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AQUATIC LIFE AMBIENT WATER QUALITY CRITERIA FOR AMMONIA – FRESHWATER

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Aquatic Life
Ambient Water Quality Criteria For
Ammonia – Freshwater
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U.S. Environmental Protection Agency
Office of Water
Office of Science and Technology
Washington, DC

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

FOREWORD

This water quality criteria update provides scientific recommendations to states and tribes authorized to establish water quality standards under the Clean Water Act (CWA), to protect aquatic life from acute and chronic effects of ammonia in freshwater ecosystems. Under the CWA, states and tribes are to establish water quality criteria to protect designated uses. State and tribal decision makers retain the discretion to adopt approaches on a case-by-case basis that differ from those used in these criteria when appropriate. While this update constitutes United States Environmental Protection Agency (EPA) scientific recommendations regarding ambient concentrations of ammonia that protect freshwater aquatic life, this update does not substitute for the CWA or EPA's regulations; nor is it a regulation itself. Thus, it cannot 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 these criteria in the future, as new scientific information becomes available. This document has been approved for publication by the Office of Science and Technology, Office of Water, U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

ACKNOWLEDGMENT

This 2013 criteria document is an update of the 1999 Update of Ambient Water Quality Criteria for Ammonia. The updates described herein were prepared by Lisa Huff (EPA Team Leader), Charles Delos, Kathryn Gallagher, and Joe Beaman with written and technical support provided by EPA contractor: Great Lakes Environmental Center, Inc. EPA received substantial input from Dave Mount, James (Russ) Hockett, Russell Erickson, and Charles Stephan of the EPA's Office of Research and Development (ORD) National Health and Environmental Effects Research Laboratory (NHEERL) Mid-Continent Ecology Division, Duluth, MN, and Cindy Roberts, ORD Office of Science Policy. Please submit comments or questions to: Lisa Huff, U.S. EPA, Mail Code 4304, Washington, DC 20460 (e-mail: huff.lisa@epa.gov).

EXECUTIVE SUMMARY

EPA has updated the freshwater ammonia aquatic life ambient water quality criteria in accord with the provisions of Section 304(a) of the Clean Water Act to revise Ambient Water Quality Criteria (AWQC) from time to time in order to reflect the latest scientific knowledge. Literature searches for laboratory toxicity tests of ammonia on freshwater aquatic life, published from1985 to 2012, identified new studies containing acute and chronic toxicity data acceptable for criteria derivation. The acute criterion dataset includes 12 species of aquatic animals Federally-listed as threatened, endangered or species of concern. In the chronic dataset for ammonia, Federally-listed species are represented by three salmonid fish species in the genus *Oncorhynchus*, including sockeye salmon, rainbow trout/steelhead, and the subspecies Lahontan cutthroat trout. Data were assessed from the perspective of EPA's "*Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*" (Stephan et al. 1985).

The 1999 recommended aquatic life criteria for ammonia were based on the most sensitive endpoints known at the time: the acute criterion was based primarily on effects on salmonids (where present) or other fish, and the chronic criterion was based primarily on reproductive effects on the benthic invertebrate *Hyalella* or on survival and growth of fish early life stages (when present), depending on temperature and season.

The 2013 recommended criteria of this document take into account data for several sensitive freshwater mussel species in the Family Unionidae that had not previously been tested. As noted in the 2009 draft ammonia criteria document, available data indicated that another freshwater mollusk taxon, non-pulmonate (gill-bearing) snails, are also sensitive to the effects of ammonia (EPA-822-D-09-001). The 2013 criteria include additional data confirming the sensitivity of freshwater non-pulmonate snails. Many states in the continental United States have freshwater unionid mussel fauna in at least some of their waters (Abell et al. 2000, Williams et al. 1993, Williams and Neves 1995). Moreover, approximately one-quarter of approximately 300 freshwater unionid mussel taxa in the United States are Federally-listed as endangered or threatened species. Freshwater mussels are broadly distributed across the U.S., as are freshwater non-pulmonate snails, another sensitive invertebrate taxon, and both of these groups are now included in the ammonia dataset. Thus, the 2013 freshwater acute and chronic aquatic life criteria for ammonia will more fully protect the aquatic community than previous criteria, and are represented by a single (non-bifurcated) value each for acute and chronic criteria.

The criteria magnitude is affected by pH and temperature. After analysis of the new data, EPA determined that the pH and temperature relationships established in the 1999 ammonia criterion document still hold. When expressed as total ammonia nitrogen (TAN), the effect concentrations for fish are normalized only for pH, reflecting the minimal influence of temperature on TAN toxicity to fish. For invertebrates, TAN effect concentrations are normalized for both pH and temperature. At water temperatures greater than 15.7°C, the 2013 acute criterion magnitude is determined primarily by effects on freshwater unionid mussels. At lower temperatures the acute criterion magnitude is based primarily on effects on salmonids and other fish. Throughout the temperature range, the 2013 chronic criterion magnitude is determined primarily by the effects on freshwater mollusks, particularly unionid mussels.

At an example pH of 7 and temperature of 20°C, the 2013 acute criterion magnitude is 17 mg TAN/L and the chronic criterion magnitude is 1.9 mg TAN/L. At pH 7 and 20°C the 2013 acute criterion magnitude is 1.4-fold lower than the 1999 acute criterion magnitude. At this pH and temperature, the 2013 chronic criterion magnitude is 2.4-fold lower than the 1999 chronic criterion magnitude. See the Criterion Statements (pages 40-49) for the criterion concentrations at other pH and temperature conditions. The decreases in acute and chronic criteria magnitudes below those of 1999 reflect the inclusion of the new data discussed above.

The acute criterion duration represents a one-hour average. The chronic criterion duration represents a 30-day rolling average with the additional restriction that the highest 4-day average within the 30 days be no greater than 2.5 times the chronic criterion magnitude. These values are not to be exceeded more than once in 3 years on average.

	1999 AWQC Update Criteria Magnitude		**2009 Draft AWQC Update Criteria[c] Magnitude**		**2013 AWQC Update Criteria Magnitude**
Criterion Duration	pH 8.0, (mg TAN/L)	pH 7.0, T=20°C (mg TAN/L)	pH 8.0, T=25°C (mg TAN/L)	pH 7.0, T=20°C (mg TAN/L)	pH 7.0, T=20°C (mg TAN/L)
Acute (1-hr average)	5.6[a]	24[a]	2.9	19	17[a]
Chronic (30-d rolling average)	1.2	4.5[b]	0.26	0.91	1.9*
*Not to exceed 2.5 times CCC or 4.8 mg TAN/L (at pH 7, 20°C) as a 4-day average within the 30-days, more than once in three years on average.					
Criteria frequency: Not to be exceeded more than once in three years on average.					

[a] Salmonids present

[b] Based on renormalization of data to pH 7 and 20°C

[c] Mussels present

ACRONYMS

ACR	Acute-Chronic Ratio
ASTM	American Society of Testing and Materials
AWQC	Ambient Water Quality Criteria
CCC	Criterion Continuous Concentration
CMC	Criterion Maximum Concentration
CV	Chronic Value (expressed in this document as an EC20 or MATC)
CWA	Clean Water Act
ECx	Effect Concentration at X Percent Effect Level
LCx	Lethal Concentration at X Percent Survival Level
EPA	Environmental Protection Agency
ESA	Endangered Species Act
FACR	Final Acute-Chronic Ratio
FAV	Final Acute Value
FCV	Final Chronic Value
GMACR	Genus Mean Acute-Chronic Ratio
GMAV	Genus Mean Acute Value
GMCV	Genus Mean Chronic Value
LOEC	Lowest Observed Effect Concentration
MATC	Maximum Acceptable Toxicant Concentration (expressed mathematically as the geometric mean of the NOEC and LOEC)
NOEC	No Observed Effect Concentration
SD	Sensitivity Distribution
SMACR	Species Mean Acute-Chronic Ratio
SMAV	Species Mean Acute Value
SMCV	Species Mean Chronic Value
TAN	Total Ammonia Nitrogen
TRAP	EPA's Statistical Program: Toxicity Relationship Analysis Program (Version 1.21)
WER	Water Effect Ratio

INTRODUCTION AND BACKGROUND

National Ambient Water Quality Criteria (AWQC) are established by the United States Environmental Protection Agency (EPA) under the Clean Water Act (CWA). EPA will review and from time to time revise 304(a) AWQC as necessary to ensure the criteria are consistent with the latest scientific information. Section 304(a) aquatic life criteria serve as recommendations to states and tribes in defining ambient water concentrations that will protect against adverse ecological effects to aquatic life resulting from exposure to a pollutant found in water from direct contact or ingestion of contaminated water and/or food. Aquatic life criteria address the CWA goals of providing for the protection and propagation of fish and shellfish. When adopted into state standards, these criteria can become a basis for establishing permit limits and Total Maximum Daily Loads (TMDLs).

EPA first published aquatic life criteria recommendations for ammonia in 1976, followed nine years later by the 1985 criteria revision, which used updated procedures and additional information. The 1985 acute ammonia criterion was calculated from acute values expressed as unionized ammonia and normalized for pH (8.0) for all freshwater aquatic animals, and temperature (20°C) for freshwater fish only. Because the fraction of total ammonia that is unionized varies with pH and temperature, the 1985 toxicity data normalizations with *unionized* ammonia were necessarily structured differently than the current document's normalizations with *total* ammonia nitrogen. Because the 1985 chronic toxicity dataset was more limited than is available now, the 1985 chronic criterion was calculated by dividing the Final Acute Value by an acute-chronic ratio (ACR). The 1985 acute and chronic criteria concentrations were 19 and 1.2 mg/L expressed as total ammonia nitrogen at pH 7 and temperature 20°C for salmonids or other coldwater species present (e.g., rainbow trout). The durations for these criteria were one-hour (acute) and four-day (chronic) averaging periods. The 1985 freshwater acute criterion dataset was composed of acute values from tests involving 41 species (29 fish and 12 invertebrate) representing 34 genera (18 fish and 16 invertebrate). The data available for invertebrates at the time indicated they were not among the more acutely-sensitive organisms to ammonia.

In 1999 EPA revised the 1985 freshwater ammonia criteria to incorporate newer data, better models, and improved statistical methods. For its acute criterion, the revision included a re-examination of the temperature and pH relationships underlying the 1985 acute criterion, reworked from the perspective of total ammonia nitrogen rather than unionized ammonia. For its chronic criterion, EPA developed relationships for formulating a seasonal, pH- and temperature-dependent relationship, in part because the chronic criterion was based on endpoints that might not be of concern during cold-season conditions (e.g., fish early life stages). EPA analyzed all of the freshwater chronic data used in the 1985 criteria document as well as newer chronic data and was able to directly calculate a chronic criterion instead of calculating it from the acute criterion with an ACR. EPA did not conduct a comprehensive literature search for and critical review of all of the acute toxicity data published after 1985, but focused on the chronic criteria, in response to scientific issues raised by the public. Thus, the 1999 acute criterion relied on acute tests reported in Table 1 in the 1985 criteria document, supplemented by a limited number of newer studies relevant to the revised pH relationship.

The 1999 criteria were based on the most sensitive endpoints known at the time: the acute criterion was based primarily on effects on fish throughout the temperature range, and the chronic criterion was based primarily on effects on benthic macroinvertebrates or fish early life stages (when present), depending on temperature and season. For the 1999 acute criterion the effect concentrations for fish were normalized for pH only, reflecting the minimal influence of temperature on total ammonia toxicity to fish. The 1999 acute criterion was not adjusted for temperature because invertebrates that were included in the dataset, mollusks included, were not among the species highly sensitive to ammonia, thus, the invertebrate temperature slope did not affect the formulation of the 1999 acute criterion. The 1999 chronic criterion was adjusted for pH for fish and for pH and temperature for invertebrates. The chronic averaging period was increased from a 4- to a 30-day average in the 1999 update; the rationale for this change was based on analysis of chronic data from fathead minnow laboratory tests of different exposure durations and exposure concentrations with “limited variability” (see detailed discussion in the Problem Formulation of this document under Chronic Measures of Effect). For chronic toxicity, the 1999 updated dataset consisted of nine values representing four invertebrate and five fish genera. Two of the four most chronically sensitive species were invertebrates (the benthic amphipod *Hyalella azteca* and the bivalve mollusk, *Musculium transversum*). Missing were representative chronic values for the genus *Oncorhynchus* (salmonid) and an insect genus, although in both of these cases the calculation of the fifth percentile directly from the GMCVs in Table 5 of the 1999 update was deemed to adequately protect the freshwater aquatic community.

In 2004 EPA published a Federal Register Notice indicating its intent to re-evaluate the freshwater ammonia criteria and requesting new information on ammonia toxicity to freshwater mussel species in the Family Unionidae. This action was taken in response to concerns from U.S. Fish and Wildlife Service (USFWS) and mussel researchers about the sensitivity of unionid mussels to ammonia (summarized by Augspurger et al. 2003). The current document takes into account all such data, new toxicity data obtained by a search of the literature for all other species, and updated analyses of tests previously included in the 1999 document.

In 2009, EPA published a draft ammonia criteria document that included all available new data on the toxicity of ammonia to freshwater mussels (EPA-822-D-09-001). The draft 2009 document incorporated new toxicity data in the acute and chronic dataset while retaining the relationships describing the influence of pH and temperature on ammonia toxicity established in the 1999 criteria. The 2009 acute dataset represented 67 genera, including 12 species of freshwater mussels, compared to only 34 genera in the 1999 AWQC. Freshwater bivalve mollusks and snails were the predominant groups of genera ranked in the lowest (most sensitive) quartile, and the four most acutely sensitive genera were all bivalves. The 2009 chronic dataset incorporated two new fish species and new data for three freshwater mussel species, which represented two of the four most sensitive genera. The draft 2009 criteria recommendations were bifurcated, with a set of acute and chronic criteria values for waters with mussels present that reflects their greater sensitivity to ammonia, and a different set of criteria values for waters where mussels are absent. Including the new acceptable data for freshwater unionid mussels, the draft 2009 acute and chronic criteria magnitudes, respectively, were 19 and 0.91 mg TAN/L adjusted to pH 7.0 and 20°C.

For this 2013 update, EPA conducted a new literature search for both acute and chronic toxicity data and reanalyzed data considered in the 1999 criteria and the 2009 draft. EPA reviewed results from this literature search and reanalysis of previously considered data to identify data from laboratory toxicity tests that quantify the adverse effects of ammonia on freshwater aquatic life (amphibians, fishes, and macroinvertebrates), with particular attention given to tests conducted with freshwater unionid mussels and non-pulmonate snails, since such data were not available for many of these species previously. While unionid mussel species are not prevalent in some waters, such as in the arid west, non-pulmonate snails are broadly distributed across the U.S. Thus, considering that freshwater unionid mussels are among the most sensitive genera in the dataset, and that all states have at least one freshwater unionid mussel or bivalve mollusk, or non-pulmonate snail species, another relatively sensitive mollusk group, native or present in at least some of their waters, EPA is recommending a single national acute and a single national chronic criterion be applied to all waters rather than different criteria based on the presence or absence of mussels.

EPA also conducted a separate search and analysis of any relevant new data specific for freshwater mussels to evaluate whether the existing pH-acute TAN toxicity relationship established in the 1999 update document similarly applies to this group of invertebrates. Based on the results of the literature review, EPA concludes that the same pH and temperature relationships used to account for the influence of these two abiotic factors on ammonia toxicity in the 1999 AWQC document are still applicable (e.g., see *Additional Explanation and Justification Supporting the 2013 Temperature and pH-Dependent Calculations and Criteria Magnitudes* section for additional details, pg. 50).

PROBLEM FORMULATION

Problem formulation provides a strategic framework for water quality criteria development by focusing the effects assessment on the most relevant chemical properties and endpoints. The structure of this effects assessment is consistent with EPA's Guidelines for Ecological Risk Assessment (U.S. EPA 1998)

This ecological effects assessment defines scientifically-defensible water quality criteria values for ammonia under section 304(a)(1) of the Clean Water Act. The goal of the Clean Water Act is to protect and restore the biological, chemical and physical integrity of waters of the U.S. Clean Water Act Section 304(a)(1) requires EPA to develop criteria for water quality that accurately reflect the latest scientific knowledge. These criteria are based solely on data and best professional scientific judgments on toxicological effects. Criteria are developed following the guidance outlined in the Agency's *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (Stephan et al. 1985).

Once Section 304(a) water quality criteria are finalized, states and authorized tribes may adopt the criteria into their water quality standards to protect designated uses of water bodies. States and tribes may also modify the criteria to reflect site-specific conditions or use other scientifically-defensible methods to develop standards. Water quality standards are subsequently approved by EPA.

Overview of Stressor Sources and Occurrence

Ammonia is considered one of the most important pollutants in the aquatic environment not only because of its highly toxic nature, but also its ubiquity in surface water systems (Russo 1985). Ammonia is produced for commercial fertilizers and other industrial applications using a reaction that converts atmospheric nitrogen to ammonia using hydrogen obtained from methane (natural gas) under high heat and pressure; the ammonia gas is then compressed under low temperature and stored in an anhydrous liquid form (Appl 1999). In agriculture, ammonia is used both directly (in anhydrous form), as well as a precursor for other nitrogen-based fertilizers such as ammonium nitrate, ammonium phosphate, urea, and ammonium sulfate (Environment Canada 2010). The agricultural industry uses approximately 90% of the U.S. annual domestic ammonia production (USGS 2004). Ammonia also has numerous industrial applications, including use as a protective atmosphere and as a source of hydrogen in metal finishing and treating applications (e.g., nitriding; Appl 1999), as well as many other uses in the chemical industry including the production of pharmaceuticals (Karolyi 1968) and dyes (Appl 1999). The petroleum industry utilizes ammonia for processing of crude oil and in corrosion protection (U.S. EPA 2004). Ammonia is also used in the mining industry for metals extraction (U.S. EPA 2004). Natural sources of ammonia include the decomposition or breakdown of organic waste matter, gas exchange with the atmosphere, forest fires, animal waste, the discharge of ammonia by biota, and nitrogen fixation processes (Environment Canada 1997; Environment Canada 2010; Geadah 1985).

Ammonia can enter the aquatic environment via anthropogenic sources or discharges such as municipal effluent discharges, agricultural runoff, and natural sources such as nitrogen fixation and the excretion of nitrogenous wastes from animals. While much of the early information regarding lethal concentrations of ammonia was driven by the consequences of ammonia buildup in aquaculture systems (i.e., fish culture ponds, hatchery raceways, and fish holding and transporting tanks), the introduction of ammonia into surface water systems from industrial processes, agricultural runoff, and sewage effluents has received considerable attention since the 1980s (Alabaster and Lloyd 1980; U.S. EPA 1985). Many effluents have to be treated extensively in order to keep the concentrations of ammonia in surface waters from being unacceptably high. In 2011, there were approximately 4.7 million pounds (lbs.) of ammonia documented as discharged from all reporting industries to surface waters (U.S. EPA 2011). In 2010, industrial releases of ammonia to ten large aquatic ecosystems (e.g., Chesapeake Bay, Puget Sound, Great Lakes) were reported to total approximately 1.3 million lbs. (U.S. EPA 2010).

Environmental Fate and Transport of Ammonia in the Aquatic Environment

Ammonia (NH_3) is formed in the natural environment by the fixation of atmospheric nitrogen and hydrogen by diazotrophic microbes, such as cyanobacteria (Latysheva et al. 2012). Trace amounts are also produced by lightning (Noxon 1976). Decomposition of manure, dead plants and animals by bacteria in the aquatic and terrestrial environments produce ammonia and other ammonium compounds through conversion of nitrogen during decomposition of tissues in a process called ammonification (ATSDR 2004; Sylvia 2005). In the aquatic environment, ammonia is also produced and excreted by fish. The chemical form of ammonia in water consists of two species, the more abundant of which is the ammonium ion (NH_4^+) and the less abundant of which is the non-dissociated or unionized ammonia (NH_3) molecule; the ratio of these species in a given aqueous solution is dependent upon both pH and temperature (Emerson et al. 1975; Erickson 1985; Thurston 1988; Whitfield 1974; Wood 1993). Chemically, ammonia in an aqueous medium behaves as a moderately strong base with pK_a values ranging from approximately 9 to slightly above 10 as a function of temperature and ionic strength (Emerson et al. 1975; Whitfield 1974). In general, the ratio of unionized ammonia to ammonium ion in fresh water increases by 10-fold for each rise of a single pH unit, and by approximately two-fold for each 10°C rise in temperature from 0-30°C (Erickson 1985). Basically, as values of pH and temperature tend to increase, the concentration of NH_3 increases and the concentration of NH_4^+ decreases.

The ionized ammonium ion (NH_4^+) and unionized ammonia molecule (NH_3) are interrelated through the chemical equilibrium NH_4^+- OH^-↔ $NH_3 \cdot H_2O$↔ NH_3+H_2O (Emerson et al. 1975; Russo 1985). The concentration of total ammonia (often expressed on the basis of nitrogen as total ammonia nitrogen or TAN) is the sum of NH_4^+ and NH_3 concentrations. It is total ammonia that is analytically measured in water samples. To estimate the relative concentrations of NH_4^+ and NH_3 from total ammonia, Emerson et al.'s (1975) formulas are recommended (Adams and Bealing 1994; Alabaster and Lloyd 1980; Richardson 1997; Russo 1985). Figure 1 (below) shows the chemical speciation of ammonia over a range of pH levels in ambient waters at 25°C. It depicts the 10-fold increase in the ratio of unionized ammonia to ammonium ion in fresh water for each rise of a single pH unit as described above. This increase in unionized ammonia with increased pH is one hypothesis explaining why toxicity of total ammonia increases as pH increases.

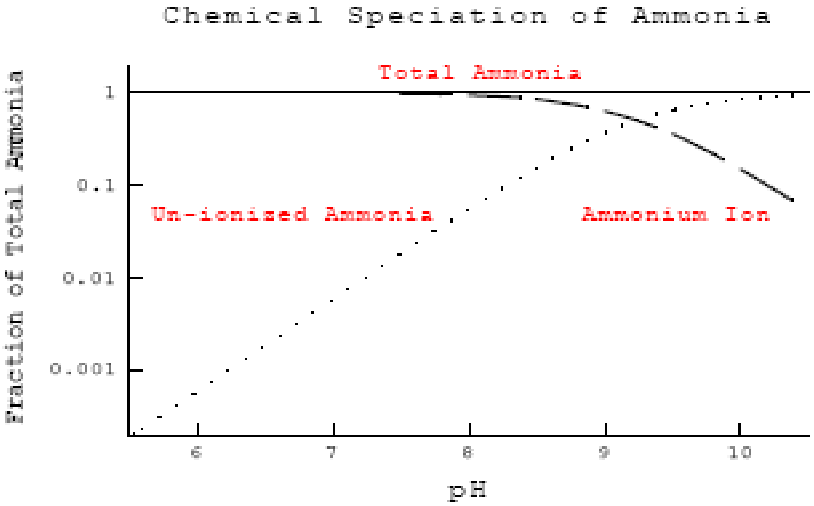

Figure 1. Fraction of Chemical Species of Ammonia Present with Change in pH (at 25°C).

