$$Y = 0.657X + 1.075 \quad (7\text{-}1)$$

Where:

X is the log10 of the ecoregional background SC
Y is the log10 of the predicted HC_{05}

The background for Ecoregion 4 (33 µS/cm) is converted to log10, replacing X in the formula with that value and Y is computed (see eq 7-2). The predicted value Y is converted from log10 to a number that is the mean modeled HC_{05} for that region. In Ecoregion 4 the mean modeled HC_{05} is 118 µS/cm after rounding to two significant figures.

$$\text{Log Predicted } HC_{05} = (0.657 \times 1.518\ \mu\text{S/cm}) + 1.075 = 2.072\ \mu\text{S/cm} \quad (7\text{-}2)$$

Then

$$\text{Predicted } HC_{05} = 10^{2.072}\ \mu\text{S/cm} = 118\ \mu\text{S/cm}$$

7.2.2. Calculation of the Lower 50% Prediction Limit

Because the available paired SC and biological data constitute <200 sites, the CCC was estimated at the lower 50% PL of the HC_{05} (see Figure 7-6). The 25th centile of background SC and the predicted mean HC_{05} of a region and variance of the B-C model is used to calculate the lower 50% PL (see eq 7-3). In this case example for Ecoregion 4, the background is 33 µS/cm and the mean modeled HC_{05} is 118 µS/cm. Both values are converted to log10 (x, y) as shown in eq 7-4. The prediction interval from the regression line for a mean predicted value $\hat{\bar{y}}$ can be calculated as follows or more conveniently using statistical software thus avoiding rounding errors:

$$\hat{\bar{y}} \pm t_{\alpha/2,n-2} S_y \sqrt{1 + \frac{1}{n} + \frac{(x^{\circ} - \bar{x})^2}{SS}} = \text{PL} \quad (7\text{-}3)$$

Symbol	Explanation	Example from the B-C model
$\widetilde{y}$	Log10 of mean predicted HC_{05}	2.072 µS/cm, log10 of 118 µS/cm
n	Number of samples in the model	$n = 24$
α	Alpha error rate for prediction interval (desired confidence level)	50% prediction interval (α = 0.5)
t_{n-2}	t-value at specific level (alpha, α) and degrees of freedom ($n - 2$) of interval	For 50% prediction interval (α = 0.5), $t_{(1-0.5)/2,24-2} = 0.686$
S_y	Residual standard error of prediction (standard deviation)	$S_y = 0.11$
SS	Sum of square of x deviation from their mean, SS = $\sum_{i=1}^{n}(x_i - \bar{x})^2$	$SS = 4.21$
$\bar{x}$	Mean x values used in the model generation	$\bar{x} = 2.15$
x°	x value for a new prediction interval	Log10 118 µS/cm = 2.072
PL	Upper and lower prediction limits of mean predicted $\widetilde{y}$	calculated in eq 6-4

Using x° = log10 (118) µS/cm and the mean predicted HC_{05} for Ecoregion 4 value, ($\widetilde{y}$ = 2.072, the log10 of 118 µS/cm) the lower 50% PL is calculated as follows in eq 7-4. Note, the upper 50% PL is not calculated but is included in the formula because it may be used to estimate a CMEC where there are insufficient data to calculate a CMEC using the method described in Section 3.2.

$$log10\ (118)\ \pm 0.686 \times\ 0.11\sqrt{1 + \frac{1}{24} + \frac{(log_{10}(118) - 2.15)^2}{4.21}} \tag{7-4}$$

So:

$$2.072 - 0.686\ \times 0.11\sqrt{\frac{25}{24} + \frac{(2.072 - 2.15)^2}{4.21}}$$

$$2.072 - 0.686\ \times 0.11 \times 1.02$$

$$\text{Log}10^{1.997} = 98\ \mu\text{S/cm}$$

The log of the lower calculated 50% PL is log10 1.997 which equals 98 µS/cm after back transformation and rounding to two significant figures yields a CCC of 98 µS/cm.