Each separate fraction of total ammonia can be calculated in freshwater from the Henderson-Hasselbach equation if the pH and p*Ka* are known:

NH_4^+ = Total ammonia/(1+ antilog (pH-pKa)) = Total ammonia – NH_3 (Wood 1993)

and,

pKa = 0.09018 + (2729.92/(273.2 + T)) (Emerson et al. 1975)

where T is temperature in °C.

Mode of Action and Toxicity

Ammonia is unique among regulated pollutants because it is an endogenously produced toxicant that organisms have developed various strategies to excrete, which is in large part by passive diffusion of unionized ammonia from internal organs, such as the gills in fish. High external unionized ammonia concentrations reduce or reverse diffusive gradients and cause the buildup of ammonia in internal tissues and blood. Unionized ammonia may cause toxicity to *Nitrosomonas* spp. and *Nitrobacter* spp. bacteria, inhibiting the nitrification process (Russo 1985). Bacterial inhibition can result in the increased accumulation of ammonia in the aquatic environment, thereby intensifying the toxicity to beneficial bacteria and aquatic animals (Russo 1985).

The toxic action of unionized ammonia on aquatic animals, particularly in sensitive fish, may be due to one or more of the following causes: (1) proliferation in gill tissues, increased ventilation rates and damage to the gill epithelium (Lang et al. 1987); (2) reduction in blood oxygen-carrying capacity due to progressive acidosis (Russo 1985); (3) uncoupling oxidative phosphorylation causing inhibition of production and depletion of adenosine triphosphate (ATP) in the brain (Camargo and Alonso 2006); (4) and the disruption of osmoregulatory and circulatory activity disrupting normal metabolic functioning of the liver and kidneys (Arillo et. al.1981; Tomasso et al. 1980).

Among invertebrates, studies testing ammonia toxicity to bivalves, and particularly studies with freshwater mussels in the family Unionidae, have demonstrated their sensitivity to ammonia (Augspurger et al. 2003; Wang et al. 2007a, b; Wang et al. 2008). Toxic effects of unionized ammonia to both freshwater and marine bivalves include reduced opening of valves for respiration and feeding (Epifanio and Srna 1975); impaired secretion of the byssus, or anchoring threads in bivalves (Reddy and Menon 1979); reduced ciliary action in bivalves (U.S. EPA 1985); depletion of lipid and carbohydrate stores leading to metabolic alteration (Chetty and Indira 1995) as well as mortality (Goudreau et al. 1993). These negative physiological effects may lead to reductions in feeding, fecundity, and survivorship, resulting in decreased bivalve populations (Alonso and Camargo 2004; Constable et al. 2003).

Assessment Endpoints

Assessment endpoints are defined as "explicit expressions of the actual environmental value that is to be protected" and are defined by an ecological entity (species, community, or other entity) and its attribute or characteristics (U.S. EPA 1998). Assessment endpoints may be identified at any level of organization (e.g., individual, population, community). In the context of the Clean Water Act, aquatic life criteria for toxics are typically determined based on the results of toxicity tests with aquatic organisms in which unacceptable effects on growth, reproduction, or survival occurred. This information is aggregated into a species sensitivity analysis that evaluates the impact on the aquatic community. Criteria are designed to be protective of the vast majority of aquatic animal species in an aquatic community (i.e., approximately 95th percentile of tested aquatic animals representing the aquatic community). As a result, health of the aquatic ecosystem may be considered as an assessment endpoint indicated by survival, growth, and reproduction. To assess potential effects on the aquatic ecosystem by a particular stressor, and develop 304(a) aquatic life criteria under the CWA, EPA typically requires the following:

- Acute toxicity test data (mortality, immobility, loss of equilibrium) for aquatic animals from a minimum of eight diverse taxonomic groups. The diversity of tested species is intended to ensure protection of various components of an aquatic ecosystem. The acute freshwater toxicity testing requirement is fulfilled with the following eight minimum data requirements:
    - the family Salmonidae in the class Osteichthyes
    - a second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)
    - a third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)
    - a planktonic crustacean (e.g., cladoceran, copepod, etc.)
    - a benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)
    - an insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)

    - a family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
    - a family in any order of insect or any phylum not already represented

- Chronic toxicity test data (longer-term survival, growth, or reproduction) are required for a minimum of three taxa in order to use acute to chronic ratios to estimate a chronic value, which involves having acceptable chronic toxicity data for the following:
    - at least one fish
    - at least one invertebrate
    - at least one chronic test being from an acutely-sensitive species

  However, since acceptable chronic values were available for ammonia for all eight minimum data requirements, the chronic criterion was derived following the same genus level sensitivity distribution (SD) approach used to calculate the acute criterion (see 1985 Guidelines for additional detail).

- The Guidelines also require at least one acceptable test with a freshwater alga or vascular plant. If plants are among the aquatic organisms most sensitive to the material, results of a plant in another phylum should also be available. The data available on the toxicity of ammonia to freshwater plants indicate that plants are approximately two orders of magnitude less sensitive than the aquatic animals tested. Therefore, plant endpoints were not used in criteria derivation.

Measures of Effect

Each assessment endpoint requires one or more “measures of ecological effect,” which are defined as changes in the attributes of an assessment endpoint itself or changes in a surrogate entity or attribute in response to chemical exposure. Ecological effect data are used as measures of direct and indirect effects to biological receptors. The measures of effect selected represent the growth, reproduction, and survival of the organisms.

The amount of toxicity testing data available for any given pollutant varies significantly, depending primarily on whether any major environmental issues are raised due to interpretation of those data. An in-depth evaluation of available data is performed by EPA to determine test acceptability.

Acute measures of effect

Acute measures of effect used for organisms in this document are the LC_{50} and EC_{50}. LC stands for “Lethal Concentration” and the LC_{50} is the concentration of a chemical that is estimated to kill 50% of the test organisms. EC stands for “Effective Concentration” and the EC_{50} is the concentration of a chemical that is estimated to produce a specific effect in 50% of the test organisms.

As part of the evaluation of new acute data for ammonia, studies submitted using glochidia, the larval life stage of freshwater mussels in the family Unionidae, were reviewed for acceptability for use in the ammonia criteria development. In 2006 a new ASTM method was published for toxicity tests with glochidia. However, at the time of the 2009 draft revised criteria for ammonia, EPA and external peer reviewers were concerned that information was unavailable to determine whether the tests with glochidia were ecologically relevant. Specifically, the appropriate duration of the tests (24, 48, or 96 hrs) was uncertain because it was unclear how the tests of various durations related to the viability of this short parasitic life stage and its ability to successfully infect a fish host upon encountering the appropriate fish species. Since that time, studies by Bringolf et al. (2013) have resulted in the recommendation of a maximum test duration of 24 hours for glochidia corresponding with the ecologically relevant endpoint of infectivity for this parasitic life stage. EPA agreed with this recommendation and decided to include glochidia tests in the criterion dataset for test data with durations of up to 24 hours with survival of glochidia at the end of 24 hours of at least 90% in the control treatment. In addition, to account for species of mussels whose glochidia might not be expected to be viable at 24 hours (i.e., potentially mantle lure strategists), EPA examined available tests with glochidia that were conducted for 24 hours that included testing for viability at 6, 12, and 18 hours. If the viability was less than 90% at 24 hours in the control animals, then the next longest duration less than 24 hours that had at least 90% survival in the control, was considered acceptable for use in deriving the ammonia criteria.

Chronic measures of effect

Chronic measures of effect are $EC_{20,}$ NOEC, LOEC, and MATC. EC_{20} values were used to estimate a low level of effect observed in chronic datasets that are available for ammonia (see U.S. EPA 1999). EC_{20} is the concentration of a chemical that is estimated to result in a 20 percent effect in a chronic endpoint (e.g., growth, reproduction, and survival) of the test organisms.

The NOEC (i.e., "No-Observed -Effect-Concentration") is the highest test concentration at which none of the observed effects are statistically different from the control. The LOEC (i.e., "Lowest-Observed- Effect-Concentration") is the lowest test concentration at which observed effects are found to be statistically different from the control. The MATC is the calculated geometric mean of the NOEC and LOEC.

For life-cycle (LC) and partial life-cycle (PLC) tests, the toxicological variables used in regression analyses were survival, embryo production, and embryo hatchability. For early life-stage (ELS) tests with fishes, the endpoints used were embryo hatchability, fry/larval survival, and fry/larval growth. If ammonia reduced both survival and growth, the product of these variables (biomass) was analyzed (when possible), rather than analyzing them separately. For other acceptable chronic and related (e.g., 28-day juvenile or adult) tests, the toxicological endpoints analyzed were survival, reproduction, hatchability, or growth as appropriate.

Regression analysis was used, both to demonstrate that a concentration-effect relationship was present, and to estimate chronic values at a consistent level of effect. Estimates of effect concentrations can generally be made with precision for a 50 percent reduction in response (EC_{50}), but at low percent reductions such precision is decreased. A major reduction, such as 50 percent, is not consistent with the intent of establishing chronic criteria to protect the population from long-term effects. In contrast, a concentration that causes a low level of reduction in response, such as an EC_5 or EC_{10}, is rarely statistically significantly different from the control treatment. EPA selected EC_{20} values to be used to estimate a low level of effect that would be statistically different from control effects, yet not so severe as to be expected to cause chronic impacts at the population level (see U.S. EPA 1999). For calculation of the chronic criterion, the EC_{20} point estimate was selected for use over a NOEC or LOEC as the measure of effect to use, as NOECs and LOECs are highly dependent on test concentrations selected. Furthermore, point estimates provide additional information that is difficult to determine using NOEC and LOEC effect measures, such as a measure of effect level across the range of tested concentrations, and the confidence intervals around those measures of effect.

The typical assessment endpoints for aquatic life criteria are based on unacceptable effects on growth, reproduction, or survival of the assessed taxa. These measures of effect on toxicological endpoints of consequence to populations are provided by results from the acute and chronic toxicity tests with aquatic plants and animals. The toxicity values (i.e., measures of effect expressed as genus means) are used in the genus sensitivity distribution of the aquatic community to derive the aquatic life criteria. Endpoints used in this assessment are listed in Table 1.

Table 1. Summary of Assessment Endpoints and Measures of Effect Used in Criteria Derivation for Ammonia.

Assessment Endpoints for the Aquatic Community	Measures of Effect
Survival, growth, and reproduction of freshwater fish, other freshwater vertebrates, and invertebrates	For acute effects: LC_{50} or EC_{50} For chronic effects: EC_{20}, NOEC and LOEC, calculated MATC
Maintenance and growth of aquatic plants from standing crop or biomass	Not relevant for ammonia because plants are substantially less sensitive than animals

MATC = maximum acceptable toxicant concentration (geometric mean of NOEC and LOEC)
NOEC = No observed effect concentration
LOEC = Lowest observed effect concentration
LC_{50} = Lethal concentration to 50% of the test population
EC_{50}/EC_{20} = Effect concentration to 50/20% of the test population

Chronic averaging period of 30 days

The 30-day averaging period for chronic effects has been retained from the 1999 chronic criterion, as is the restriction that the highest 4-day average within the 30 days may be no greater than 2.5 times the chronic concentration (CCC) more than once every three years on average. This is based on analysis of chronic data from fathead minnow laboratory tests of different exposure durations and starting with different age test organisms as summarized below and described in greater detail in the 1999 ammonia criteria update.

The 1985 ammonia criteria document specified a CCC averaging period of 4 days as recommended in the 1985 Guidelines (Stephan et al. 1985), except that an averaging period of 30 days could be used when exposure concentrations were shown to have "limited variability". For ammonia, the toxicity data on the fathead minnow demonstrate how long the averaging period should be when concentrations have limited variability, and what restriction applies in terms of the maximum concentration that can be reached and for how long within that averaging period. Based on 7-day tests, EC_{20}s of 29.34 and 24.88 mg TAN/L were calculated from the data of Willingham (1987), adjusted to pH 7. Chronic values of 20.32 mg TAN/L at pH 7 and 20.99 mg TAN/L similarly adjusted to pH 7 were reported by Camp Dresser and McKee (1997). The geometric mean of the four values is 23.62 mg TAN/L. This is approximately 2.5 times the geometric mean EC_{20} (i.e., 9.396 mg TAN/L at pH 7) for the 30-day early life-stage tests conducted on the same species by Swigert and Spacie (1983) and Mayes et al. (1986), [see also Appendix B].

Thus, in the 1999 criteria document, EPA determined that because the mean chronic value from the shorter 7-day toxicity tests with slightly older (< 1 day old) fish is substantially higher than the mean chronic value from the longer 30-day ELS tests initiated with newly fertilized embryos, the CCC averaging period under this "limited variability" can be 30 days, as long as excursions above the CCC are restricted sufficiently to not exceed the mean chronic value from the 7-day tests. As indicated in the 1999 AWQC document, a more rigorous definition of this excursion restriction is not possible with the data available, especially because the information is not available concerning the effects to fish or other animals of variations in ammonia concentration within a 7-day test period. It is useful, however, to base the excursion restriction on a 4-day period, because this period is the default that already has to be considered in calculations of water quality-based effluent limits, and because it provides a substantial limitation of variability relative to the 7-day chronic values. While it may be uncertain how much higher than the CCC the 4-day average can be, based on the fathead minnow test results summarized above, 2.5 -fold higher concentrations should be acceptable. Other data and justification supporting the use of a longer averaging period for ammonia and the excursion restriction is provided in the 1999 AWQC document under Chronic Averaging Period (page 81).

Ammonia toxicity data fulfilling minimum data requirements

Table 2 provides a summary of the number of toxicity data currently available for genera and species that fulfill the 1985 Guidelines minimum requirements for calculation of acute and chronic criteria for freshwater species exposed to ammonia.

Table 2. 1985 Guidelines Minimum Data Requirements Summary Table Reflecting the Number of Species and Genus Level Mean Values Represented in the Acute and Chronic Toxicity Datasets for Ammonia in Freshwater.

	Genus Mean Acute Value (GMAV)	**Species Mean Acute Value (SMAV)**	**Genus Mean Chronic Value (GMCV)**	**Species Mean Chronic Value (SMCV)**
Freshwater				
Family Salmonidae in the class Osteichthyes	4	11	1	3
Second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species	22	33	6	7
Third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)	3	4	1	1
Planktonic Crustacean	4	6	2	3
Benthic Crustacean	6	8	1	1
Insect	9	11	1	1
Family in a phylum other than Arthropoda or Chordata (*e.g.*, Rotifera, Annelida, or Mollusca)	17	23	4	5
Family in any order of insect or any phylum not already represented	4	4	1[a]	1[a]
Total	69	100	17	22

[a] In the absence of other chronic data to fulfill this MDR for another phylum not already represented in the chronic dataset, the acute data for species within the phylum Annelida were used to calculate a surrogate chronic value, by applying a geometric mean ACR from the available invertebrate ACRs.

Since the data available regarding the toxicity of ammonia to freshwater phytoplankton and vascular plants reported in the 1985 AWQC document indicate that aquatic plants appear to be two orders of magnitude less sensitive than the aquatic animals tested, it is assumed that any ammonia criterion appropriate for the protection of freshwater aquatic animals will also be protective of aquatic vegetation (U.S. EPA 1985, 1999, 2009). The greater tolerance of these taxa to ammonia is due in part to the fact that ammonia is a readily available and energy-efficient source of nitrogen for plants; although ammonia can be toxic when present at high concentrations. For example, the experimental data concerning the toxicity of ammonia to freshwater phytoplankton show negative effects occurring in the green alga, *Scenedesmus obliquus*, ranging from approximately 26.88 to 70.14 mg TAN/L with regards to oxygen evolution and reduction in carbon dioxide photoassimilation (Abeliovich and Azov 1976). Additionally, ammonia caused growth inhibition and cell death of the green alga, *Chlorella vulgaris*, at concentrations ranging from 326 to 1,330 mg TAN/L (Przytocka-Jusiak 1976); and for another algal species, *Ochromonas sociabilis*, a concentration of 256 mg TAN/L was algicidal while a concentration of approximately half that (128 mg TAN/L) reduced population development (assuming pH 6.5 and 30˚C; see Bretthauer 1978). Furthermore, Champ et al. (1973) investigated the effects of treating a Texas pond with a mean ammonia concentration of 25.6 mg/L NH_3 (unionized ammonia) for two weeks. A diverse population of dinoflagellates, diatoms, desmids, and blue-green algae had been reduced by 95% at the end of the experiment. At the same time, the pond was virtually eradicated of all rooted aquatic vegetation. Compared to the 2013 chronic criterion magnitude of 1.9 mg TAN/L, the results from these plant tests, which are considered as chronic effects according to the 1985 Guidelines, indicate that the 2013 CCC for ammonia will be protective of aquatic plants.

Much of the early work concerning the response of freshwater vegetation to high ammonia concentrations is not quantitative or the result of research exploring the possible use of ammonia as an aquatic herbicide (U.S. EPA 1985). There is no new evidence to suggest that freshwater phytoplankton and vascular species are more sensitive to ammonia than invertebrates or fish. Until such a time as those data are produced, EPA will continue to assume that any ammonia criterion appropriate for the protection of freshwater aquatic animals will also be protective of aquatic vegetation.

Conceptual Model

A conceptual model consists of a written description and diagram (U.S. EPA 1998) that illustrates the relationships between human activities, stressors, and ecological effects on assessment endpoints. The conceptual model links exposure characteristics with the ecological endpoints important for management goals. Under the CWA, these management goals are established by states and tribes as designated uses of waters of the United States (for example, aquatic life support). In deriving aquatic life criteria, EPA is developing acceptable thresholds for pollutants that, if not exceeded, are expected to protect designated uses. A state and/or tribe may implement these criteria by adopting them into their respective water quality standards.

Conceptual diagram

Environmental exposure to ammonia, while ultimately determined by various site specific conditions and processes, occurs from human activities related to agricultural practices, urbanization and industrial processes, or from natural sources. Point and non-point sources contribute to elevated concentrations in ambient surface water. The environmental fate properties of ammonia indicate that direct discharge, runoff, groundwater transport, and atmospheric deposition represent the pathways of greatest transport to the ambient surface waters which serve as habitat for aquatic organisms. These sources and transport mechanisms are depicted in the conceptual model below (Figure 2). The model also depicts exposure pathways for biological receptors of concern (e.g., aquatic animals) and the potential attribute changes (i.e., effects such as reduced survival, growth and reproduction) in the ecological receptors due to ammonia exposure.

The conceptual model provides a broad overview of how aquatic organisms can potentially be exposed to ammonia. Transport mechanisms and exposure pathways are not quantitatively considered in the derivation of aquatic life criteria, which are effects assessments, not risk assessments. Derivation of criteria focuses on effects on survival, growth and reproduction of aquatic organisms. However, the pathways, receptors, and attribute changes depicted in Figure 2 may be helpful for states and tribes as they adopt criteria into standards and need to evaluate potential exposure pathways affecting designated uses.

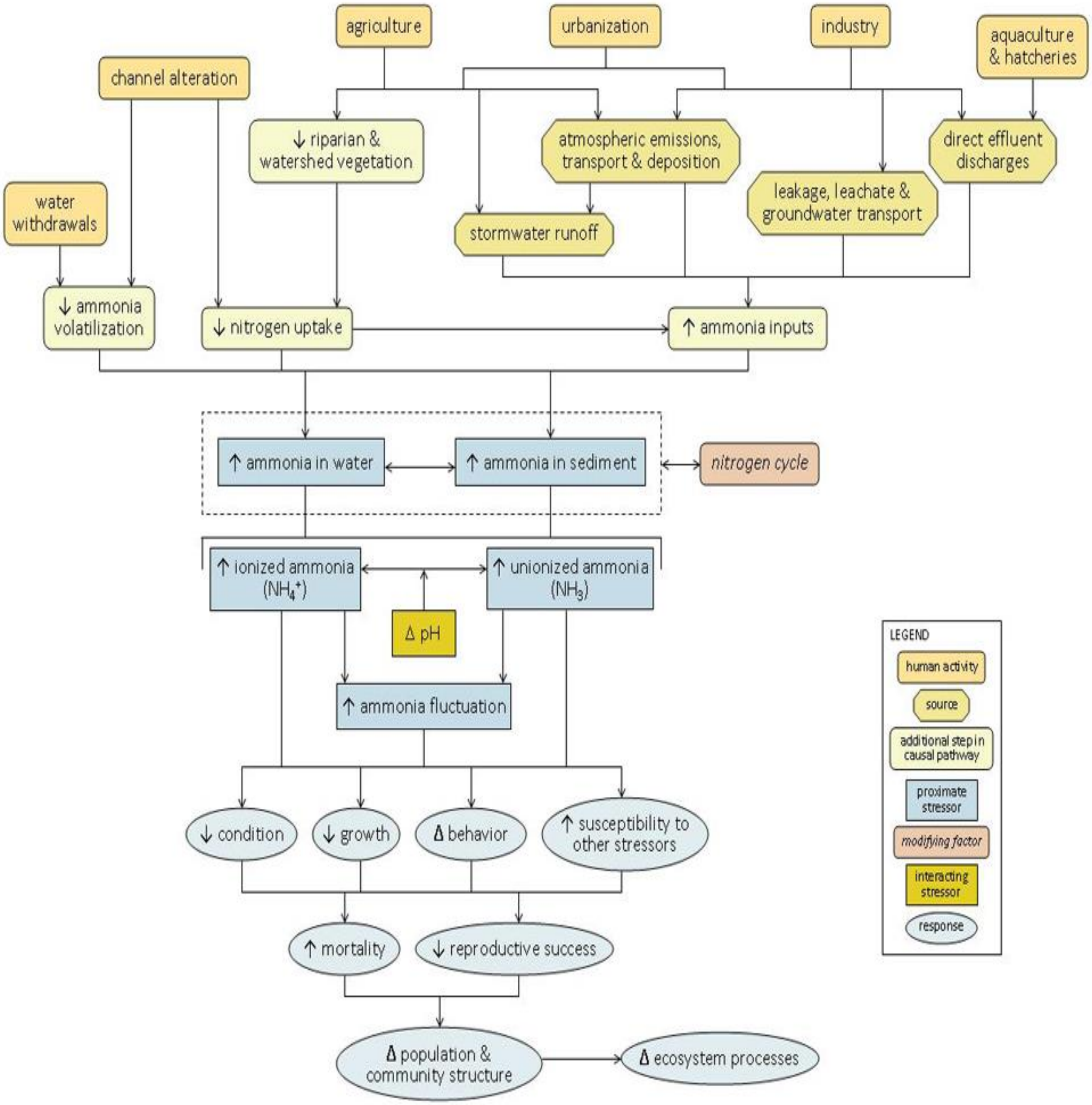

Figure 2: Conceptual Model for Ammonia Effects on Aquatic Animals.
(Available at: http://www.epa.gov/caddis/ssr_amm_int.html)

Analysis Plan

During development of CWA section 304(a) criteria, EPA assembles all available toxicity test data and considers which data are relevant that also meet data quality acceptance standards for all genera. Where data allow, two to four criterion values are developed (acute and chronic freshwater, acute and chronic saltwater). If plants are the most sensitive relative to vertebrates and invertebrates, plant criteria are developed. This criteria update document is specific to ammonia in fresh water, and thus, only two criterion values (freshwater acute and chronic) are derived in this document. Available data indicate freshwater plants are not more sensitive to ammonia than freshwater animals, thus, plant criteria are not developed. Finally, ammonia does not bioaccumulate in aquatic animals, thus, final tissue values are not developed.

These criteria are based on a sensitivity distribution (SD) comprised of ranked genus mean acute values (GMAVs), calculated from combined species mean acute values (SMAVs within each genus) for acceptable data. SMAVs are calculated using the geometric mean for all acceptable measures of effect based on the results of toxicity tests within a given species (e.g., all EC_{50}s from acceptable acute tests for *Daphnia magna*). GMAVs are then calculated using the geometric means of all SMAVs within a given genus (e.g., all SMAVs for genus Daphnia, such as *Daphnia pulex, Daphnia magna*). If only one SMAV is available for a genus, then the GMAV is represented by that value. GMAVs are then rank-ordered by sensitivity from most sensitive to least sensitive. The final acute value (FAV) is determined by regression analysis using a log-triangular fit based on the four most sensitive genera (reflected as GMAVs) in the data set to interpolate or extrapolate (as appropriate) to the 5th percentile of the distribution represented by the tested genera. If there are 59 or more GMAVs, as is the case with ammonia, the four GMAVs closest to the 5th percentile of the distribution are used to calculate the FAV. The acute criterion magnitude is the FAV divided by two, in order to provide an acute criterion magnitude protective of nearly all individuals in 95% of all genera, since the effect endpoint is a 50th percentile effect (e.g., LC_{50} or EC_{50}) (see 1985 Guidelines, Section XI. Criterion, B.).

Although the aquatic life criteria derivation process relies on selected toxicity endpoints from the sensitive species tested, it does not necessarily mean that the selected toxicity endpoints reflect the sensitivity of the most sensitive species existing in a given environment. The intent of the eight minimum data requirements is to serve as a sample representative of the aquatic community. These minimum data requirements represent different ecological, trophic, taxonomic and functional differences observed in the natural aquatic ecosystem. The use of the four most sensitive genera to determine the final criterion value is a censored statistical approach than improves estimation of the lower tail (most sensitive) of the distribution when the shape of the overall distribution, particularly in the less sensitive part of the distribution, is uncertain.

The chronic criterion may be determined by one of two methods. If all eight minimum data requirements are met with acceptable chronic test data (as is the case with ammonia), then the chronic criterion is derived using the same method used for the acute criterion. Genus Mean Chronic Values (GMCVs) are derived from available Species Mean Chronic Values (SMCVs) and are then rank-ordered from least to most sensitive, and the Final Chronic Value (FCV) is calculated based on regression analysis of a censored distribution using the four most sensitive GMCVs, similar to calculation of the FAV. Unlike the FAV, however, the FCV directly serves as the basis for the chronic criterion without further adjustment because the endpoint measured represents a low level (e.g., EC_{20} or NOEC) of effect (see 1985 Guidelines).

In addition, whenever adequately justified, a state can develop a site-specific criterion in lieu of the use of a national recommended criterion (U.S. EPA 1983). The site-specific criterion may include not only site-specific criterion concentrations, but also site-specific durations or averaging periods, site-specific frequencies of allowed excursions, and representative species present at a given site, where supported by sound science (U.S. EPA 1991). The *Revised Deletion Process for the Site-Specific Recalculation Procedure for Aquatic Life Criteria* (U.S. EPA 2013) provides guidance on revising the taxonomic composition of the toxicity data set used for the sensitivity distribution upon which a site-specific criterion is based, in order to better reflect the assemblage of organisms that resides at the site. For more information on criteria derivation, see: *http://water.epa.gov/scitech/swguidance/waterquality/standards/upload/2009_01_13_criteria_85guidelines.pdf*.

The criteria presented are the Agency’s best estimate of maximum ambient concentrations of ammonia to protect most freshwater aquatic organisms from unacceptable short- or long-term effects. Results of intermediate calculations such as Species Mean Acute Values (see in Appendix A) and chronic values (see in Appendix B) are specified to four significant figures to prevent rounding error in subsequent calculations, not to reflect the precision of the value. All of the ammonia acute values (LC_{50}s and EC_{50}s) in Appendix A of this document were converted to TAN acute values using the reported temperatures and pHs as described using an example in Appendix D (Conversion of Acute Results of Toxicity Tests). Similarly, all of the ammonia chronic values (EC_{20}s) in Appendix B were converted to TAN chronic values as described in Appendix E (Conversion of Chronic Results of Toxicity Tests).

EFFECTS ANALYSES FOR FRESHWATER AQUATIC ORGANISMS

The acute and chronic ammonia toxicity data used here to update the acute and chronic criteria for ammonia (freshwater) were collected via literature searches of EPA's ECOTOX database, EPA's Ambient Aquatic Life Water Quality Criteria for Ammonia (U.S. EPA 1985, 1998, 1999), data provided by the U.S. Fish and Wildlife Service and the National Oceanic and Atmospheric Administration (NOAA) National Marine Fisheries Service (collectively known as the Services), and EPA regional and field offices. Relevant papers were identified, by title and abstract, and their data screened according to data quality criteria described in the 1985 Guidelines. All available, reliable acute and chronic toxicity values published since 1985 were incorporated into the appropriate ammonia AWQC tables and used to recalculate the CMC and the CCC, as outlined in detail in the 1985 Guidelines. The most recent literature search covered the period from 1985 through October 2012.

Acute Toxicity to Aquatic Animals

All available data relating to the acute effects of ammonia on aquatic animals were considered in deriving the ammonia criteria and were subjected to a data quality review per the 1985 Guidelines. The acute effects concentrations are all normalized to pH 7.0 (for all organisms) and temperature 20°C (for invertebrates) as indicated via the equations provided in Appendix D. The pH and temperature conditions to which these data are normalized were deemed to be generally representative of ambient surface water. Data that were suitable for the derivation of a freshwater FAV are presented in Appendix A**.**

The GMAVs ranked according to sensitivity, as well as the new (2013) and previous (1999) acute criterion values (CMCs), are shown in Figure 3. The GMAVs represent LC_{50}s or EC_{50}s, whereas the CMC (the FAV/2) values represent concentrations that are expected to be lethal to less than 50% of the individuals in either the fifth percentile genus, or, a sensitive commercially or recreationally important species (e.g., adult rainbow trout).

For this 2013 AWQC document, results from acute toxicity tests that met test acceptability and quality (according to the 1985 Guidelines) were available for 44 species of fish, 52 species of invertebrates and four species of amphibians. This data includes ammonia toxicity test data on 52 new species of aquatic animals not previously included in the 1999 acute criterion dataset. There are now 69 genera represented in the freshwater acute toxicity dataset for ammonia, and of the 69 genera (represented in Appendix A and listed according to sensitivity in Table 3), approximately half are invertebrates. The acute dataset more than fulfills the eight minimum data requirements outlined in the 1985 Guidelines with between three and 22 genera represented for each taxa category specified (see Table 2 above). The acute criterion dataset now includes 12 species of aquatic animals Federally-listed as threatened, endangered or species of concern. Freshwater invertebrates in the Phylum Mollusca, particularly freshwater mussels in the family Unionidae, freshwater clams, and some non-pulmonate snails, are the predominant group of aquatic organisms ranked in the lowest quartile. The four most acutely sensitive genera are all freshwater bivalve mussels (Table 3). GMAVs for freshwater mollusks in general, are now among the most influential in the 2013 acute criterion dataset.

Data for glochidia and juvenile life stages of freshwater unionid mussels were evaluated for acceptability based on the 1985 Guidelines, the approved ASTM protocol for toxicity testing with these life stages of unionid mussels (ASTM 2006), and recent studies on the most ecologically relevant toxicological endpoint(s) and exposure duration(s) for glochidia tests by Bringolf et al. (2013). The acute unionid mussel dataset for ammonia now includes acceptable data for 11 genera, totaling 16 species of freshwater mussels, as well as two sensitive species of non-pulmonate snails. Of these, four of the 18 mollusk species included in 2013 acute dataset are Federally-listed as threatened or endangered (as identified in Table 3).

Nearly all states in the continental United States have freshwater unionid mussel fauna in at least some of their waters (Abell et al. 2000; Williams et al. 1993; Williams and Neves 1995). While the number of freshwater unionid mussel species is less and the distribution is sparse in the dry western states, even New Mexico and Arizona have at least one native mussel species (Williams et al. 1993). Moreover, approximately one-quarter of nearly 300 freshwater unionid mussel taxa in the USA are Federally-listed as endangered, threatened or of special concern. In addition, non-pulmonate snails are relatively ubiquitous compared to mussels and of the 650 freshwater snail species, 25 species are Federally-listed. Every state in the continental U.S. has at least one family of non-pulmonate snail in at least some of their waters. Thus, considering that freshwater unionid mussels are among some of the most sensitive genera in the dataset, and that all states have at least one freshwater unionid mussel or bivalve mollusk, or non-pulmonate snail species, another relatively sensitive mollusk group, native or present in at least some of their waters, EPA is recommending a single national acute criterion to be applied to all waters rather than different criteria based on the presence or absence of mussels.

The most sensitive fish SMAV is for mountain whitefish, *Prosopium williamsoni* (SMAV of 51.93 mg TAN/L), representing one of the four genera of salmonids in the acute dataset, followed by the second most sensitive fish, the Lost River sucker (SMAV of 56.62 mg TAN/L), which is an endangered species (Table 3). The mountain whitefish GMAV is ranked eighth most sensitive after seven more sensitive GMAVs for freshwater mussel species, thus, salmonids should be adequately protected by the new acute criterion. The next most sensitive salmonid genus is Oncorhynchus, represented by data for six different species, three of which are threatened or endangered, with SMAVs ranging from 78.92 mg TAN/L for Cutthroat trout, *O. clarkii*, to 180.7 mg TAN/L for pink salmon, *O. gorbuscha.* The GMAV for Oncorhynchus (99.15 mg TAN/L) is ranked #25 in acute sensitivity rank at pH 7 and temperature 20°C (Table 3).

The four lowest GMAVs in this 2013 ammonia AWQC update are for invertebrate species (specifically, freshwater bivalve mollusks dominated by mussels in the family Unionidae). Because the most sensitive GMAVs are all represented by invertebrate species, the CMC is both pH-dependent, in accordance with the acute pH-toxicity relationship for all aquatic organisms, and temperature-dependent, due to the invertebrate acute-temperature relationship.

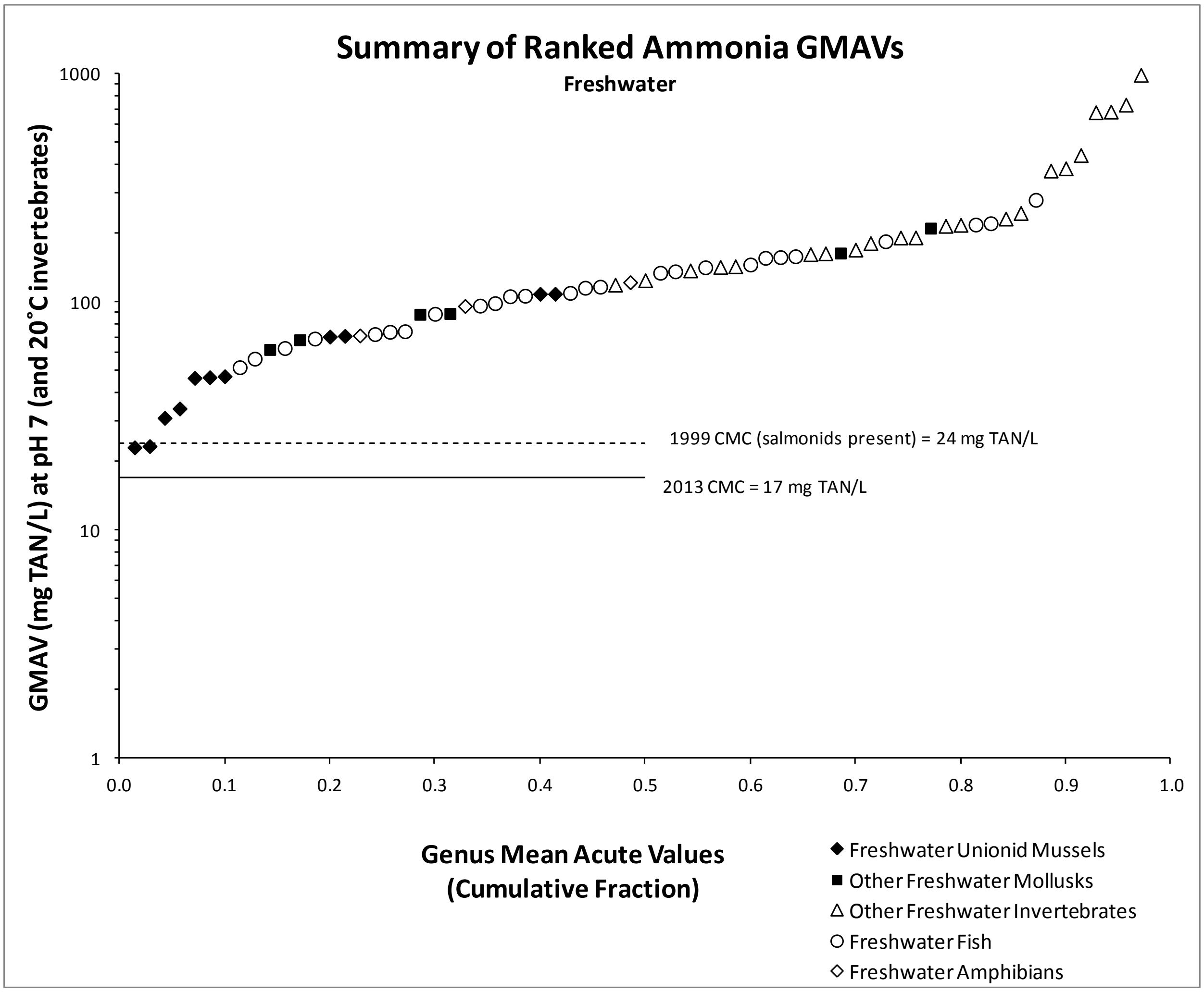

Figure 3. Ranked Freshwater Genus Mean Acute Values (GMAVs) with Criterion Maximum Concentrations (CMCs).

Summaries of studies used in acute criterion determination

Presented in this section are brief summaries of the results of acute toxicity tests that meet the data quality acceptability criteria and that are used directly for deriving the FAV (i.e., serve as the basis for the SMAV or GMAV of one of the most sensitive genera). As per the 1985 Guidelines, whenever there are 59 or more GMAVs in the acute criteria dataset, the FAV is calculated using the four GMAVs closest to the 5th percentile of the distribution.

The four species and associated endpoints (SMAV or GMAV) used in calculating the acute criterion (sensitivity rank 2-5) are ranked below from most to least sensitive:

2. *Lasmigona subviridis*, Green Floater (GMAV= 23.41 mg TAN/L)
3. *Epioblasma capsaeformis*, Oyster mussel (GMAV= 31.14 mg TAN/L)
4. *Villosa iris*, Rainbow Mussel (GMAV= 34.23 mg TAN/L)
5. *Lampsilis* sp. (GMAV=46.63 mg TAN/L)

The most sensitive species *Venustaconcha ellipsiformis* (SMAV=23.12 mg TAN/L) is not included in the criteria numeric calculation, because it falls below the 5th percentile in sensitivity in the distribution of 69 genera included in the dataset.

Summaries are provided on the basis of individual species or genera (in cases where more than one species is included in the calculation of the GMAV). All values are provided in terms of total ammonia nitrogen (TAN), either as reported by the authors or as converted from the reported values for unionized ammonia, pH, and temperature (using the speciation relationship) applied in the 1999 AWQC document (i.e., Emerson et al. 1975). In the special cases where the result of a test is considered an upper limit on an acute value, the value is ascribed a greater than ("\>") sign indicating as much.

Lasmigona subviridis (green floater)

The GMAV/SMAV for the green floater, a freshwater bivalve mollusk, of 23.41 mg TAN/L is based on the geometric mean of three 96-hr EC_{50}s from tests using less than two-month old juveniles as reported in Black (2001). Test solutions were renewed after 48 hours. The mean pH and test temperature for two of the tests was 7.73 and 24°C, and for the third, 7.92 and 24.8°C. Control survival exceeded 90 percent in all three tests. The reported EC_{50}s at test temperature and pH expressed on the basis of TAN were 6.613, 6.613 and 3.969 mg TAN/L, respectively. Adjusted to pH 7 and 20°C, the EC_{50}s are 24.24, 24.24 and 21.84 mg TAN/L, respectively (Appendix A). The GMAV for juvenile green floaters of 23.41 mg TAN/L represents the second lowest in the acute dataset, and the lowest of the four GMAVs used to calculate the FAV (Table 3).

Epioblasma capsaeformis (oyster mussel)

The GMAV/SMAV for the endangered oyster mussel (31.14 mg TAN/L) is the third lowest in the acute dataset (Table 3), and is based on the geometric mean of a 96-hr EC_{50} from a renewal test using less than five-day old juveniles, and two 6-hr EC_{50}s from static tests conducted with two-hour old glochidia (Wang et al. 2007b). The mean pH and test temperature for all three tests was 8.5 and 20°C. Control survival exceeded 90 percent in all tests. The estimated measured EC_{50} for juvenile oyster mussels at test temperature and pH was 4.760 mg TAN/L, after adjusting the reported nominal EC_{50} by multiplying by a factor of 0.835 (i.e., measured total ammonia concentrations were 83.5 percent of nominal concentrations for 96 hour juvenile exposures). The reported EC_{50}s for glochidia were 3.4 and 5.0 mg TAN/L, respectively (no further adjustment necessary). These EC_{50}s normalized to pH 7 and 20°C are 53.63, 17.81 and 31.61 mg TAN/L, for the two glochidia and juveniles respectively (Appendix A).

Villosa iris (rainbow mussel)

Ten EC_{50}s from several studies (Goudreau et al. 1993; Scheller 1997; Mummert et al. 2003; Wang et al. 2007b) using two different life stages (glochidia and juvenile) and range of ages within each life-stage were used to calculate the GMAV/SMAV for rainbow mussel (Appendix A). All tests were either static or static renewal where concentrations were measured. The GMAV of 34.23 mg TAN/L is the fourth lowest in the acute dataset (Table 3), and is composed of individual EC_{50} values (expressed as TAN and normalized to pH 7 and 20°C) ranging from 12.62 to 99.28 mg TAN/L (Appendix A). The difference in pH and test temperature among the 10 different tests ranged from 7.29 to 8.40 and 12.6 to 25.0°C, respectively. Control survival exceeded 90 percent in all tests regardless of life-stage tested. The glochidia were not substantially more sensitive than the juveniles (less than a factor of 2 difference).

Mussels in Genus *Lampsilis*

Freshwater unionid mussels within the Genus *Lampsilis* represent the most widely tested genus to date. The GMAV of 46.63 mg TAN/L reflects the geometric mean of SMAVs for six species, two (*Lampsilis abrupta* and *L. higginsii*) which are endangered and a third (*L. rafinesqueana*) that is a Federal species of concern (Table 3). The SMAVs for this genus range from 26.03 mg TAN/L (*L. abrupta*) to 69.97 mg TAN/L (*L. rafinesqueana*), and are composed of anywhere from one (*L. abrupta*) to fourteen (*L. siliquoidea*) individual EC_{50}s (Appendix A). The range of EC_{50}s used to calculate the FAV, normalized to pH 7 and 20°C across all species of

Lampsilis is from 24.30 to 160.5 mg TAN/L (see Appendix A). The GMAV for *Lampsilis* is the fifth most sensitive in the acute dataset, and the highest of the four GMAVs used to calculate the FAV (Table 3). Both glochidia and juvenile data were available for three of the six *Lampsilis* species, showing an inconsistent pattern of relative sensitivity.

Table 3. Ranked Genus Mean Acute Values.