7.2.3. Example Criterion Maximum Exposure Concentration

At sites meeting the CCC of 98 µS/cm, 90% of the SC observations are estimated to occur below the CMEC (see Section 3.2). The CMEC was derived using a combined USGS (6,258 samples collected between 1959−2016) and State data sets (3,073 samples collected between 1990−2015). These data sets were used because they contained multiple measures of SC within a year whereas the EPA survey data set consisted of single measurements at each site. Of the 9,331 samples in this ecoregion, there are 6,130 samples in a year representing 312 station years, 69 unique stations, with at least 1 sample from March to June and one from July to October and at least 6 samples within a year. Note that inclusion of samples is not contingent on biological data.

Of the 312 station years (69 unique stations) with multiple SC measurements, the variability of within station SC was slightly differed for streams with different mean SC (see Figure 7-7). However, the LOWESS and confidence bounds for any detectable change points indicated that the average variability (residual standard deviation for a station) was relatively stable generally between 0.05 and 0.1 (see Figure 7-7); therefore, the entire data set was used to estimate the standard deviation components of the annual mean SC (98 µS/cm). The proposed CCC and standard deviation of this data set was determined, and the CMEC was calculated. The example calculation of the CMEC for Ecoregion 4 is shown below (see eq 7-5) using eq 3-2 from Section 3.2:

$$10^{\log10(98) + 1.28 * 0.234} = 196\ \mu\text{S/cm} \qquad (7\text{-}5)$$

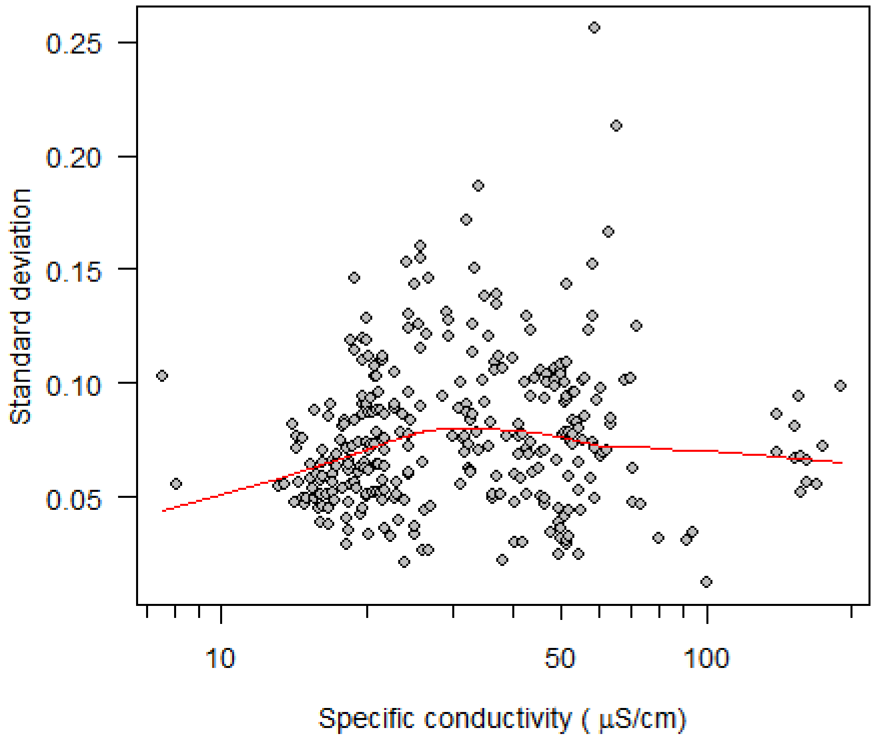

Figure 7-7. Illustration of within site variability (residual standard deviation for each station) along the specific conductivity gradient (station means) in Ecoregion 4.
The *x*-axis is log annual mean specific conductivity. Each dot represents a station. The fitted line is a locally weighted scatterplot smoothing spline (LOWESS, span = 0.75, linear polynomial model).