Table 3. Ranked Genus Mean Acute Values

Rank	**GMAV (mg TAN/L)**	**Species**	**SMAV (mg TAN/L)**
69	2515	Insect, *Erythromma najas*	2515
68	994.5	Caddisfly, *Philarctus quaeris*	994.5
67	735.9	Beetle, *Stenelmis sexlineata*	735.9
66	686.2	Crayfish, *Orconectes immunis*	1550
		Crayfish, *Orconectes nais*	303.8
65	681.8	Midge, *Chironomus riparius*	1029
		Midge, *Chironomus tentans*	451.8
64	442.4	Mayfly, *Drunella grandis*	442.4
63	387.0	Aquatic sowbug, *Caecidotea racovitzai*	387.0
62	378.2	Isopod, *Asellus aquaticus*	378.2
61	281.5	Threespine stickleback, *Gasterosteus aculeatus*	281.5
60	246.5	Mayfly, *Callibaetis skokianus*	364.6
		Mayfly, *Callibaetis* sp.	166.7
59	233.0	Dragonfly, *Pachydiplax longipennis*	233.0
58	222.2	Mottled sculpin, *Cottus bairdii*	222.2
57	219.3	Western mosquitofish, *Gambusia affinis*	219.3
56	218.7	Oligochaete worm, *Lumbriculus variegatus*	218.7
55	216.5	Tubificid worm, *Tubifex tubifex*	216.5
54	211.6	Marsh ramshorn snail, *Planorbella trivolvis*	211.6

Table 3. Ranked Genus Mean Acute Values

Rank	GMAV (mg TAN/L)	Species	SMAV (mg TAN/L)
53	192.6	Scud, *Hyalella azteca*	192.6
52	192.4	Stonefly, *Skwala americana*	192.4
51	185.2	Mozambique tilapia, *Oreochromis mossambicus*	185.2
50	181.8	Amphipod, *Crangonyx pseudogracilis*	270.5
		Amphipod, *Crangonyx* sp.	122.2
49	170.2	Tubificid worm, *Limnodrilus hoffmeisteri*	170.2
48	164.5	Pouch snail, *Physa gyrina*	164.5
47	164.0	Damselfly, *Enallagma* sp.	164.0
46	162.6	Water flea, *Chydorus sphaericus*	162.6
45	159.2	Fathead minnow, *Pimephales promelas*	159.2
44	157.8	Brook trout, *Salvelinus fontinalis*	156.3
		Lake trout, *Salvelinus namaycush*	159.3
43	156.7	Shortnose sturgeon, *Acipenser brevirostrum (LS)*	156.7
42	146.5	White sucker, *Catostomus commersonii*	157.5
		Mountain sucker, *Catostomus platyrhynchus*	136.2
41	143.9	Water flea, *Ceriodaphnia acanthine*	154.3
		Water flea, *Ceriodaphnia dubia*	134.2
40	142.9	Water flea, *Simocephalus vetulus*	142.9
39	142.4	Channel catfish, *Ictalurus punctatus*	142.4
38	138.0	Red swamp crayfish, *Procambarus clarkii*	138.0
37	136.7	Atlantic salmon, *Salmo salar (LS)*	183.3
		Brown trout, *Salmo trutta*	102.0
36	134.8	White perch, *Morone americana*	132.7
		White bass, *Morone chrysops*	144.0

Table 3. Ranked Genus Mean Acute Values

Rank	GMAV (mg TAN/L)	Species	SMAV (mg TAN/L)
		Striped bass, *Morone saxatilis*	246.2
		Sunshine bass, *Morone saxatilis x chrysops*	70.22
35	125.0	Water flea, *Daphnia magna*	157.7
		Water flea, *Daphnia pulicaria*	99.03
34	122.5	Clawed toad, *Xenopus laevis*	122.5
33	119.5	Flatworm, *Dendrocoelum lacteum*	119.5
32	117.1	Walleye, *Sander vitreus*	117.1
31	115.9	Central stoneroller, *Campostoma anomalum*	115.9
30	110.0	Rainbow dace, *Cyprinella lutrensis*	196.1
		Spotfin shiner, *Cyprinella spiloptera*	83.80
		Steelcolor shiner, *Cyprinella whipplei*	80.94
29	109.0	Dwarf wedgemussel, *Alasmidonta heterodon (LS)*	109.0
28	109.0	Pink papershell, *Potamilus ohiensis*	109.0
27	106.9	Green sunfish, *Lepomis cyanellus*	150.8
		Pumpkinseed, *Lepomis gibbosus*	77.53
		Bluegill, *Lepomis macrochirus*	104.5
26	106.3	Common carp, *Cyprinus carpio*	106.3
25	99.15	Golden trout, *Oncorhynchus aguabonita*	112.1
		Cutthroat trout, *Oncorhynchus clarkii*	78.92
		Pink salmon, *Oncorhynchus gorbuscha*	180.7
		Coho salmon, *Oncorhynchus kisutch (LS)*	87.05
		Rainbow trout, *Oncorhynchus mykiss (LS)*	82.88
		Chinook salmon, *Oncorhynchus tshawytscha (LS)*	82.39
24	96.72	Topeka shiner, *Notropis topeka (LS)*	96.72

Table 3. Ranked Genus Mean Acute Values

Rank	GMAV (mg TAN/L)	Species	SMAV (mg TAN/L)
23	96.38	Leopard frog, *Rana pipiens*	96.38
22	89.36	Long fingernailclam, *Musculium transversum*	89.36
21	89.06	Smallmouth bass, *Micropterus dolomieu*	150.6
		Largemouth bass, *Micropterus salmoides*	86.02
		Guadalupe bass, *Micropterus treculii*	54.52
20	88.62	Great pond snail, *Lymnaea stagnalis*	88.62
19	74.66	Guppy, *Poecilia reticulata*	74.66
18	74.25	Johnny darter, *Etheostoma nigrum*	71.45
		Orangethroat darter, *Etheostoma spectabile*	77.17
17	72.55	Rio Grande silvery minnow, *Hybognathus amarus*	72.55
16	71.56	Spring peeper, *Pseudacris crucifer*	61.18
		Pacific tree frog, *Pseudacris regilla*	83.71
15	71.25	Mucket, *Actinonaias ligamentina*	63.89
		Pheasantshell, *Actinonaias pectorosa*	79.46
14	70.73	Giant floater mussel, *Pyganodon grandis*	70.73
13	69.36	Shortnose sucker, *Chasmistes brevirostris*	69.36
12	68.54	Pagoda hornsnail, *Pleurocera uncialis*	68.54
11	63.02	Golden shiner, *Notemigonus crysoleucas*	63.02
10	62.15	Pebblesnail, *Fluminicola* sp.	62.15
9	56.62	Lost River sucker, *Deltistes luxatus(LS)*	56.62
8	51.93	Mountain whitefish, *Prosopium williamsoni*	51.93
7	47.40	Atlantic pigtoe, *Fusconaia masoni*	47.40
6	46.93	Pondshell mussel, *Utterbackia imbecillis*	46.93
5	46.63	Pink mucket, *Lampsilis abrupta (LS)*	26.03

Table 3. Ranked Genus Mean Acute Values			
Rank	GMAV (mg TAN/L)	Species	SMAV (mg TAN/L)
		Plain pocketbook, *Lampsilis cardium*	50.51
		Wavy-rayed lampmussel, *Lampsilis fasciola*	48.11
		Higgin's eye, *Lampsilis higginsii (LS)*	41.90
		Neosho mucket, *Lampsilis rafinesqueana (LS)*	69.97
		Fatmucket, *Lampsilis siliquoidea*	55.42
4	34.23	Rainbow mussel, *Villosa iris*	34.23
3	31.14	Oyster mussel, *Epioblasma capsaeformis (LS)*	31.14
2	23.41	Green floater, *Lasmigona subviridis*	23.41
1	23.12	Ellipse, *Venustaconcha ellipsiformis*	23.12
FAV = 33.52			
CMC = 17			

LS = Federally-listed as threatened or endangered species

Chronic Toxicity to Freshwater Aquatic Animals

Freshwater chronic toxicity data that meet the test acceptability and quality assurance/control criteria are presented in Appendix B. All tests were conducted with measured concentrations of ammonia. Ammonia chronic toxicity data are available for 21 species of freshwater organisms: ten invertebrate species (mussels, clam, snail, cladocerans, daphnid, and insect) and 11 fish species, including three Federally-listed salmonid species. The chronic dataset includes data for three freshwater unionid mussel species, one freshwater non-pulmonate snail species, and two fish species not included in the 1999 criteria (see Appendix B). It also includes an estimate of chronic effects for the Phylum Annelida, to meet the data requirement of a species in “a family in any order of insect or any phylum not already represented,” as described below.

Each chronic test was reviewed to determine acceptability based on the dilution water, control mortality, experimental design, organism loading, etc., as consistent with ASTM standards, including for freshwater mussels via E2455-06 (ASTM 2006). The concentration of dissolved oxygen was also reviewed to determine acceptability based on the general limits specified in the 1999 AWQC document. The mean measured dissolved oxygen concentration and the lower limit for dissolved oxygen concentration required to be protective varies based on taxa group. The mean dissolved oxygen concentration for toxicity tests should be at least 6.5 mg/L for salmonids, 6.0 mg/L for invertebrates, and the lower limit of dissolved oxygen should be 5.0 mg/L to be protective of both of these groups of organisms (U.S. EPA 1999).

Based on the determination that the test methodology used was acceptable, the studies were evaluated to determine whether the ammonia caused a reduction in (a) survival (if over a period of at least seven days), (b) growth, or (c) reproduction. If the test demonstrated reduction in any of these toxicological endpoints, the test could be accepted for use in calculating the chronic value (CV).

Acceptable 28-day survival tests using juvenile freshwater mussels and juvenile freshwater snails and growth tests using juvenile freshwater snails were evaluated for inclusion in the derivation of the chronic aquatic life criterion when the test concentration caused a reduction in survival or growth of 20 percent or more of these types of organisms at those life stages. Based on evaluation of the individual studies (Wang et al. 2007a; Wang et al. 2011), growth data for juvenile mussels was not used in the derivation of the chronic criterion due to uncertainty in method of measurement for the growth endpoint (see Effects Characterization for further discussion).

All chronic data in individual studies were analyzed using regression analysis to demonstrate the presence of a concentration-effect relationship within the test. For those studies that demonstrated a concentration-effect relationship, EPA used regression analysis to estimate the EC_{20}.

Sixteen GMCVs are presented in Appendix B and ranked according to sensitivity in Table 4. The four lowest values were used to calculate the FCV, because values for fewer than 59 genera exist. EPA calculated the chronic criterion based on fifth percentile of the GMCVs in Table 4. The GMCVs for the four most sensitive species are ranked below from most to least sensitive:

1. *Lampsilis* spp, Wavy-rayed lamp mussel and Fatmucket (GMCV=2.126 mg TAN/L*)*
2. *Villosa iris*, Rainbow mussel (GMCV= 3.501 mg TAN/L)

3. *Lepomis* spp., Bluegill and Green sunfish (GMCV= 6.920 mg TAN/L)
4. *Musculium transversum,* Long fingernailclam (GMCV= 7.547 mg TAN/L)

The chronic criterion magnitude is 1.9 mg TAN/L at 20°C and pH 7. The four most sensitive species are predominantly mollusks although *Lepomis* species (bluegill and green sunfish) comprise the third most sensitive GMCV. Figure 4 shows the GMCVs ranked according to sensitivity and shows the 2013 chronic criteria magnitude as well as the 1999 criterion value (based on fish early life stages) for comparative purposes.

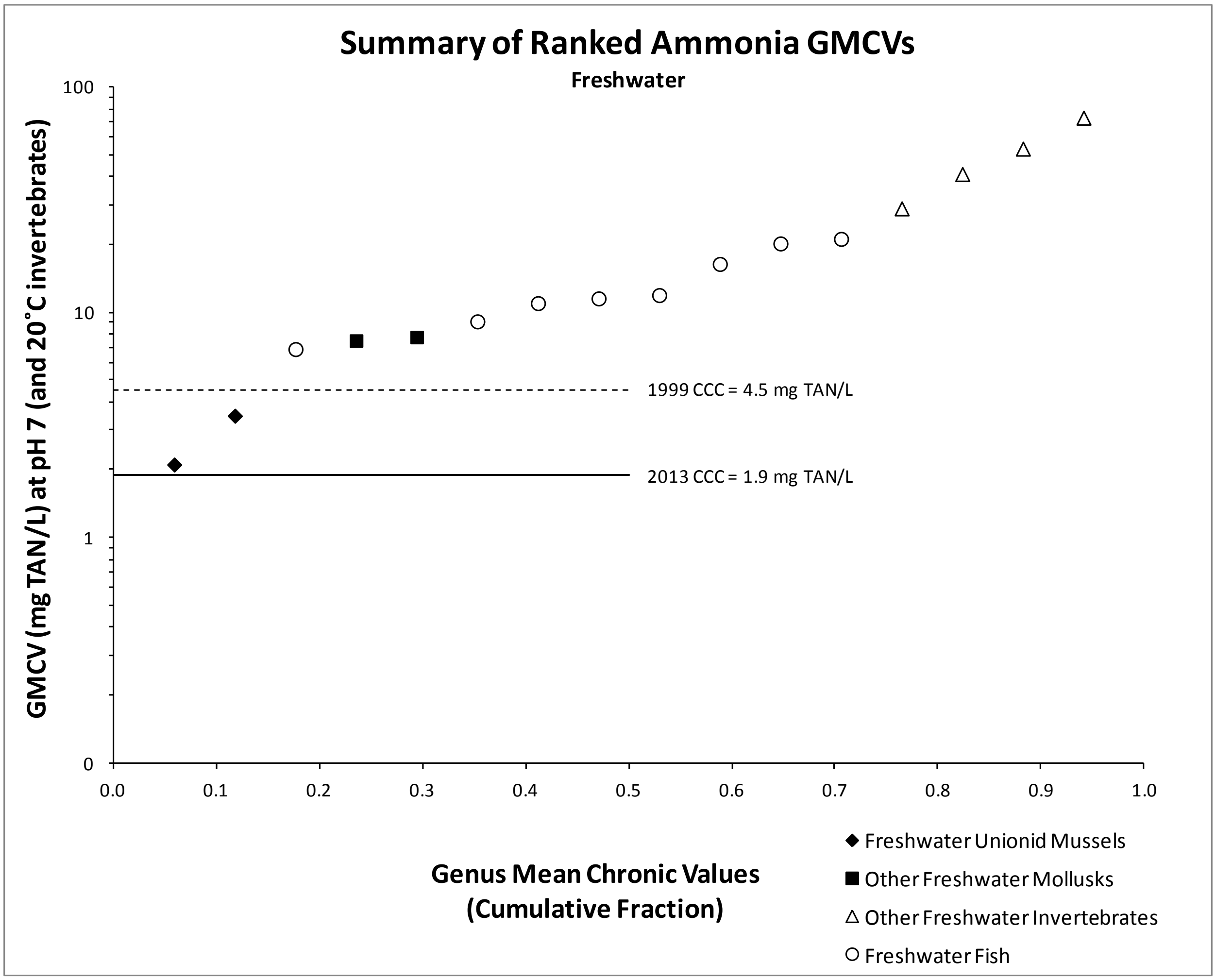

Figure 4. Ranked Freshwater Genus Mean Chronic Values (GMCVs) with Criterion Continuous Concentrations (CCCs).

Summaries of studies used in chronic criterion determination

The following presents a species-by-species discussion of freshwater chronic data used in deriving the chronic criterion magnitude for ammonia. All analyses were conducted in terms of TAN, either as reported by the authors or as converted from the reported values for unionized ammonia, pH, and temperature (using the speciation relationship in Emerson et al. 1975). EC_{20} values were adjusted to pH 7, and for invertebrates, also adjusted to a temperature of 20°C. SMCVs were used when data were available for only one species. When data for more than one species in a taxon were available, GMCVs were calculated from the SMCVs. All of the CVs (EC_{20} values), SMCVs, and GMCVs derived are tabulated and included in Appendix B. For some of the new chronic data, authors reported EC_{20} values on the basis of TAN. In such cases these reported CVs were normalized to pH 7 and 20°C (temperature normalization for invertebrates only), and utilized for the analysis. The results of all intermediate calculations such as ECs, SMCVs and GMCVs are given to four significant figures to prevent round-off error in subsequent calculations, not to reflect the precision of the value.

Lampsilis species

Lampsilis fasciola (wavy-rayed lampmussel)

Wang et al. (2007a) published results of the effect of ammonia on survival and growth of 2-month old juvenile freshwater unionid mussels. The 28-day juvenile test was part of a series of studies designed to refine the methods for conducting acute and chronic toxicity tests with early life stages of freshwater mussels. Dissolved oxygen was maintained above 7.0 mg/L during the 28-day test. Survival in the control treatment and lowest ammonia test concentration (0.13 mg TAN/L) were 100 and 83 percent, respectively. Survival decreased to 30 percent at 1.02 mg TAN/L, and zero at 1.98 mg TAN/L. There was no concentration-response relationship for either length at 28 days or change in length after 28 days. Using EPA's TRAP model (see Appendix G), the survival EC_{20} for this freshwater unionid mussel species is 0.4272 mg TAN/L at test temperature (20°C) and pH (8.2), or 1.408 mg TAN/L when adjusted to pH 7 and 20°C (Appendix B).

Lampsilis siliquoidea (fatmucket)

In a recent study, Wang et al. (2011) evaluated the influence of substrate on the sensitivity of two-month-old juvenile mussels to ammonia in 28-day water-only exposure and substrate exposure. The methods used were similar to those in an earlier study (Wang et al. 2007a) except for how the organisms were exposed. In this study, the organisms were housed in a glass tube with a screen bottom that was suspended in a beaker. The authors conducted two exposure conditions simultaneously for comparison of the water-only and substrate exposure. The organisms used in the water-only exposure were simply placed on the screen at the bottom of the tube. The substrate treatment involved substrate that was screened, eliminating both large and small particles, with only particles between 300-500 microns retained, which is essentially the grain size of medium sand. A layer of substrate was placed on the screen and the organisms were placed on top of the relatively inert substrate. In the substrate treatment, the water actively flowed past the organisms and through the substrate. Water chemistry was characterized before and after passing through the substrate and found not to be substantially altered. Furthermore, the pH was maintained consistently at approximately pH 8.25 in overlying water and porewater.

The survival response between the water-only and substrate treatments was similar with a reported LOEC of 0.53 mg TAN/L in the water-only and 0.88 mg TAN/L for the substrate treatment at the test pH 8.25 and temperature 20°C. Mean control survival in both the water-only and substrate treatments was 95% at the end of the 28-day exposures, which met acceptability requirements. Dry weight measurements of the mussels increased by 165% in the water-only exposure compared to 590% increase in the substrate exposure suggesting that the presence of the substrate increased food availability, as noted by the authors.

Using TRAP threshold sigmoid regression of the survival response results in an EC_{20} of 0.5957 mg TAN/L for the water-only and EC_{20} 0.8988 mg TAN/L for the substrate exposure at the test pH and temperature, or adjusted to pH 7 and 20°C, chronic values equivalent to 2.128 and 3.211 mg TAN/L, respectively (Appendix B). Based on the apparent improved health of the test organisms in the substrate exposures, and the lack of any significant alteration of water chemistry in the exposure, the SMCV 3.211 mg TAN/L, based on survival of juvenile fatmucket from the substrate exposures is used to calculate the CCC rather than the water-only exposure.

The geometric mean of SMCVs for fatmucket and wavy-rayed lamp mussel of 3.211 and 1.408 mg TAN/L, respectively, results in a GMCV of 2.126 mg TAN/L for the genus *Lampsilis* (Table 4).

Villosa iris (rainbow mussel)

The effect of ammonia on survival and growth of this freshwater unionid mussel species was also reported in the study by Wang et al. (2007a). Juvenile (2-month-old) rainbow mussels were tested via a 28-day test under similar conditions as described above. Survival was ≥ 98 percent up to the 0.81 mg TAN/L exposure, but fell to 15 percent at 1.67 mg TAN/L and zero percent at 3.45 and 7.56 mg TAN/L. EPA's TRAP was used to generate a chronic value for this species based on survival resulting in EC_{20} of 1.063 mg TAN/L at test temperature (20°C) and pH (8.2) – (Appendix G), or 3.501 mg TAN/L adjusted to pH 7.0. Wang et al. (2007a) elected to exclude length estimates for concentrations above those where significant survival effects were measured (or in this case, 1.67 mg TAN/L). As a result, growth data are available for only three effect concentrations, even though there was 15% survival at the 1.67 mg TAN/L treatment level. Due to the uncertainties in the limited growth data for this test the growth data was not used in the calculation of the GMCV.

The SMCV and GMCV for this freshwater unionid mussel species is 3.501 mg TAN/L when adjusted to pH 7 and 20°C (Appendix B).

Lepomis species

Lepomis cyanellus (green sunfish)

Reinbold and Pescitelli (1982a) conducted a 31-day early life-stage (ELS) test that started with <24-hour-old embryos. No information was reported concerning the DO concentration, but it averaged 70 to 76 percent of saturation (5.7 to 6.2 mg/L) in a similar test in the same report with another fish species at about the same temperature. The weight data were not used in the calculation of an EC_{20} because of the greater weight of the fish in test chambers containing fewer fish, which indicated that weight was density-dependent. Although overflows resulted in loss of fish from some chambers, survival was 96 percent in one of the chambers affected by overflow, indicating that the survival data were either adjusted or not affected by the overflows. Survival by the end of the test was reduced at test concentrations of 6.3 mg TAN/L and above. TRAP analysis of the survival data resulted in an EC_{20} of 5.840 mg TAN/L at pH 8.16 and 25.4°C (U.S. EPA 1999). Adjusted to pH 7, the EC_{20} is 18.06 mg TAN/L (Appendix B).

McCormick et al. (1984) conducted a 44-day ELS test starting with <24-hour-old embryos. During this test, no effect was found on percent hatch, but survival and growth were both reduced at measured test concentrations of 14 mg TAN/L and above. TRAP analysis using biomass resulted in an EC_{20} of 5.61 mg TAN/L at pH 7.9 and 22.0°C for the test (U.S. EPA 1999). Adjusted to pH 7, the EC_{20} calculated using the data as previously reported in U.S. EPA (1999) is 11.85 mg TAN/L (Appendix B).

The pH-adjusted EC_{20}s of 18.06 mg TAN/L from Reinbold and Pescitelli (1982a) and 11.85 mg TAN/L from McCormick et al. (1984) agree well with one another. It is possible that the second value is lower because it was based on survival and growth, whereas the first value was based only on survival. The results of the tests were deemed acceptable for use in calculating a SMCV for the species, which is 14.63 mg TAN/L (Table 4) at pH 7.

Lepomis macrochirus (bluegill)

Similar to the studies summarized above for *L. cyanellus*, Smith et al. (1984) conducted a 30-day ELS test starting with <28-hour old embryos of *L. macrochirus*. No information was reported concerning the DO concentration, but the flow-rate was kept high during the test. In this study, the authors found no significant reduction in percent hatch up to a test concentration of 37 mg TAN/L, but hatched larvae were deformed at this concentration and died within six days. By the end of the test, both survival and growth were greatly reduced at measured test concentrations ranging from 3.75 to 18 mg TAN/L. TRAP analysis of biomass resulted in calculation of an EC_{20} of 1.85 mg TAN/L at pH 7.76 and 22.5°C (U.S. EPA 1999). The EC_{20} adjusted to pH 7 is 3.273 mg TAN/L (Appendix B).

The SMCV for the bluegill is 3.273 mg TAN/L, which, when calculated as a geometric mean with the SMCV of 14.63 mg TAN/L for green sunfish, results in a GMCV of 6.920 for the genus *Lepomis* (Table 4).

Musculium transversum

Anderson et al. (1978) conducted two 42-day tests of the effect of ammonia on survival of field-collected juvenile clams whose length averaged 2.2 mm. The results of the two tests were similar so the data were pooled for analysis. Survival in the control treatment and low ammonia concentrations (<5.1 mg TAN/L) ranged from 79 to 90%, but decreased to zero at 18 mg TAN/L. TRAP analysis of the survival data resulted in a calculated EC_{20} of 5.820 mg TAN/L at 23.5°C and pH 8.15. The EC_{20} is 22.21 mg TAN/L when adjusted to pH 7 and 20°C (Appendix B).

Sparks and Sandusky (1981) conducted a test similar to Anderson et al. (1978) with field-collected juvenile clams whose average length was 2.1 mm. The test was conducted in the same laboratory and used test organisms from the same location in the Mississippi River as Anderson et al. (1978), but employed a feeding regime and food for the test that was deemed by the authors to be better suited to maintaining the health of fingernail clams during chronic toxicity testing. Survival in the control treatment was 92% and decreased with increasing concentration of ammonia to 17% at 18 mg TAN/L. Effects on survival were evident at lower concentrations, resulting in an EC_{20} of only 1.23 mg TAN/L at 21.8°C and pH 7.80 when calculated using TRAP. The EC_{20} adjusted to pH 7 and 20°C is 2.565 mg TAN/L (Appendix B).

Although this latter EC_{20} determined for the test reported by Sparks and Sandusky (1981) is substantially lower than that obtained by Anderson et al. (1978), the difference is less than a factor of 10, and thus, the SMCV for this species (at pH 7 and 20°C) is the geometric mean of the two values, or 7.547 mg TAN/L (Table 4).

Table 4. Ranked Genus Mean Chronic Values.

Rank	GMCV (mg TAN/L)	Species	SMCV (mg TAN/L)
16	73.74	Stonefly, *Pteronarcella badia*	73.74
15	53.75	Water flea, *Ceriodaphnia acanthina*	64.10
		Water flea, *Ceriodaphnia dubia*	45.08
14	41.46	Water flea, *Daphnia magna*	41.46
13	29.17	Amphipod, *Hyalella azteca*	29.17
12	21.36	Channel catfish, *Ictalurus punctatus*	21.36
11	20.38	Northern pike, *Esox lucius*	20.38
10	16.53	Common carp, *Cyprinus carpio*	16.53
9	12.02	Lahontan cutthroat trout, *Oncorhynchus clarkii henshawi (LS)**	25.83
		Rainbow trout, *Oncorhynchus mykiss (LS)*	6.663
		Sockeye salmon, *Oncorhynchus nerka (LS)*	10.09
8	11.62	White sucker, *Catostomus commersonii*	11.62
7	11.07	Smallmouth bass, *Micropterus dolomieu*	11.07
6	9.187	Fathead minnow, *Pimephales promelas*	9.187
5	7.828	Pebblesnail, *Fluminicola* sp.	7.828
4	7.547	Long fingernailclam, *Musculium transversum*	7.547
3	6.920	Green sunfish, *Lepomis cyanellus*	14.63
		Bluegill, *Lepomis macrochirus*	3.273
2	3.501	Rainbow mussel, *Villosa iris*	3.501
1	2.126	Fatmucket, *Lampsilis siliquoidea*	3.211
		Wavy-rayed lamp mussel, *Lampsilis fasciola*	1.408
FCV = 1.887 mg TAN/L			
CCC = 1.9 mg TAN/L			

LS= Federally-listed species as threatened or endangered

*LS** = Listed at the subspecies only for specific populations

The National Criteria for Ammonia in Fresh Water

This ammonia criteria update document recommends an acute criterion magnitude of 17 mg TAN/L and a chronic criterion magnitude of 1.9 mg TAN/L at pH 7 and 20°C, with the stipulation that the chronic criterion cannot exceed 4.8 mg TAN/L as a 4-day average. All criteria magnitudes are recommended not to be exceeded more than once in three years on average.

2013 Final Aquatic Life Criteria for Ammonia (Magnitude, Frequency, and Duration) (mg TAN/L) pH 7.0, T=20°C	
Acute (1-hour average)	17
Chronic (30-day rolling average)	1.9*
*Not to exceed 2.5 times the CCC as a 4-day average within the 30-days, i.e. 4.8 mg TAN/L at pH 7 and 20°C, more than once in three years on average.	
Criteria frequency: Not to be exceeded more than once in three years on average.	

The available data for ammonia indicate that, except possibly where an unusually sensitive species is important at a site, freshwater aquatic life will be protected if these criteria are met. Tables 5a and 5b below provide the temperature and pH-dependent values of the CMC (acute criterion magnitude) and Table 6 provides the temperature and pH-dependent values of the CCC (chronic criterion magnitude) based on the following recommended criterion calculations derived for this update.

Acute criterion calculations

The one-hour average concentration of total ammonia nitrogen (in mg TAN/L) is not to exceed, more than once every three years on the average, the CMC (acute criterion magnitude) calculated using the following equation:

$$CMC = MIN\left(\left(\frac{0.275}{1+10^{7.204-pH}}+\frac{39.0}{1+10^{pH-7.204}}\right),\right.$$
$$\left.\left(0.7249\times\left(\frac{0.0114}{1+10^{7.204-pH}}+\frac{1.6181}{1+10^{pH-7.204}}\right)\times\left(23.12\times\ 10^{0.036\times(20-T)}\right)\right)\right)$$

The 2013 CMC equation is predicated on the following:

1. The lowest GMAV in this criterion update is for an invertebrate species; thus, the CMC is both pH- **and** temperature-dependent and varies with temperature according to the invertebrate acute temperature relationship. The lowest GMAV is 23.12 mg TAN/L for *Venustaconcha* (Table 3). The updated CMC (rounded to 4 significant figures) of 16.76 mg TAN/L at pH 7 and 20°C is 27.5 percent lower than this value. The CMC divided by the lowest GMAV is 0.7249.

2. Where salmonids in the Genus *Oncorhynchus* are present, EPA's recommended acute criterion magnitude is protective of the commercially and recreationally important adult rainbow trout, which becomes the most sensitive endpoint at lower temperatures (see footnotes pertaining to the 1999 FAV in Table 7 and Appendix A). Vertebrate sensitivity to ammonia is independent of temperature, while invertebrate sensitivity to ammonia decreases as temperature decreases. Therefore, across all temperatures the CMC equals the lower of: a) 0.7249 times the temperature adjusted lowest invertebrate GMAV (for Ellipse 23.12 mg TAN/L times 0.7249, or 16.76 mg TAN/L at pH 7.0 and 20ºC), or (b) the FAV protective of adult rainbow trout (48.21 mg TAN/L) divided by two, or 24.10 mg TAN/L at pH 7.0 and across all temperatures, according to the following temperature relationship:

$$CMC(at\ pH\ 7) = MIN\left(24.10, \left(0.7249\times 23.12\ \left(10^{0.036\times(20-T)}\right)\right)\right)$$

Thus, the CMC increases with decreasing temperature as a result of increased invertebrate insensitivity until it reaches a plateau of 24.10 mg TAN/L at 15.7°C and below, where the most sensitive taxa is the temperature invariant rainbow trout (Table 5a; see also *Oncorhynchus* present line in Figure 5a).

3. Where *Oncorhynchus* species are absent, EPA retains all tested species in the order Salmoniformes as tested surrogate species representing untested freshwater fish resident in the U.S. from another order, but does not lower the criterion to protect them as commercially and recreationally important species. The lowest GMAV for a freshwater fish is 51.93 mg TAN/L for mountain whitefish *(Prosopium williamsoni)* (Table 3). Therefore, in this case, the CMC equals the lower of: a) 0.7249 times the temperature adjusted lowest invertebrate GMAV (for Ellipse 23.12 mg TAN/L times 0.7249, or 16.76 mg TAN/L at pH 7.0 and 20ºC), or (b) 0.7249 times the lowest freshwater fish GMAV (51.93 mg TAN/L at pH 7.0 and all temperatures), according to the following temperature relationship:

$$CMC(at\, pH\ 7) = 0.7249 \times MIN\left(51.93, 23.12 \times 10^{0.036 \times (20-T)}\right)$$

Thus, the CMC increases with decreasing temperature as a result of increased invertebrate insensitivity until it reaches a plateau of 37.65 mg TAN/L at 10.2°C and below (51.93 mg TAN/L x 0.7249), where the most sensitive taxa switches to the temperature invariant fish genus *Prosopium* (Table 5b; see also *Oncorhynchus* absent line in Figure 5a). Note: while the mountain whitefish (*Prosopium williamsoni*) is a species in the same family as *Oncorhynchus* species (i.e., Family: Salmonidae), it is also an appropriately sensitive surrogate species amongst all freshwater fish in the Class Actinopterygii.

The CMC, where *Oncorhynchus* species are absent, extrapolated across both temperature and pH is as follows:

$$CMC = 0.7249 \times \frac{0.0114}{1 + 10^{7.204-pH}} + \frac{1.6181}{1 + 10^{pH-7.204}} \times MIN\left(51.93, 23.12 \times 10^{0.036 \times (20-T)}\right)$$

4. When a threatened or endangered species occurs at a site and sufficient data indicate that it is sensitive at 1-hour average concentrations below the CMC, it is appropriate to consider deriving a site-specific criterion magnitude. It should be noted that the dataset used to derive the 2013

ammonia criteria magnitudes included some threatened or endangered species, none of which were the most sensitive of the species tested.

In summary, at pH 7 and 20°C the CMC is 17 mg TAN/L, as primarily determined by the sensitivity of invertebrates. As temperature decreases to 15.7°C and below, invertebrates no longer are the most sensitive taxa, and thus in this range the CMC is 24 mg TAN/L. Where recreationally and/or commercially important *Oncorhynchus* species are not present, the CMC is determined according to statement three above. Below 15.7°C, if *Oncorhynchus* species are not present the criterion continues to increase with decreasing temperature to 10.2°C and below, where the CMC is 38 mg TAN/L.

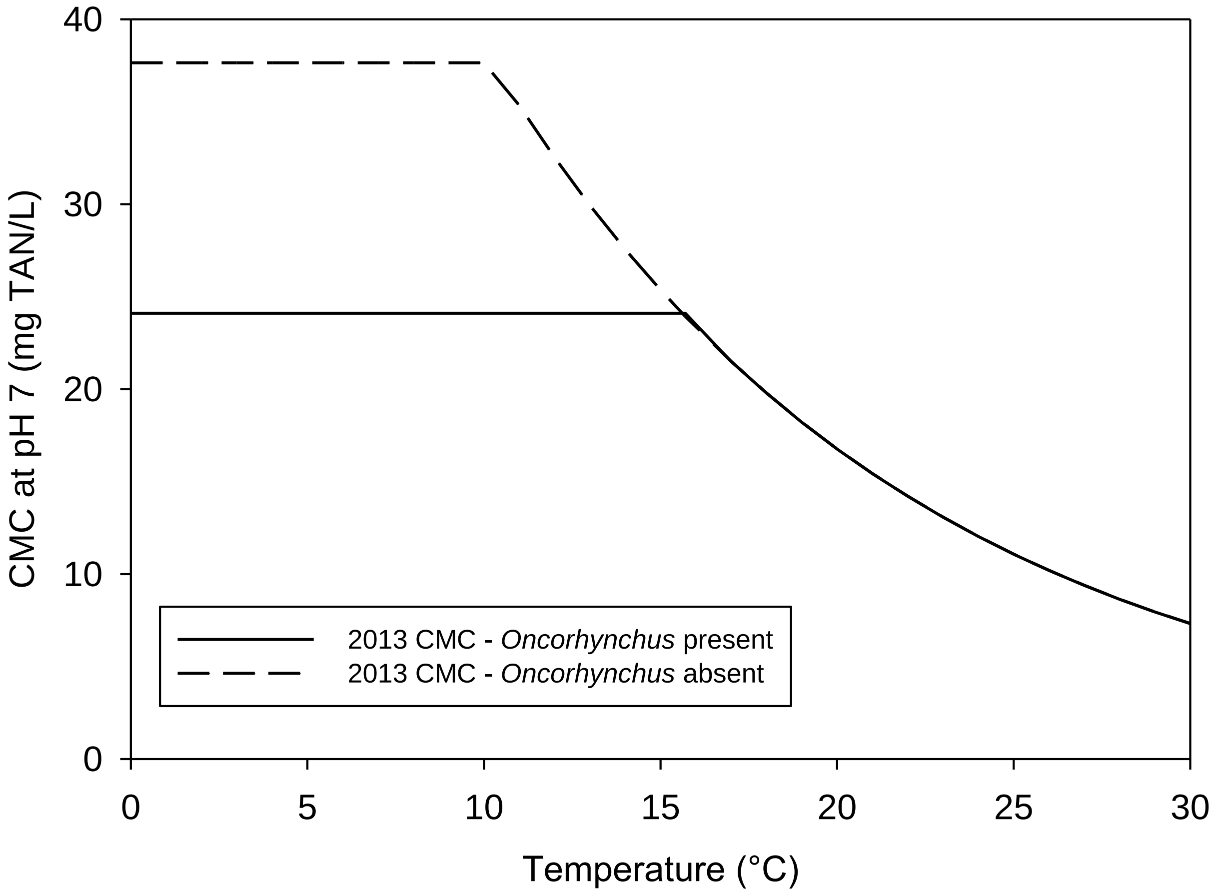

Figure 5a. 2013 Acute Criterion Magnitudes Extrapolated Across a Temperature Gradient at pH 7.

Table 5a. Temperature and pH-Dependent Values of the CMC (Acute Criterion Magnitude) – *Oncorhynchus spp.* Present.

	Temperature (°C)																
pH	0-14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
6.5	33	33	32	29	27	25	23	21	19	18	16	15	14	13	12	11	9.9
6.6	31	31	30	28	26	24	22	20	18	17	16	14	13	12	11	10	9.5
6.7	30	30	29	27	24	22	21	19	18	16	15	14	13	12	11	9.8	9.0
6.8	28	28	27	25	23	21	20	18	17	15	14	13	12	11	10	9.2	8.5
6.9	26	26	25	23	21	20	18	17	15	14	13	12	11	10	9.4	8.6	7.9
7.0	24	24	23	21	20	18	**17**	15	14	13	12	11	10	9.4	8.6	8.0	7.3
7.1	22	22	21	20	18	17	15	14	13	12	11	10	9.3	8.5	7.9	7.2	6.7
7.2	20	20	19	18	16	15	14	13	12	11	9.8	9.1	8.3	7.7	7.1	6.5	6.0
7.3	18	18	17	16	14	13	12	11	10	9.5	8.7	8.0	7.4	6.8	6.3	5.8	5.3
7.4	15	15	15	14	13	12	11	9.8	9.0	8.3	7.7	7.0	6.5	6.0	5.5	5.1	4.7
7.5	13	13	13	12	11	10	9.2	8.5	7.8	7.2	6.6	6.1	5.6	5.2	4.8	4.4	4.0
7.6	11	11	11	10	9.3	8.6	7.9	7.3	6.7	6.2	5.7	5.2	4.8	4.4	4.1	3.8	3.5
7.7	9.6	9.6	9.3	8.6	7.9	7.3	6.7	6.2	5.7	5.2	4.8	4.4	4.1	3.8	3.5	3.2	3.0
7.8	8.1	8.1	7.9	7.2	6.7	6.1	5.6	5.2	4.8	4.4	4.0	3.7	3.4	3.2	2.9	2.7	2.5
7.9	6.8	6.8	6.6	6.0	5.6	5.1	4.7	4.3	4.0	3.7	3.4	3.1	2.9	2.6	2.4	2.2	2.1
8.0	5.6	5.6	5.4	5.0	4.6	4.2	3.9	3.6	3.3	3.0	2.8	2.6	2.4	2.2	2.0	1.9	1.7
8.1	4.6	4.6	4.5	4.1	3.8	3.5	3.2	3.0	2.7	2.5	2.3	2.1	2.0	1.8	1.7	1.5	1.4
8.2	3.8	3.8	3.7	3.5	3.1	2.9	2.7	2.4	2.3	2.1	1.9	1.8	1.6	1.5	1.4	1.3	1.2
8.3	3.1	3.1	3.1	2.8	2.6	2.4	2.2	2.0	1.9	1.7	1.6	1.4	1.3	1.2	1.1	1.0	0.96
8.4	2.6	2.6	2.5	2.3	2.1	2.0	1.8	1.7	1.5	1.4	1.3	1.2	1.1	1.0	0.93	0.86	0.79
8.5	2.1	2.1	2.1	1.9	1.8	1.6	1.5	1.4	1.3	1.2	1.1	0.98	0.90	0.83	0.77	0.71	0.65
8.6	1.8	1.8	1.7	1.6	1.5	1.3	1.2	1.1	1.0	0.96	0.88	0.81	0.75	0.69	0.63	0.59	0.54
8.7	1.5	1.5	1.4	1.3	1.2	1.1	1.0	0.94	0.87	0.80	0.74	0.68	0.62	0.57	0.53	0.49	0.45
8.8	1.2	1.2	1.2	1.1	1.0	0.93	0.86	0.79	0.73	0.67	0.62	0.57	0.52	0.48	0.44	0.41	0.37
8.9	1.0	1.0	1.0	0.93	0.85	0.79	0.72	0.67	0.61	0.56	0.52	0.48	0.44	0.40	0.37	0.34	0.32
9.0	0.88	0.88	0.86	0.79	0.73	0.67	0.62	0.57	0.52	0.48	0.44	0.41	0.37	0.34	0.32	0.29	0.27

Table 5b. Temperature and pH-Dependent Values of the CMC (Acute Criterion Magnitude) – *Oncorhynchus spp.* Absent.

	Temperature (°C)																				
pH	0-10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
6.5	51	48	44	41	37	34	32	29	27	25	23	21	19	18	16	15	14	13	12	11	9.9
6.6	49	46	42	39	36	33	30	28	26	24	22	20	18	17	16	14	13	12	11	10	9.5
6.7	46	44	40	37	34	31	29	27	24	22	21	19	18	16	15	14	13	12	11	9.8	9.0
6.8	44	41	38	35	32	30	27	25	23	21	20	18	17	15	14	13	12	11	10	9.2	8.5
6.9	41	38	35	32	30	28	25	23	21	20	18	17	15	14	13	12	11	10	9.4	8.6	7.9
7.0	38	35	33	30	28	25	23	21	20	18	**17**	15	14	13	12	11	10	9.4	8.6	7.9	7.3
7.1	34	32	30	27	25	23	21	20	18	17	15	14	13	12	11	10	9.3	8.5	7.9	7.2	6.7
7.2	31	29	27	25	23	21	19	18	16	15	14	13	12	11	9.8	9.1	8.3	7.7	7.1	6.5	6.0
7.3	27	26	24	22	20	18	17	16	14	13	12	11	10	9.5	8.7	8.0	7.4	6.8	6.3	5.8	5.3
7.4	24	22	21	19	18	16	15	14	13	12	11	9.8	9.0	8.3	7.7	7.0	6.5	6.0	5.5	5.1	4.7
7.5	21	19	18	17	15	14	13	12	11	10	9.2	8.5	7.8	7.2	6.6	6.1	5.6	5.2	4.8	4.4	4.0
7.6	18	17	15	14	13	12	11	10	9.3	8.6	7.9	7.3	6.7	6.2	5.7	5.2	4.8	4.4	4.1	3.8	3.5
7.7	15	14	13	12	11	10	9.3	8.6	7.9	7.3	6.7	6.2	5.7	5.2	4.8	4.4	4.1	3.8	3.5	3.2	2.9
7.8	13	12	11	10	9.3	8.5	7.9	7.2	6.7	6.1	5.6	5.2	4.8	4.4	4.0	3.7	3.4	3.2	2.9	2.7	2.5
7.9	11	9.9	9.1	8.4	7.7	7.1	6.6	3.0	5.6	5.1	4.7	4.3	4.0	3.7	3.4	3.1	2.9	2.6	2.4	2.2	2.1
8.0	8.8	8.2	7.6	7.0	6.4	5.9	5.4	5.0	4.6	4.2	3.9	3.6	3.3	3.0	2.8	2.6	2.4	2.2	2.0	1.9	1.7
8.1	7.2	6.8	6.3	5.8	5.3	4.9	4.5	4.1	3.8	3.5	3.2	3.0	2.7	2.5	2.3	2.1	2.0	1.8	1.7	1.5	1.4
8.2	6.0	5.6	5.2	4.8	4.4	4.0	3.7	3.4	3.1	2.9	2.7	2.4	2.3	2.1	1.9	1.8	1.6	1.5	1.4	1.3	1.2
8.3	4.9	4.6	4.3	3.9	3.6	3.3	3.1	2.8	2.6	2.4	2.2	2.0	1.9	1.7	1.6	1.4	1.3	1.2	1.1	1.0	0.96
8.4	4.1	3.8	3.5	3.2	3.0	2.7	2.5	2.3	2.1	2.0	1.8	1.7	1.5	1.4	1.3	1.2	1.1	1.0	0.93	0.86	0.79
8.5	3.3	3.1	2.9	2.7	2.4	2.3	2.1	1.9	1.8	1.6	1.5	1.4	1.3	1.2	1.1	0.98	0.90	0.83	0.77	0.71	0.65
8.6	2.8	2.6	2.4	2.2	2.0	1.9	1.7	1.6	1.5	1.3	1.2	1.1	1.0	0.96	0.88	0.81	0.75	0.69	0.63	0.58	0.54
8.7	2.3	2.2	2.0	1.8	1.7	1.6	1.4	1.3	1.2	1.1	1.0	0.94	0.87	0.80	0.74	0.68	0.62	0.57	0.53	0.49	0.45
8.8	1.9	1.8	1.7	1.5	1.4	1.3	1.2	1.1	1.0	0.93	0.86	0.79	0.73	0.67	0.62	0.57	0.52	0.48	0.44	0.41	0.37
8.9	1.6	1.5	1.4	1.3	1.2	1.1	1.0	0.93	0.85	0.79	0.72	0.67	0.61	0.56	0.52	0.48	0.44	0.40	0.37	0.34	0.32
9.0	1.4	1.3	1.2	1.1	1.0	0.93	0.86	0.79	0.73	0.67	0.62	0.57	0.52	0.48	0.44	0.41	0.37	0.34	0.32	0.29	0.27

Chronic criterion calculations

The thirty-day rolling average concentration of total ammonia nitrogen (in mg TAN/L) is not to exceed, more than once every three years on the average, the chronic criterion magnitude (CCC) calculated using the following equation:

$$CCC = 0.8876 \times \left(\frac{0.0278}{1 + 10^{7.688-pH}} + \frac{1.1994}{1 + 10^{pH-7.688}}\right) \times \left(2.126 \times 10^{0.028\times(20-MAX(T,7))}\right)$$

In addition, the highest four-day average within the 30-day averaging period should not be more than 2.5 times the CCC (e.g., 2.5 x 1.9 mg TAN/L at pH 7 and 20°C or 4.8 mg TAN/L) more than once in three years on average.

The 2013 CCC equation is predicated on the following:

1. The lowest GMCV in this criteria update is for an invertebrate species; thus, the CCC is both pH- **and** temperature-dependent (based on the invertebrate chronic temperature relationship). The lowest GMCV is 2.126 mg TAN/L for *Villosa iris* (Table 4). The updated CCC (rounded to 4 significant figures) of 1.887 mg TAN/L at pH 7and 20°C is 11.2 percent lower than the lowest GMCV. The CCC to lowest GMCV ratio is 0.8876.