The example CMEC (see Table 7-6) rounded to two significant figures yields a CMEC of 200 µS/cm for Ecoregion 4. At this level, where the annual geometric mean SC <98 µS/cm, 90% of the observations are expected to be less than the CMEC.

Table 7-6. Summary data related to the calculation of the example criterion maximum exposure concentration (CMEC) for Ecoregion 4

Number of samples July to June prior to biological sampling	6,130
Number of unique stations/rotation year	69/312
CCC	98 µS/cm
CMEC	200 µS/cm

7.3. EXAMPLE CRITERION CHARACTERIZATION FOR ECOREGION 4 BASED ON A BACKGROUND-TO-CRITERION MODEL

The case example for Ecoregion 4 includes an annual geometric mean (i.e., CCC) and a 1-day mean (i.e., CMEC), not to be exceeded more than once in 3 years on average. Both of these distinct expressions of the example SC criteria would need to be met in order to adequately protect aquatic life. These values indicate that freshwater animals are protected if the annual geometric mean SC concentration in flowing waters does not exceed 98 µS/cm and the 1-day mean does not exceed 200 µS/cm more than once every 3 years on average. These example criteria would apply to all flowing freshwaters (ephemeral, intermittent, and perennial streams) in Ecoregion 4 inclusive of portions of Washington, Oregon, and California. On a site-by-site basis, these example ecoregional criteria apply if the ionic mixture is dominated by anions of bicarbonate and sulfate and cations calcium and magnesium. For streams crossing into Ecoregion 4 from ecoregions with either lower or higher background SC, professional judgment may be needed to assess the potential effect of different ionic composition or concentration. Professional judgment is recommended when applying to sites with a catchment area greater than 1,000 km^2 (386 mi^2) owing to lesser representation in the data set by this class of stream in the development of the B-C model. On a site by site basis, alternative SC criteria may be more appropriate if the natural background of a site is shown to be lower or higher than its regional background SC.

The Cascades ecoregion has less sources of ionic inputs and the igneous geology leads to very low stream SC (background SC of 33 µS/cm), which represents minimally affected conditions with respect to SC. Reference sites were not identified in the data sets so a comparison with the 75th centile SC in any data set was not possible. About 88% of the sampled sites (537) meet the CCC and more than 99% of all samples (7,855) meet the CMEC calculated for this example. Owing to the very low conductivity, there is very little difference between the lower 50% prediction interval or the mean modeled HC_{05}, 98 versus 118 µS/cm, respectively. Two samples from the USGS data set were in the 1,000 µS/cm range and were associated with salt springs at Lake Paulina and Longmire Meadow mineral springs and were removed from the data set (Ingebritsen et al., 2014). Six sites (421−1,030 µS/cm) were sampled on the flanks of Mount St. Helen after the 1980 eruption, and they were also removed before the analysis. The example criterion would not apply to these areas with naturally higher background SC. The weight-of-evidence method described in Appendix C could be used to evaluate subregions or stream classes that may have different background SC in this large ecoregion. In particular, the isolated area in Northern California may have a naturally higher background SC based on the USGS measurements (see Figure 7-3) and the mean predicted baseflow (see Figure 7-4). Also, potential unique sources of salt such as fumaroles and salt springs may naturally raise SC.

7.4. PROTECTION OF FEDERALLY-LISTED SPECIES AND OTHER HIGHLY VALUED TAXA

Although the example criteria were derived using XC_{95} values for the macroinvertebrate taxa represented in the data sets used to develop the B-C model, the available evidence indicates that other taxa in the streams would likely be protected as well (see Section 2.6 and Appendix G). Hence, no adjustment was made for unanalyzed taxa. However, on a site-specific basis, the example criterion could be adjusted or recalculated to protect important species, highly valued aquatic communities, or specially protected waters.

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