2. The most sensitive freshwater fish to chronic ammonia exposure are early life stages of *Lepomis* with a GMCV of 6.920 mg TAN/L (Table 4). At a pH of 7 and temperature of 7°C and below, the CCC plateaus (see Figure 5b) at 4.363 mg TAN/L, which is lower than the GMCV for *Lepomis*, the most sensitive fish, multiplied by the CCC to lowest GMCV ratio (or 6.920 mg TAN/L x 0.8876 = 6.142 mg TAN/L); thus, at pH 7, the CCC is expressed as:

$$CCC = 0.8876 \times \left(2.126 \times 10^{0.028\times(20-MAX(T,7))}\right)$$

This function increases steadily with decreasing temperature (T), until it reaches a maximum at 7°C, below which it remains constant (see Table 6; also shown graphically in Figure 5b). The rationale for the 7°C plateau in extrapolated invertebrate sensitivities is described in detail in

Appendix M. The assumption of invertebrate insensitivity to temperatures of 7°C and below is based on an interpretation of the empirical relationship between acute ammonia toxicity of invertebrates and temperature, first described by Arthur et al. (1987), and in Appendix M).

3. All new chronic fish data added to this update of the freshwater AWQC for ammonia are from early life-stage tests of the species (see new data for *Oncorhynchus clarkii henshawi*, *Oncorhynchus mykiss*, *Esox lucius*, and *Cyprinus carpio* in Appendix B), and since the new chronic criterion magnitude lies far below all chronic values for these fishes (as well as for *Lepomis* spp.), the early life stage of fish no longer warrants special consideration.

4. Where a threatened or endangered species occurs at a site and sufficient data indicate that it is sensitive at concentrations below the CCC, it is appropriate to consider deriving a site-specific criterion magnitude.

In summary, at pH 7 and 20°C the CCC of 1.9 mg TAN/L is determined by the sensitivity of invertebrates. As temperature decreases, invertebrate sensitivity to ammonia decreases until the CCC reaches a maximum of 4.4 mg TAN/L at pH 7 and temperature of 7°C and below.

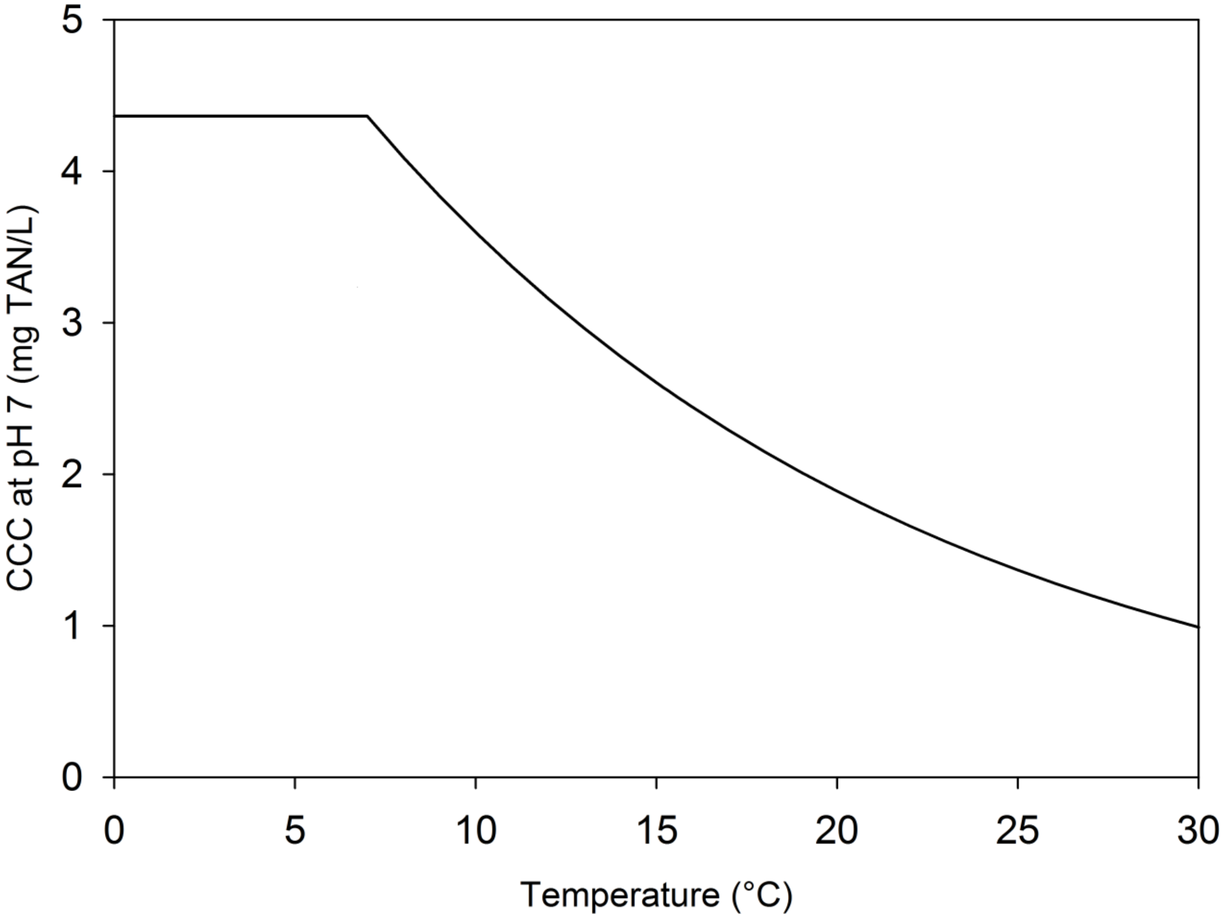

Figure 5b. 2013 Chronic Criterion Magnitudes Extrapolated Across a Temperature Gradient at pH 7.

Table 6. Temperature and pH-Dependent Values of the CCC (Chronic Criterion Magnitude).

	Temperature (°C)																							
pH	0-7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
6.5	4.9	4.6	4.3	4.1	3.8	3.6	3.3	3.1	2.9	2.8	2.6	2.4	2.3	2.1	2.0	1.9	1.8	1.6	1.5	1.5	1.4	1.3	1.2	1.1
6.6	4.8	4.5	4.3	4.0	3.8	3.5	3.3	3.1	2.9	2.7	2.5	2.4	2.2	2.1	2.0	1.8	1.7	1.6	1.5	1.4	1.3	1.3	1.2	1.1
6.7	4.8	4.5	4.2	3.9	3.7	3.5	3.2	3.0	2.8	2.7	2.5	2.3	2.2	2.1	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.2	1.1
6.8	4.6	4.4	4.1	3.8	3.6	3.4	3.2	3.0	2.8	2.6	2.4	2.3	2.1	2.0	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.1	1.1
6.9	4.5	4.2	4.0	3.7	3.5	3.3	3.1	2.9	2.7	2.5	2.4	2.2	2.1	2.0	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.2	1.1	1.0
7.0	4.4	4.1	3.8	3.6	3.4	3.2	3.0	2.8	2.6	2.4	2.3	2.2	2.0	**1.9**	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.1	1.1	0.99
7.1	4.2	3.9	3.7	3.5	3.2	3.0	2.8	2.7	2.5	2.3	2.2	2.1	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.2	1.1	1.0	0.95
7.2	4.0	3.7	3.5	3.3	3.1	2.9	2.7	2.5	2.4	2.2	2.1	2.0	1.8	1.7	1.6	1.5	1.4	1.3	1.3	1.2	1.1	1.0	0.96	0.90
7.3	3.8	3.5	3.3	3.1	2.9	2.7	2.6	2.4	2.2	2.1	2.0	1.8	1.7	1.6	1.5	1.4	1.3	1.3	1.2	1.1	1.0	0.97	0.91	0.85
7.4	3.5	3.3	3.1	2.9	2.7	2.5	2.4	2.2	2.1	2.0	1.8	1.7	1.6	1.5	1.4	1.3	1.3	1.2	1.1	1.0	0.96	0.90	0.85	0.79
7.5	3.2	3.0	2.8	2.7	2.5	2.3	2.2	2.1	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.2	1.1	1.0	0.95	0.89	0.83	0.78	0.73
7.6	2.9	2.8	2.6	2.4	2.3	2.1	2.0	1.9	1.8	1.6	1.5	1.4	1.4	1.3	1.2	1.1	1.1	0.98	0.92	0.86	0.81	0.76	0.71	0.67
7.7	2.6	2.4	2.3	2.2	2.0	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.1	1.1	1.0	0.94	0.88	0.83	0.78	0.73	0.68	0.64	0.60
7.8	2.3	2.2	2.1	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.2	1.1	1.0	0.95	0.89	0.84	0.79	0.74	0.69	0.65	0.61	0.57	0.53
7.9	2.1	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.2	1.1	1.0	0.95	0.89	0.84	0.79	0.74	0.69	0.65	0.61	0.57	0.53	0.50	0.47
8.0	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.1	1.1	1.0	0.94	0.88	0.83	0.78	0.73	0.68	0.64	0.60	0.56	0.53	0.50	0.44	0.44	0.41
8.1	1.5	1.5	1.4	1.3	1.2	1.1	1.1	0.99	0.92	0.87	0.81	0.76	0.71	0.67	0.63	0.59	0.55	0.52	0.49	0.46	0.43	0.40	0.38	0.35
8.2	1.3	1.2	1.2	1.1	1.0	0.96	0.90	0.84	0.79	0.74	0.70	0.65	0.61	0.57	0.54	0.50	0.47	0.44	0.42	0.39	0.37	0.34	0.32	0.30
8.3	1.1	1.1	0.99	0.93	0.87	0.82	0.76	0.72	0.67	0.63	0.59	0.55	0.52	0.49	0.46	0.43	0.40	0.38	0.35	0.33	0.31	0.29	0.27	0.26
8.4	0.95	0.89	0.84	0.79	0.74	0.69	0.65	0.61	0.57	0.53	0.50	0.47	0.44	0.41	0.39	0.36	0.34	0.32	0.30	0.28	0.26	0.25	0.23	0.22
8.5	0.80	0.75	0.71	0.67	0.62	0.58	0.55	0.51	0.48	0.45	0.42	0.40	0.37	0.35	0.33	0.31	0.29	0.27	0.25	0.24	0.22	0.21	0.20	0.18
8.6	0.68	0.64	0.60	0.56	0.53	0.49	0.46	0.43	0.41	0.38	0.36	0.33	0.31	0.29	0.28	0.26	0.24	0.23	0.21	0.20	0.19	0.18	0.16	0.15
8.7	0.57	0.54	0.51	0.47	0.44	0.42	0.39	0.37	0.34	0.32	0.30	0.28	0.27	0.25	0.23	0.22	0.21	0.19	0.18	0.17	0.16	0.15	0.14	0.13
8.8	0.49	0.46	0.43	0.40	0.38	0.35	0.33	0.31	0.29	0.27	0.26	0.24	0.23	0.21	0.20	0.19	0.17	0.16	0.15	0.14	0.13	0.13	0.12	0.11
8.9	0.42	0.39	0.37	0.34	0.32	0.30	0.28	0.27	0.25	0.23	0.22	0.21	0.19	0.18	0.17	0.16	0.15	0.14	0.13	0.12	0.12	0.11	0.10	0.09
9.0	0.36	0.34	0.32	0.30	0.28	0.26	0.24	0.23	0.21	0.20	0.19	0.18	0.17	0.16	0.15	0.14	0.13	0.12	0.11	0.11	0.10	0.09	0.09	0.08

Additional explanation and justification supporting the 2013 temperature and pH-dependent calculations and criteria magnitudes

Part of a criterion magnitude derivation is the estimation of the CMC or CCC based on the set of toxicity values available for different genera. The CMC or CCC estimate is intended to be equivalent to what would be obtained by simple inspection if many genera had been tested. Generally, the CMC or CCC is below the lowest value. For small datasets (<19) it is assumed that the fifth percentile is lower than the lowest toxicity value. Because the CMC is one half of the fifth percentile (i.e., FAV/2), it is frequently lower than the lowest GMAV even in large datasets. Because the extrapolation procedure used to calculate the FAV or FCV/CCC is based on the slope of the four most sensitive genera, when there are data for less than 59 genera, if the genera vary widely in sensitivity, the extrapolated criterion value is further below the lowest value than if the criteria were tightly grouped. This is statistically appropriate because when variance is high (i.e., values are widely spaced), the fifth percentile of the distribution would be expected to lie further below the lowest value of a small dataset than if the variance was low.

This extrapolation procedure, while appropriate for criteria derivations across chemicals with different variances for genus sensitivities, is not necessarily appropriate when the genera are following different temperature or life stage dependencies. Sensitivities can change with temperature or life stage, and as a result, the spread of the four lowest GMAVs or GMCVs, and the resulting degree of extrapolation to the fifth percentile of sensitivity, can also change. Rather than develop separate sets of GMAVs and GMCVs for each temperature and re-computing iteratively the CMC or CCC from the four most sensitive GMAVs or GMCVs at each temperature-pH combination, the extrapolation approach described below was used.

This issue of extrapolation to different temperatures and pH values with regard to chronic toxicity was addressed in the 1999 AWQC document for ammonia by first calculating the ratio of the CCC to the lowest GMCV, and then applying that ratio to subsequent criteria calculations for all possible pH and temperature combinations. The rationale of this approach was that it offered a degree of extrapolation that was modest and reasonable given the relatively low number of tested genera, and that it was a preferable approach to the alternative procedure of calculating CCCs directly from new sets of GMCVs for each pH-temperature combination, as each combination could result in different degrees of extrapolation, some of which could be more than 50 percent below the lowest GMCV.

In the 1999 AWQC document, the temperature extrapolations for the CCC determination described above were conducted separately for adult fish, fish early life stages, and invertebrates. This was because fish GMCVs are not affected by temperature, and because the most sensitive fish species was an early life stage of *Lepomis*. As a consequence, even though the lowest GMCV at 20°C was for an invertebrate, as temperature decreases, invertebrates, but not fish, become less sensitive to ammonia, and below 14.6°C, fish genera become the most sensitive. However, the above scenario is not applicable now because at the new recommended CCC (1.9 mg TAN/L), invertebrate genera are the most sensitive across the entire temperature range.

In the 1999 AWQC document, the most sensitive GMAVs were for fish, and because the sensitivities of fish to ammonia did not vary with temperature, no temperature extrapolation was performed. In contrast, the lowest GMAVs in this document are for invertebrates, and as a consequence, the temperature extrapolation procedure is similarly applied to the CMC as well as the CCC.

For the reassessment of the pH-TAN acute toxicity relationship, EPA has determined that the current pH-TAN acute toxicity relationship equation effectively represents the pH-TAN toxicity relationship for *L. siliquoidea* (as determined by Wang et al. 2008), as well as for other invertebrates, *Potamopyrgus antipodarum (snail)*, *Macrobrachium rosenbergii (freshwater shrimp)*, and *H. azteca (amphipod)*, as tested by Hickey and Vickers (1994), Straus et al. (1991), and Borgmann and Borgmann (1997), respectively. Also, for the reassessment of the temperature-TAN acute toxicity relationship, EPA similarly determined that the current temperature-TAN acute toxicity relationship equation effectively represents the temperature-TAN toxicity relationship for other invertebrates, *P. antipodarum (snail)*, *Branchiura sowerbyi (oligochaete)*, and *Viviparus bengalensis (snail)* as tested by Hickey and Vickers (1994) and Sarkar (1997), respectively.

Protection of downstream waters

EPA regulations at 40 CFR 131.10(b) provide that "[i]n designating uses of a water body and the appropriate criteria for those uses, the state shall take into consideration the water quality standards of downstream waters and ensure that its water quality standards provide for the attainment and maintenance of the water quality standards of downstream waters." In cases where downstream waters are characterized by higher pH and/or temperature, or harbor more sensitive species, ammonia criteria more stringent than those required to protect in-stream uses may be necessary in order to ensure that water quality standards provide for the attainment and maintenance of the water quality standards of downstream waters.

Considerations for site-specific criteria derivation

At water temperatures above 15.7°C, the 2013 acute criteria magnitude is based on effects to freshwater unionid mussels. However, when the temperature is below 15.7°C, and salmonids are present (even when mussels or other sensitive mollusk species are present), salmonid sensitivity determines the acute criterion (regardless of pH), similar to the 1999 acute criterion, which was based on effects on salmonids (i.e., adult rainbow trout). Where unionid mussels and other sensitive related mollusk species are absent, the commercially and recreationally important adult rainbow trout is the most sensitive species. Site-specific criteria derivation must take into account the temperature at the site. As an example, the acute criterion magnitude at pH 7 and temperature 20°C cannot exceed 24 mg TAN/L (the rainbow trout SMAV of 48.21 mg TAN/L, divided by two, used in this 2013 update as being representative of the most sensitive fish in general).

The 1999 chronic criterion (CCC) magnitude was based on the effects on fish early life stages, whereas based on the new data, the 2013 CCC magnitude is based on the effects on sensitive invertebrate genera, including unionid mussels. When mussels are present, the 2013 CCC magnitude is protective of fish early life stages regardless of temperature. See Appendix N for additional information on site-specific criteria for ammonia.

EFFECTS CHARACTERIZATION

The purpose of this section is to characterize the potential effects of ammonia on aquatic life considering available test data and to describe additional lines of evidence not used directly in the criteria calculations, but which support the 2013 aquatic life criteria values. This section will also provide a summary of the uncertainties and assumptions, as well as provide explanations for decisions regarding data acceptability and usage in the effects assessment. In addition, this section will describe substantive differences between the 1999 ammonia AWQC and the 2013 update resulting from the incorporation of the latest scientific knowledge.

All acceptable acute and chronic values for freshwater aquatic animal species, including those from the 1999 AWQC document (re-normalized to pH 7 and 20°C in the case of invertebrates), are presented in Appendices A (acute) and B (chronic). These tables include new acute and chronic ammonia toxicity data for freshwater mussels in the Family Unionidae and reflect the latest science informing the determinations regarding acceptable test conditions and associated data for glochidia and juvenile mussels.

Freshwater Acute Toxicity Data

Acute toxicity data for freshwater mussels and non-pulmonate (gill-bearing) snails

Prior to publishing the 2009 draft ammonia AWQC, concerns had been raised about the appropriateness of using data obtained from tests conducted with the parasitic glochidia life-stage of freshwater unionid mussels. Glochidia of different species have different life history strategies for finding an appropriate fish host; glochidia may be free living in the water column (and potentially exposed to pollutants) for a duration ranging from seconds to days, depending on the particular species. EPA concluded it was useful to consider potential adverse effects on glochidia, because effects of chemicals on this early life stage of mussels could potentially have broad impacts on mussel populations. In order for the toxicity test results with glochidia to be ecologically relevant, the duration of the acute toxicity test must be comparable to the duration of the free-living stage of the glochidia prior to attaching to a host. Supported by research conducted by Bringolf et al. (2013) demonstrating the appropriate duration of exposure for this life stage, acceptable acute toxicity data for glochidia with an exposure duration of 24 hours or less have been included in this 2013 AWQC Update, with the stipulation that control survival for the time period used is at least 90%.

In addition to the four sensitive bivalve mollusk species in Table 7, there are three other unionid mussel species among the seven genera found to be most acutely sensitive to ammonia as well as two non-pulmonate snail species are ranked tenth and twelfth in sensitivity. These GMAVs represent mollusk toxicity data that were not in the 1999 acute criteria dataset.

New test data for the ellipse (*Venustaconcha ellipsiformis),* the most sensitive species tested, was not directly used in the acute criterion calculation because the methodology used calculated the acute value using the second through fifth most sensitive species to approximate effects for a 5th percentile of species as noted in the effects assessment. The GMAV for ellipse

(23.12 mg TAN/L at pH 7 and 20°C) is greater than the acute criterion of 17 mg TAN/L and thus provides additional evidence supporting the protectiveness of the calculated acute criterion (Table 3).

Available data on non-pulmonate snails show that they are another taxon within the Phylum Mollusca sensitive to ammonia in freshwater ecosystems. The calculated GMAV of 62.15 mg TAN/L for *Fluminicola* sp. (pebblesnail) is the tenth most sensitive in the acute dataset (Table 3). Another non-pulmonate snail species *Pleurocera uncialis* (pagoda hornsnail) was ranked 12th in acute sensitivity. The LC_{50} for *P. uncialis* (reported in Goudreau et al. 1993), normalized to pH 7 and 20°C, is 68.54 mg TAN/L (Appendix A). To date, few studies have been attempted with this group of species; additional testing would improve understanding of their relative sensitivity to ammonia compared to other aquatic animals.

The draft 2009 acute criterion magnitude recommended for waters with mussels present was slightly higher than the 2013 acute criteria (19 vs. 17 mg TAN/L at pH 7, T 20°C) due to a number of differences in the data used in the CMC derivations. In response to comments received on the 2009 draft criteria, EPA removed the six invasive species from the acute dataset for ammonia; one of the invasive species removed from the dataset was Asiatic clam, which had been ranked as the fourth most sensitive GMAVs in the 2009 draft AWQC. Because the acute dataset for ammonia is extensive and contains toxicity data for other bivalves that are native North American species, the Asiatic clam was not needed as a bivalve surrogate. Also in the 2013 CMC, the most sensitive GMAV used to derive the CMC is for *Lasmigona subviridis* (green floater mussel) (GMAV=23.41 mg TAN/L) which is lower than the lowest GMAV (32.73 mg TAN/L) in the draft 2009 CMC used in the derivation of the CMC. Based on new scientific information regarding determination of test acceptability, EPA included acceptable data for glochidia (mussel larvae) and *Hyalella azteca*, which added five GMAVs for derivation of the CMC. Since the number of GMAVs is a factor in the equation used to derive the CMC, a change to the number of GMAVs results in a change in the resulting FAV and CMC.

Table 7. Comparison of the Four Taxa Used to Calculate the FAV and CMC in the 1999, 2009 Draft and 2013 AWQC.

1999 Update CMC Magnitude			**2009 Draft Update CMC Magnitude**			**2013 Final CMC Magnitude**	
Species	**pH 8.0, T=25°C (mg TAN/L)**	**pH 7.0, T=20°C (mg TAN /L)**	**Species**	**pH 8.0, T=25°C (mg TAN/L)**	**pH 7.0, T=20°C (mg TAN/L)**	**Species**	**pH 7.0, T=20°C (mg TAN/L)**
Oncorhynchus sp. (salmonids), includes: *O. aquabonita*, *O. clarkii*, *O. gorbuscha*, *O. kisutch*, *O. mykiss*, and *O. tshawytscha*	21.95	99.15	Oyster mussel, *Epioblasma capsaeformis*	6.037	39.24	*Lampsilis* sp. (Unionidae), includes: *L. abrupta*, *L. cardium*, *L. fasciola*, *L. higginsii*, *L. rafinesqueana*, and *L. siliquoidea*	46.63
Orangethroat darter, *Etheostoma spectabile*	17.96	74.25	Asiatic clam, *Corbicula fluminea*	6.018	39.12	Rainbow mussel, *Villosa iris*	34.23
Golden shiner, *Notemigonus crysoleucas*	14.67	63.02	*Lampsilis* sp. (Unionidae), includes: *L. abrupta*, *L. cardium*, *L. fasciola*, *L. higginsii*, *L. rafinesqueana*, and *L. siliquoidea*	5.919	38.48	Oyster mussel, *Epioblasma capsaeformis*	31.14
Mountain whitefish, *Prosopium williamsoni*	12.11	51.93	Rainbow mussel, *Villosa iris*	5.036	32.73	Green floater, *Lasmigona subviridis*	23.41
FAV[1]	11.23	**48.21**	**FAV**	5.734	**37.27**	**FAV**	**33.52**
CMC	5.6	**24**	**CMC**	2.9	**19**	**CMC**	**17**

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[1] The FAV in the 1999 AWQC document of 11.23 mg TAN/L at pH 8 was lowered to the geometric mean of these seven LC50 values at the time in order to protect large rainbow trout which were shown in Thurston and Russo (1983) to be measurably more sensitive than other life stages. The FAV prior to adjusting it to protect the commercially and recreationally important adult rainbow trout was calculated to be 14.32 mg TAN/L (CMC = 7.2 mg TAN/L) at pH 8. This FAV based on protection of adult rainbow trout at pH 7 is 48.21 mg TAN/L (see also Appendix A in this document).

Freshwater Chronic Toxicity Data

Use of 28-day juvenile unionid mussel data

EPA decided that growth data from 28-day tests with juvenile unionid mussels presented in the Wang et al. studies from 2007 and 2011 would not be used in calculating the 2013 chronic criterion. The decision not to use the growth data was based on the uncertainty in the test methods for assessing the growth endpoint and the need, as stated by the authors, for additional research "to optimize feeding conditions, to conduct longer-term exposures (e.g., 90 d), and to compare growth effect to potential reproductive effect in partial life-cycle exposure" (Wang et al. 2011). The growth endpoint showed a high degree of variability, and the test methods for assessing growth, based on substrate or water-only exposures, are currently being evaluated. For these reasons, the survival data for 28-day juvenile mussels were used in the calculation of the CCC and not the growth data. Appendix G provides the TRAP EC_{20}s for survival for rainbow mussel and both *Lampsilis* species, and a comparison to the growth of fatmucket mussel from 28-day tests reported by Wang et al. (2007a, 2011), which shows the additional uncertainty in the concentration-response relationship based on growth.

28-day toxicity data for freshwater snails

As noted in the 2009 draft ammonia criteria document, non-pulmonate snails have been demonstrated to be sensitive to ammonia in freshwater ecosystems, in addition to other taxa within the Phylum Mollusca. Besser et al. (2010) data from a repeat test with pebblesnail (referred to in this document as Besser 2011) support the conclusion that non-pulmonate snails may be slightly less sensitive to ammonia than freshwater mussels. Additional toxicity tests are recommended for non-pulmonate snails and other freshwater mollusks to further substantiate the findings from the 28-day tests summarized in Appendices H and I. The calculated EC_{20} values using TRAP for *P. idahoensis*, *F. aldrichi*, and *T. serptenticola*, and the recommended 28-day ammonia survival effects concentration of <7.667 mg TAN/L for *P. canaliculata*, are considered representative of non-pulmonate snail sensitivity in general and are included in Appendix C for the purpose of comparison.

Based on the 28-day toxicity test results for the gill-bearing, non-pulmonate snail species (Appendices B and C), EPA concludes that the overall sensitivity of this particular group of snail species (Sub-class Prosobranchia, Order Neotaenioglossa) appears high. Furthermore, the sensitivity of juvenile and adult mixed-age non-pulmonate snails to ammonia may be greater than that of their air-breathing, pulmonate counterparts such as *L. stagnalis*.

Although the GMCV for *Fluminicola* species is not ranked as one of the four most sensitive used to calculate the FCV, the value is ranked the 5th most sensitive in the chronic criterion dataset. The 28-day growth EC_{20} for this freshwater non-pulmonate snail species, calculated using EPA's TRAP, is 2.281 mg TAN/L at test pH (8.22) and temperature (20.1°C), or 7.828 mg TAN/L after adjustment to pH 7 and 20°C (see Appendix B). Appendix H includes a summary of the 28-day toxicity test results for *Fluminicola* species which are acceptable for use quantitatively in the chronic dataset. The TRAP output for this test is provided in Appendix H.

28-day toxicity data for *Hyalella azteca*: Minimum Data Requirement Number 5

Literature data indicate that the response of *Hyalella azteca* is influenced not only by pH, but also by sodium concentration in the dilution water. Borgmann and Borgmann (1997) demonstrated that increasing sodium decreased the toxicity of ammonia to *Hyalella*, and applied these findings to explain differences in toxicity observed by Ankley et al. (1995), which were originally attributed to water hardness. Further unpublished experiments by EPA's Office of Research and Development confirm Borgmann's assertion that sodium concentration plays a key role in determining the acute response of *Hyalella* to ammonia (personal communication, D.R. Mount, EPA, ORD). Because sodium is not known to affect ammonia toxicity to other species, this criterion does not consider sodium concentration, and this variation is not explicitly addressed. For purposes of deriving a GMAV for *Hyalella*, tests were selected that had a moderate sodium concentration (e.g., "moderately hard" water tests from Ankley et al. 1995, see Appendix A), and tests with extremely low sodium concentrations were excluded (e.g., "soft" water tests from Ankley et al. 1995; data from Whiteman et al. 1996, see Appendix J). The available acute data for ammonia did not include tests conducted in natural waters with a sodium concentration below about 3 mg/L; at that sodium concentration, the acute values for *Hyalella* were near the FAV reported in this document. Whether acute toxicity of ammonia to *Hyalella* would occur below the FAV in waters with less than 3 mg/L sodium is unknown (Appendix H).

For the 2013 chronic criterion, EPA re-evaluated the available data for *Hyalella azteca* based on recent research regarding the appropriate test conditions, including water chemistry (e.g., appropriate concentrations for specific ions such as chloride) and feeding regimes. The concentrations of sodium are important to *H. azteca* health as discussed previously and the sodium concentrations in the chronic test used in the CCC represent approximately the mid-range of U.S. waters. Based on this re-evaluation, EPA determined that certain tests met the new recommended conditions that would support healthy test organisms, and accepted those data for use in the calculation of the CCC. The specific tests used were from Borgmann (1994); details on these tests are included in Appendix H under Chronic Toxicity Tests with Juvenile *Hyalella azteca*. As a result of inclusion of acceptable *H. azteca* data, the minimum data requirement (MDR) for a benthic crustacean is fulfilled for the chronic criterion provided in this document. The GMCV of 29.17 mg TAN/L ranks *Hyalella azteca* as the thirteenth (of 16) most sensitive GMCV.

Reconsideration of the chronic toxicity data available for aquatic insects: Minimum Data Requirement Number 6

EPA chose not to include a chronic value for the stonefly, *Pteronarcella badia*, from Thurston et al. (1984a) in the 1999 AWQC update document because this aquatic insect species is relatively insensitive. Upon further consideration, EC_{20}s for 30-day nymph mortality were calculated for field collected *Pteronarcella badia* for two separate partial life cycle tests in consecutive years, in order to develop a GMCV for insects to fulfill the sixth minimum data requirement (MDR), and to clearly specify the expected lack of sensitivity of insects to ammonia based on available data. EC_{20}s were calculated for each test, and, as the authors themselves noted, the results were variable between the two tests. The normalized EC_{20} for the test conducted with *P. badia* collected from the Gallatin R. was 207.0 mg TAN/L, and was 26.27 mg TAN/L for the test conducted with *P. badia* collected from the Rocky River (Appendix B). The geometric mean for these two tests is 73.74 mg TAN/L. It is not known if these tests were conducted using the most sensitive life stage; however, the authors did note that the length of individuals used in both tests was similar. EPA used the two EC_{20}s based on 30-day nymph mortality to calculate a GMCV of 73.74 mg TAN/L for this species. The stonefly is listed as sixteenth GMCV in chronic sensitivity, fulfilling the sixth MDR. Additional data on insect sensitivity to ammonia would be useful in confirming the conclusion that insects are relatively insensitive to ammonia in freshwater environments, given the limited data available.

New chronic toxicity data for salmonid species and derivation of a GMCV for *Oncorhynchus:* Minimum Data Requirement Number 1

Chronic values from two additional studies with *Oncorhynchus* species (salmonids) are included in this AWQC document that were not included in the 1999 document (see Appendix H for more detailed descriptions of the results from these studies). Koch et al. (1980) exposed Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*), an endangered species, for 103 days in an ELS test. There were no successful hatches at exposure levels of 148 mg TAN/L or higher and no significant mortality at exposure levels below 32.9 mg TAN/L. Regression analysis of the survival data resulted in a calculated EC_{20} value of 17.89 mg TAN/L at 13.7°C and pH 7.57. The EC_{20} value is 25.83 mg TAN/L when adjusted to pH 7 (Appendix B).

The results of a more recent 90-day ELS test using a wild strain of rainbow trout exposed to ammonia were reported by Brinkman et al. (2009), and are included in this criteria document. The test was initiated with newly fertilized embryos exposed under flow-through conditions through hatch, swim-up, and early fry development. Survival, growth and biomass of swim-up fry were significantly reduced at 16.8 mg TAN/L compared to controls, but unaffected at 7.44 mg TAN/L. The EC_{20} calculated for biomass using TRAP and normalized to pH 7 is 15.60 mg TAN/L (Appendix B).

In the 1999 AWQC document, the results of six chronic tests conducted with ammonia for *Oncorhynchus mykiss* and *Oncorhynchus nerka* were included in Table 5 of that document as "acceptable" chronic tests for criteria development. A GMCV was not derived for *Oncorhynchus* at that time, however, because of the degree of variability among test results, as well as a preponderance of "greater than" or "less than" values, preventing the calculation of definitive SMCVs within the genus. The results of these chronic tests were only used at that time to assess the appropriateness of the CCC.

For this 2013 document, these six studies and the data from the two additional studies summarized above, have been re-evaluated and re-considered for inclusion in deriving a new chronic criterion for ammonia in order to consider and include all available, reliable toxicity test information for this recreationally, commercially and ecologically important taxon. The data were re-examined with specific consideration of whether unbounded (greater or less than) values add relevant information to determination of final SMCVs for *Oncorhynchus clarkii, O. mykiss, and O. nerka*. A decision rule was developed for evaluating chronic values (EC_{20}s) for potential use in deriving an SMCV for a salmon species. In developing the decision rule, it was noted that "greater than" values for concentrations of low magnitude, and "less than" values for concentrations of high magnitude do not add significant information to the analysis. That is, if a researcher only tested very low concentrations and found no chronic effects or very high concentrations and found 100% response for a chronic endpoint, those data do not significantly enhance understanding of the toxicity of ammonia. Conversely, if a researcher only tested very low concentrations and found significant chronic effects, indicating the test material was highly toxic, or relatively high concentrations and found incomplete response for a chronic endpoint, indicating low toxicity of the materials, those data do significantly enhance understanding of the toxicity of ammonia. Thus, the decision rule was applied as follows: "greater than" (>) low CVs and “less than” (<) high CVs were not used in the calculation of the SMCV; but “less than” (<) low CVs and a “greater than” (>) high CVs were included in the SMCV.

Following this decision rule, the SMCV for *O. clarkii* is the normalized EC_{20} of 25.83 mg TAN/L from Koch et al. (1980). The SMCV for *O. mykiss* is 6.663 mg TAN/L at pH 7, which is the geometric mean of the <3.246 mg TAN/L value from Calamari et al. (1977, 1981), the <3.515 mg TAN/L value from Solbe and Shurben (1989), the >11.08 mg TAN/L value from Thurston et al. (1984b), and the 15.60 mg TAN/L value from Brinkman et al. (2009). With respect to the SMCV for *O. mykiss*, both the Calamari et al. (1977, 1981) and the Solbe and Shurben (1989) values are low “less than” values, indicating demonstrated toxicity at low concentrations of ammonia, while the Thurston et al. (1984b) value is a relatively high “greater than” value, indicating lower toxicity to *O. mykiss* in this test, compared to the Calamari (1977, 1981) and Solbe and Shurben (1989) values with respect to the SMCV for *O. mykiss.* Finally, the SMCV for *O. nerka* is <10.09 mg TAN/L (Rankin 1979), and has been included as a relatively low “less than” value, indicating relative sensitivity to the effects of ammonia in this test. The <48 mg TAN/L value (at pH 7) from Thurston et al. (1978) re-calculated for *O. clarkii* (see Appendix C; value represents the geometric mean of four values) and the <45.50 mg TAN/L value from Burkhalter and Kaya (1977) for *O. mykiss* (retained in Appendix B) are not included in the SMCV calculations because they are high “less than” values, and do not add important information to the analyses. The new GMCV for *Oncorhynchus* in this 2013 Update document is 12.02 mg TAN/L, which is the geometric mean of the three SMCVs for *Oncorhynchus clarkii* (25.83 mg TAN/L)*, O. mykiss* (6.663 mg TAN/L)*, and O. nerka* (<10.09 mg TAN/L) (Appendix B), resulting in *Oncorhynchus* as being seventh most sensitive GMCV (Table 4).

Another order of insect or a phylum not already represented: Minimum Data Requirement Number 8

For the MDR identified earlier as “#8,” “another order of insect or a phylum not already represented,” there are no additional chronic toxicity data for any freshwater animal that would fulfill this MDR in the chronic dataset (the acute dataset fulfills all eight MDRs). Therefore, EPA developed a surrogate ammonia CV for the Phylum Annelida by using the geometric mean acute value from the four available genera (*Dendrocoelus, Limnodrilus, Lumbriculus, and Tubifex*) and applying an ACR from other invertebrate groups. There is less than a factor of two difference between the GMAVs for the most (*Dendrocoelus*, 119.5 mg TAN/L) and least (*Lumbriculus*, 218.7 mg TAN/L) sensitive genus (see Table 3). A surrogate chronic value was derived by dividing the GMAV for all four annelids (176.2 mg TAN/L) by a geometric mean species level invertebrate acute to chronic ratio (6.320), represented by pelagic crustaceans (cladocerans), a benthic crustacean (amphipod) and prosobranch snail (see Appendix F Acute-Chronic Ratios). The resulting surrogate CV for Phylum Annelida is 27.88 mg TAN/L (at pH 7 and 20°C).

Protection of Endangered Species

The dataset for ammonia is particularly extensive and includes data representing species that are Federally-listed as threatened or endangered by the U.S. Fish and Wildlife Service and/or NOAA National Marine Fisheries Service. Summaries are provided here describing the data for the listed species and demonstrating that the 2013 ammonia criteria update is protective of these species, based on best available scientific data.

Key acute toxicity data for listed species

The acute criterion dataset for ammonia now includes 12 aquatic species that are Federally-listed as threatened, endangered or of concern.

For unionid mussels, the 2013 criterion acute dataset includes acceptable data for 16 freshwater species across 11 genera. Of these, five of the mussel species are Federally-listed as threatened or endangered (as identified in Table 3). The oyster mussel (*Epioblasma capsaeformis*) is a Federally-listed species and is the third most sensitive in the acute dataset with a GMAV, based on a single SMAV, of 31.14 mg TAN/L. The SMAV/2 for the oyster mussel is approximately 16 mg TAN/L, similar to the 2013 acute criterion value of 17 mg TAN/L. The SMAV/2 is a value considered statistically indistinguishable from control mortality or effect based on analysis of 219 acute toxicity tests on a range of chemicals, as described in the *Federal Register* on May 18, 1978 (43 FR 21506-18). Thus, the magnitude of acute effects to this species at the SMAV/2 are not expected to significantly impact the species, because it is expected to be statistically indistinguishable from effects to control (unexposed) organisms. Furthermore, the acute criterion specifies that this concentration should not be exceeded for more than one hour once every three years on average, providing further protection through the limitation of any excursions above the criterion. Thus, the 2013 recommended CMC for ammonia of 17 mg TAN/L should be protective of oyster mussels. In waters where this listed species is present, a site-specific criterion could be considered using the SMAV for that species as the FAV from which to derive the CMC.

The *Lampsilis* GMAV of 46.63 mg TAN/L reflects the geometric mean of SMAVs for six mussel species, two (*L. abrupta* and *L. higginsii*) of which are endangered and a third (*L. rafinesqueana*) that is a Federal species of concern (Table 3). The SMAVs for this genus range from 26.03 mg TAN/L (*L. abrupta*) to 69.97 mg TAN/L (*L. rafinesqueana*) (Appendix A). Given the range of sensitivity within this genus with listed species at both the most and least sensitive ends of the range, the CMC of 17 mg TAN/L should be protective of the genus as a whole, with SMCVs/2 ranging from 13 to 34 mg TAN/L. Again, the acute criterion specifies that a concentration of 17 mg TAN/L should not be exceeded for more than one hour once every three years on average, providing further protection of species, through the limitation of any excursions above the criterion. In waters where the listed species are present, a site-specific criterion could be considered using the SMAV for that species as the FAV from which to derive the CMC.

Also among the ten most sensitive GMAVs in the acute dataset is the GMAV for the endangered Lost River sucker (*Deltistes luxatis*) endemic to the Klamath Basin of northern California and southern Oregon (Appendix A). The reported LC_{50}s at test temperature 20°C and pH 8.0 were 10.35 and 16.81 mg TAN/L for larval and juvenile fish, expressed as total ammonia (Appendix A). The LC_{50}s normalized to pH 7 and 20°C are 44.42 and 72.18 mg TAN/L, respectively (Appendix A). The GMAV for Lost River sucker is calculated as the geometric mean of the two normalized LC_{50}s, or 56.62 mg TAN/L (Table 3), with an SMAV/2, or expected low mortality level, of approximately 28 mg TAN/L, significantly above the CMC. Lost River sucker represents the ninth most sensitive genus in the acute dataset, and second most sensitive fish species (following mountain whitefish which is the most sensitive fish GMAV), and thus is expected to be protected by the CMC of 17 mg TAN/L.

The second most acutely sensitive salmonid genus (after *Prosopium,* represented by the mountain whitefish, *Prosopium williamsoni,* acute sensitivity rank 8) is *Oncorhynchus*, represented by data for six different species, three of which are threatened or endangered, with SMAVs ranging from 78.92 mg TAN/L for Cutthroat trout, *O. clarkii*, to 180.7 mg TAN/L for pink salmon, *O. gorbuscha*. The GMAV for *Oncorhynchus* (99.15 mg TAN/L) is ranked #25 in acute sensitivity (Table 3). All SMAV/2 values for the threatened or endangered species tested in this genus are significantly above the acute criterion magnitude. Thus, the acute criterion is expected to be protective of threatened and endangered salmonid species.

Key chronic toxicity data for listed species

In the chronic dataset for ammonia, the Federally-listed species are represented by three salmonid species in the genus *Oncorhynchus*, including sockeye salmon, rainbow trout, and the subspecies Lahontan cutthroat trout. The GMCV for *Oncorhynchus* of 12.02 mg TAN/L includes the three SMCVs ranging from 6.663 (rainbow trout) to 25.83 mg TAN/L (Lahontan cutthroat trout) (Table 4). The CCC for ammonia of 1.9 mg TAN/L is expected to be protective of this genus as a whole. At pH 7, the CCC is 3.5 times lower than the chronic value for the most sensitive tested listed salmonid species, *O. mykiss,* which includes populations of rainbow trout and steelhead trout.

In addition, three other studies provide useful information with which to assess the protectiveness of the CCC for threatened and endangered fish species (data included in Appendix C). All three studies indicate that the chronic criterion is expected to be protective of the endangered or listed species tested by the researchers, as described below.

Meyer and Hansen (2002) conducted a 30-day toxicity test with late-stage larvae (0.059 g) of Lost River suckers (*Deltistes luxatus*) at pH 9.5. The exposure duration and pH were chosen to represent the period of combined elevated unionized ammonia concentrations and elevated pH that occur during cyanobacterial blooms in surface waters of Upper Klamath Lake, which have been shown to last for several weeks to a month. Survival decreased significantly at 1.23 and 2.27 mg TAN/L, whereas the highest NOEC for all endpoints (survival, growth, body ions, and swimming performance) was 0.64 mg TAN/L. Control survival was > 90 percent. The calculated LOEC of 1.23 mg TAN/L at test pH and temperature when normalized to pH 7 corresponds to a value of 25.31 mg TAN/L, again, substantially higher than the 2013 chronic criterion value (Appendix C).

In order to determine if whole effluent toxicity testing is protective of threatened and endangered fish species, Dwyer et al. (2005) conducted 7-day chronic toxicity tests with *Ceriodaphnia dubia* (neonates, <24 h old) and fathead minnow larvae (*Pimephales promelas,*

<24 h) in addition to the following six threatened and endangered fish species: bonytail chub (*Gila elegans*), spotfin chub (*Erimonax*, formerly *Cyprinella, monachus*), Cape Fear shiner (*Notropis mekistocholas*), gila topminnow (*Poeciliopsis occidentalis*), Colorado pikeminnow (*Ptychocheilus lucius*), and razorback sucker (*Xyrauchen texanus*). The age of the six threatened and endangered fish species used during the 7-day ammonia exposures ranged from <1 to 7 days. The combined effect on test species survival and growth were determined as EC_{25} values. The six endangered species, presented in the same order as they are listed above, have reported EC_{25} values of: 11.0, 15.8, 8.80, 24.1, 8.90 and 13.4 mg TAN/L; or 23.24, 33.37, 18.59, 50.91, 18.80 and 28.30 mg TAN/L when adjusted to a pH of 7.0 (Appendix C). These values are all substantially higher than the 2013 chronic criterion concentration value of 1.9 mg TAN/L. Based on the results, the two species typically used for whole effluent toxicity testing (*C. dubia and P. promelas)* were more sensitive to ammonia and are protective of the six listed fish species when used as surrogate test species.

Fairchild et al. (2005) conducted 28-day toxicity tests with early life stages of Colorado pikeminnow (*Ptychocheilus lucius*) and razorback sucker (*Xyrauchen texanus*), and compared the results of those tests with a test using a surrogate fish species, the fathead minnow (*Pimephales promelas*). Effect concentrations based on the survival and growth endpoints of the fathead minnow and razorback sucker tests were not different; however, growth was the more sensitive endpoint for the Colorado pikeminnow test. The 28-day growth LOEC for the Colorado pikeminnow was 8.60 mg TAN/L, or 29.75 mg TAN/L at pH 7, substantially greater than the 2013 chronic criterion. The 28-day survival LOEC for the razorback sucker was 13.25 mg TAN/L, or 46.58 mg TAN/L at pH 7. Both endangered fish species exhibited similar sensitivity to ammonia as the fathead minnow (LOEC of 32.71 mg TAN/L at pH 7; see Appendix C). The same can be said for the Lost River sucker, which indicates that these particular endangered fish species are expected to be protected by the CCC value calculated in this 2013 AWQC Update.

Comparison of 1999, 2009, and 2013 Criteria Values

Table 8 provides a comparison of the four most sensitive taxa used to calculate the CCC in this 2013 AWQC Update document compared to the four most sensitive taxa used to calculate the CCC in the 1999 AWQC document.

The 2013 CCC is about twice the magnitude of the draft 2009 CCC recommended for waters with mussels present (1.9 vs. 0.91 mg TAN/L, respectively, at pH 7, T=20°C) as a result of differences in the data used in the CCC derivations. Based on a new study by Wang et al. (2011) described in the *Effects Analysis* section under *Summaries of Studies Used in Chronic Criterion Determination*, pg. 34, above, the lowest GMCV for the mussel genus *Lampsilis* increased from 1.154 mg TAN/L in the 2009 draft AWQC to 2.216 mg TAN/L in the 2013 AWQC. As a result, compared to the four lowest GMCVs in the 2009 draft CCC, the four lowest GMCVs in the 2013 CCC have a smaller range of variation in values (2.216 to 7.547 mg TAN/L) which decreases the uncertainty of the 5th percentile GMCV estimation. Also in the 2009 draft CCC, there were only 13 GMCVs in the dataset used to derive the CCC while in the 2013 CCC, there are 16 GMCVs used to derive the CCC, because of the addition of the GMCVs for *Hyalella azteca*, the insect *Pteronarcella badia*, and salmonids (*Oncorhynchus spp.*). The new GMCVs affect the chronic species sensitivity distribution. The cumulative probability (P) decreases as a function of the increased number of GMCVs and results in an increase in the FCV.

Table 8. Comparison of the Four Taxa Used to Calculate the FCV and CCC in the 1999 Update, 2009 Draft and the 2013 AWQC.

1999 Update CCC Magnitude			**2009 Draft Update CCC Magnitude**			**2013 Final CCC Magnitude**	
Species	**pH 8.0, T=25°C (mg TAN/L)**	**pH 7.0, T=20°C (mg TAN/L)**	**Species**	**pH 8.0, T=25°C (mg TAN/L)**	**pH 7.0, T=20°C (mg TAN/L)**	**Species**	**pH 7.0, T=20°C (mg TAN/L)**
Fathead minnow, *Pimephales promelas*	3.09	7.503	Long fingernailclam, *Musculium transversum*	<2.260	7.552	Long fingernailclam, *Musculium transversum*	7.547
Lepomis sp. (Centrarchidae), includes: Bluegill sunfish, *L. macrochirus*, and Green sunfish, *L. cyanellus*	2.85	6.92	*Lepomis* sp. (Centrarchidae), includes: Bluegill sunfish, *L. macrochirus*, and Green sunfish, *L. cyanellus*	2.852	6.924	*Lepomis* sp. (Centrarchidae), includes: Bluegill sunfish, *L. macrochirus*, and Green sunfish, *L. cyanellus*	6.92
Long fingernailclam, *Musculium transversum*	<2.26	7.547	Rainbow mussel, *Villosa iris*	<0.9805	3.286	Rainbow mussel, *Villosa iris*	3.501
Amphipod, *Hyalella azteca*	<1.45	4.865	*Lampsilis* sp. (Unionidae), includes: Wavy-rayed lamp mussel, *L. fasciola* and Fatmucket, *L. siliquoidea*	<0.3443	1.154	*Lampsilis* sp. (Unionidae), includes: Wavy-rayed lamp mussel, *L. fasciola* and Fatmucket, *L. siliquoidea*	2.216
CCC	1.2	**4.5**	**CCC**	0.26	**0.91**	**CCC**	**1.9**

Comparison of statistical approaches to develop the chronic criterion: EC20 vs. MATC

In this 2013 ammonia criteria update, the CCC is based on a 20 percent reduction in survival, growth, or reproduction, which is a risk management decision made by EPA in 1999 and also retained for this document. When an EC_{20} was not provided in a study, the EPA's TRAP program was used to estimate the EC_{20} as the basis for the GMCV and included the resultant CCC derivation of 1.9 mg TAN/L. An alternative chronic measure of effect that is commonly used is the MATC, which is the geometric mean of the NOEC and LOEC. In the case of the current ammonia dataset, using MATCs to derive the chronic criteria would result in an FCV of 1.972 and CCC of 2.0 mg TAN/L. This comparison demonstrates that, for the current ammonia chronic dataset, the use of TRAP to estimate EC_{20} values does not result in a significant difference from the MATC, another statistical approach frequently used to develop chronic effects assessments and criteria.

The concentrations of TAN affecting freshwater animals in this 2013 AWQC update are normalized to pH 7.0 for all aquatic organisms and 20°C for invertebrates. In contrast, the concentrations of TAN affecting freshwater animals in the 1999 AWQC were normalized to pH 8.0 for all organisms and temperature 25°C for invertebrates. The current pH (7) and temperature (20°C) used are considered to more closely reflect ambient pH and temperatures found generally in surface waters in the U.S. The acute and chronic criterion values can be adjusted to reflect local pH and temperature using the values in Tables 5a, 5b, and 6 derived from the equations presented in *The National Criteria for Ammonia in Fresh Water* section (pages 40-49).

UNUSED DATA

For this 2013 criteria update document, EPA considered and evaluated all available data that could possibly be used to derive the new acute and chronic criteria for ammonia in fresh water. A substantial amount of those data were associated with studies that did not meet the basic QA/QC requirements described in the 1985 Guidelines (see Stephan et al. 1985). In such cases, EPA further scrutinized those studies where either: (1) the study included tests with a species associated with one of the four most sensitive GMAVs or GMCVs used to derive the 2013 criterion; or (2) the study provided results of tests where the normalized acute or chronic value for the test was within a factor of approximately two of the fourth ranked most sensitive GMAV or GMCV, and thus might be considered potentially influential to the acute or chronic criterion. For each study that was potentially influential, but did not meet the additional data quality requirements for its use in deriving criteria for ammonia, the study and its results were included in Appendix J (acute studies) and K (chronic studies), and a rationale is provided for its exclusion. A list of all other studies considered but removed from consideration for use in deriving the criteria is provided in Appendix L with a code (and in some cases comments) indicating the reason(s) for exclusion.

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Appendix A. Acute Toxicity of Ammonia to Aquatic Animals.

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	Chemical Name	Duration	Methods[a]	pH	Temp. (˚C)	Total Ammonia (mg TAN/L)	Total Ammonia[b] (mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)	SMAV (mg TAN/L)	GMAV (mg TAN/L)	Reference
Insect (8th-10th instar), *Erythromma najas*	Ammonium chloride	4 d	R,U	7.5	25	589	**1618**			Beketov 2002
Insect (8th-10th instar), *Erythromma najas*	Ammonium chloride	4 d	R,U	8.7	25	168	**4163**			Beketov 2002
Insect (8th-10th instar), *Erythromma najas*	Ammonium chloride	4 d	R,U	9.1	25	49.2	**2361**	2515	2515	Beketov 2002
Caddisfly, *Philarctus quaeris*	Ammonium chloride	4 d	F,M	7.8	21.9	296.5	**1032**			Arthur et al. 1987
Caddisfly, *Philarctus quaeris*	Ammonium chloride	4 d	F,M	7.8	13.3	561.7	**958.4**	994.5	994.5	West 1985; Arthur et al. 1987
Beetle, *Stenelmis sexlineata*	Ammonium chloride	4 d	F,M	8.7	25	29.7	**735.9**	735.9	735.9	Hazel et al. 1979
Crayfish, *Orconectes immunis*	Ammonium chloride	4 d	F,M	7.9	17.1	488.1	**1367**			Arthur et al. 1987
Crayfish (adult), *Orconectes immunis*	Ammonium chloride	4 d	F,M	8.2	4.6	999.4	**1757**	1550		West 1985; Arthur et al. 1987
Crayfish (2.78 cm), *Orconectes nais*	Ammonium chloride	4 d	F,M	8.3	26.5	23.15	**303.8**	303.8	686.2	Evans 1979
Midge (10 d old, 2-3 instar), *Chironomus riparius*	Ammonium chloride	4 d	R,M	7.7	21.7	357.7	**1029**	1029		Monda et al. 1995
Midge, *Chironomus tentans*	Ammonium chloride	4 d	S,M	6.69	23	430	443.0			Besser et al. 1998
Midge, *Chironomus tentans*	Ammonium chloride	4 d	S,M	7.56	23	564	1439			Besser et al. 1998
Midge (2nd instar), *Chironomus tentans*	Ammonium chloride	4 d	F,M	6.5	25	371	**415.1**			Schubauer-Berigan et al. 1995

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	**Chemical Name**	**Duration**	**Methods**[a]	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia**[b] **(mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Midge (2nd instar), *Chironomus tentans*	Ammonium chloride	4 d	F,M	8.1	25	78.1	**614.0**			Schubauer-Berigan et al. 1995
Midge (2nd instar), *Chironomus tentans*	Ammonium chloride	4 d	F,M	6.5	25	368	**411.7**			Schubauer-Berigan et al. 1995
Midge (2nd instar), *Chironomus tentans*	Ammonium chloride	4 d	F,M	8.1	25	50.5	**397.0**	451.8	681.8	Schubauer-Berigan et al. 1995
Mayfly (middle to late instar), *Drunella grandis*	Ammonium chloride	4 d	F,M	7.84	12.8	259.1	**455.5**			Thurston et al. 1984b
Mayfly (middle to late instar), *Drunella grandis*	Ammonium chloride	4 d	F,M	7.84	13.2	195.6	**355.6**			Thurston et al. 1984b
Mayfly (middle to late instar), *Drunella grandis*	Ammonium chloride	4 d	F,M	7.85	12	319	**534.5**	442.4	442.4	Thurston et al. 1984b
Aquatic sowbug, *Caecidotea racovitzai* (previously *Asellus racovitzai*)	Ammonium chloride	4 d	F,M	7.8	22	148.8	**522.3**			Arthur et al. 1987
Aquatic sowbug (adult), *Caecidotea racovitzai*	Ammonium chloride	4 d	F,M	8	4	357.8	**407.7**			West 1985; Arthur et al. 1987
Aquatic sowbug, *Caecidotea racovitzai*	Ammonium chloride	4 d	F,M	7.81	11.9	176	**272.2**	387.0	387.0	Thurston et al. 1983
Isopod (adult), *Asellus aquaticus*	Ammonium chloride	4 d	F,M	7.05	12	2.60	**575.2**			Dehedin et al. 2012
Isopod (adult), *Asellus aquaticus*	Ammonium chloride	4 d	F,M	7.05	12	1.25	**276.6**			Dehedin et al. 2012
Isopod (adult), *Asellus aquaticus*	Ammonium chloride	4 d	F,M	7.05	12	1.70	**376.1**			Dehedin et al. 2012
Isopod (adult), *Asellus aquaticus*	Ammonium chloride	4 d	F,M	7.05	18	2.61	**603.8**			Dehedin et al. 2012
Isopod (adult), *Asellus aquaticus*	Ammonium chloride	4 d	F,M	7.05	18	1.40	**323.9**			Dehedin et al. 2012
Isopod (adult), *Asellus aquaticus*	Ammonium chloride	4 d	F,M	7.05	18	1.95	**451.1**			Dehedin et al. 2012
Isopod (adult), *Asellus aquaticus*	Ammonium chloride	4 d	F,M	7.05	24	1.00	**246.6**			Dehedin et al. 2012

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals										
Species	**Chemical Name**	**Duration**	**Methods**[a]	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia**[b] **(mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Isopod (adult), *Asellus aquaticus*	Ammonium chloride	4 d	F,M	7.05	24	1.00	**246.6**			Dehedin et al. 2012
Isopod (adult), *Asellus aquaticus*	Ammonium chloride	4 d	F,M	7.05	24	2.00	**493.1**	378.2	378.2	Dehedin et al. 2012
Threespine stickleback (juvenile-adult, 32-60 mm), *Gasterosteus aculeatus*	Ammonium chloride	4 d	S,M	7.1	23.3	198.1	**216.5**			Hazel et al. 1971
Threespine stickleback (juvenile-adult, 32-60 mm), *Gasterosteus aculeatus*	Ammonium chloride	4 d	S,M	7.15	15	577	**667.4**			Hazel et al. 1971
Threespine stickleback (juvenile-adult, 32-60 mm), *Gasterosteus aculeatus*	Ammonium chloride	4 d	S,M	7.25	23.3	203.8	**264.0**			Hazel et al. 1971
Threespine stickleback (juvenile-adult, 32-60 mm), *Gasterosteus aculeatus*	Ammonium chloride	4 d	S,M	7.5	15	143.9	**261.1**			Hazel et al. 1971
Threespine stickleback (juvenile-adult, 32-60 mm), *Gasterosteus aculeatus*	Ammonium chloride	4 d	S,M	7.5	23.3	78.7	**142.8**			Hazel et al. 1971
Threespine stickleback (juvenile-adult, 32-60 mm), *Gasterosteus aculeatus*	Ammonium chloride	4 d	S,M	7.5	23.3	115.4	**209.5**			Hazel et al. 1971
Threespine stickleback (juvenile-adult, 32-60 mm), *Gasterosteus aculeatus*	Ammonium chloride	4 d	S,M	7.5	15	259	**470.0**	281.5	281.5	Hazel et al. 1971
Mayfly, *Callibaetis skokianus*	Ammonium chloride	4 d	F,M	7.7	10.8	263.5	**307.2**			Arthur et al. 1987
Mayfly, *Callibaetis skokianus*	Ammonium chloride	4 d	F,M	7.9	13.3	211.7	**432.7**	364.6		West 1985; Arthur et al. 1987
Mayfly (middle to late instar), *Callibaetis* sp.	Ammonium chloride	4 d	F,M	7.81	11.9	107.8	**166.7**	166.7	246.5	Thurston et al. 1984b

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	**Chemical Name**	**Duration**	**Methods**[a]	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia**[b] **(mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Dragonfly (<233 d old), *Pachydiplax longipennis*	Ammonium chloride	4 d	F,M	8	12	76.92	**170.1**			Diamond et al. 1993
Dragonfly (<140 d old), *Pachydiplax longipennis*	Ammonium chloride	4 d	F,M	8	20	74.37	**319.2**	233.0	233.0	Diamond et al. 1993
Mottled sculpin (1.8 g, 5.4 cm), *Cottus bairdii*	Ammonium chloride	4 d	F,M	8.02	12.4	49.83	**222.2**	222.2	222.2	Thurston and Russo 1981
Western mosquitofish, *Gambusia affinis*	-	4 d	S,U	7.75	19	129.6	**352.9**			Wallen et al. 1957
Western mosquitofish, *Gambusia affinis*	-	4 d	S,U	8.2	19.5	34.54	**217.7**			Wallen et al. 1957
Western mosquitofish, *Gambusia affinis*	-	4 d	S,U	8.5	23	14.64	**165.0**			Wallen et al. 1957
Western mosquitofish, *Gambusia affinis*	-	4 d	S,U	8	24	42.53	**182.6**	219.3	219.3	Wallen et al. 1957
Oligochaete worm, *Lumbriculus variegatus*	Ammonium chloride	4 d	S,M	7.56	23	286	729.5			Besser et al. 1998
Oligochaete worm, *Lumbriculus variegatus*	Ammonium chloride	4 d	S,M	6.69	23	302	311.1			Besser et al. 1998
Oligochaete worm (10-25 mm), *Lumbriculus variegatus*	Ammonium chloride	4 d	R,M	8.2	15	13.66	56.88			Hickey and Vickers 1994
Oligochaete worm (adult), *Lumbriculus variegatus*	-	4 d	F,M	6.5	25	100	**111.9**			Schubauer-Berigan et al. 1995
Oligochaete worm (adult), *Lumbriculus variegatus*	-	4 d	F,M	6.5	25	200	**223.8**			Schubauer-Berigan et al. 1995
Oligochaete worm (adult), *Lumbriculus variegatus*	-	4 d	F,M	8.1	25	34	**267.3**			Schubauer-Berigan et al. 1995
Oligochaete worm (adult), *Lumbriculus variegatus*	-	4 d	F,M	8.1	25	43.5	**342.0**	218.7	218.7	Schubauer-Berigan et al. 1995
Tubificid worm, *Tubifex tubifex*	Ammonium chloride	4 d	S,U	8.2	12	66.67	**216.5**	216.5	216.5	Stammer 1953

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	Chemical Name	Duration	Methods[a]	pH	Temp. (˚C)	Total Ammonia (mg TAN/L)	Total Ammonia[b] (mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)	SMAV (mg TAN/L)	GMAV (mg TAN/L)	Reference
Marsh ramshorn snail, *Planorbella trivolvis* (previously *Helisoma trivolvis*)	Ammonium chloride	4 d	F,M	7.9	22	47.73	**200.7**			Arthur et al. 1987
Marsh ramshorn snail, *Planorbella trivolvis*	Ammonium chloride	4 d	F,M	8.2	12.9	63.73	**223.0**	211.6	211.6	Arthur et al. 1987
Scud (7-14 d old), *Hyalella azteca*	Ammonium chloride	4 d	R,M	8.3	25	39.8	**461.2**			Ankley et al. 1995
Scud (7-14 d old), *Hyalella azteca*	Ammonium chloride	4 d	R,M	7.31	25	64	**135.1**			Ankley et al. 1995
Scud (7-14 d old), *Hyalella azteca*	Ammonium chloride	4 d	R,M	6.43	25	105	**114.6**	192.6	192.6	Ankley et al. 1995
Stonefly, Little golden stonefly (middle to late instar), *Skwala americana*	Ammonium chloride	4 d	F,M	7.81	13.1	109.3	**186.7**			Thurston et al. 1984b
Stonefly, Little golden stonefly (middle to late instar), *Skwala americana*	Ammonium chloride	4 d	F,M	7.76	13.8	119.6	**198.3**	192.4	192.4	Thurston et al. 1984b
Mozambique tilapia (juvenile), *Oreochromis mossambicus*	Ammonium chloride	4 d	R,U	7.2	28	151.5	**185.2**	185.2	185.2	Rani et al. 1998
Amphipod (4-6 mm), *Crangonyx pseudogracilis*	Ammonium chloride	4 d	S,U	7.5	12	43.36	40.54			Prenter et al. 2004
Amphipod, *Crangonyx pseudogracilis*	Ammonium chloride	4 d	F,M	8	4	199.5	**227.3**			West 1985; Arthur et al. 1987
Amphipod, *Crangonyx pseudogracilis*	Ammonium chloride	4 d	F,M	8	12.1	216	**481.7**			West 1985; Arthur et al. 1987
Amphipod, *Crangonyx pseudogracilis*	Ammonium chloride	4 d	F,M	8	13.3	115.3	**284.1**			West 1985; Arthur et al. 1987
Amphipod, *Crangonyx pseudogracilis*	Ammonium chloride	4 d	F,M	8	24.9	25.1	**161.7**			West 1985; Arthur et al. 1987
Amphipod, *Crangonyx pseudogracilis*	Ammonium chloride	4 d	F,M	8.2	13	81.6	**287.9**	270.5		West 1985; Arthur et al. 1987

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	Chemical Name	Duration	Methods[a]	pH	Temp. (˚C)	Total Ammonia (mg TAN/L)	Total Ammonia[b] (mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)	SMAV (mg TAN/L)	GMAV (mg TAN/L)	Reference
Amphipod (13 d), *Crangonyx* sp.	Ammonium chloride	4 d	F,M	8	12	79.23	**175.3**			Diamond et al. 1993
Amphipod (8-42 d), *Crangonyx* sp.	Ammonium chloride	4 d	F,M	8	20	19.83	**85.13**	122.2	181.8	Diamond et al. 1993
Tubificid worm (30-40 mm), *Limnodrilus hoffmeisteri*	-	4 d	F,M	7.9	11.5	96.62	**170.2**	170.2	170.2	Williams et al. 1986
Pouch snail, *Physa gyrina*	Ammonium chloride	4 d	F,M	8	4	114.9	**131.0**			West 1985; Arthur et al. 1987
Pouch snail, *Physa gyrina*	Ammonium chloride	4 d	F,M	8.2	5.5	85.13	**161.3**			West 1985; Arthur et al. 1987
Pouch snail, *Physa gyrina*	Ammonium chloride	4 d	F,M	8.1	12.1	76.29	**205.9**			West 1985; Arthur et al. 1987
Pouch snail, *Physa gyrina*	Ammonium chloride	4 d	F,M	8.2	12.8	50.25	**174.4**			West 1985; Arthur et al. 1987
Pouch snail, *Physa gyrina*	Ammonium chloride	4 d	F,M	8	13.3	62.39	**153.7**			West 1985; Arthur et al. 1987
Pouch snail, *Physa gyrina*	Ammonium chloride	4 d	F,M	8	24.9	26.33	**169.7**	164.5	164.5	West 1985; Arthur et al. 1987
Damselfly (8-10 mm), *Enallagma* sp.	-	4 d	F,M	7.9	11.5	93.1	**164.0**	164.0	164.0	Williams et al. 1986
Water flea (<24 hr), *Chydorus sphaericus*	Ammonium chloride	4 d	S,M	8	20	37.88	**162.6**	162.6	162.6	Dekker et al. 2006
Fathead minnow (larva, 14 d), *Pimephales promelas*	-	4 d	S,U	7.6	20	37.56	79.59			Markle et al. 2000
Fathead minnow, *Pimephales promelas*	Ammonium chloride	4 d	S,M	7.52	20.25	36.73	68.17			EA Engineering 1985
Fathead minnow, *Pimephales promelas*	Ammonium chloride	4 d	S,M	7.48	19.85	40.93	72.10			EA Engineering 1985
Fathead minnow, *Pimephales promelas*	Ammonium chloride	4 d	S,M	7.52	20.25	37.49	69.59			EA Engineering 1985

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals										
Species	**Chemical Name**	**Duration**	**Methods[a]**	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia[b] (mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Fathead minnow, *Pimephales promelas*	Ammonium chloride	4 d	S,M	7.48	19.85	41.79	73.61			EA Engineering 1985
Fathead minnow, *Pimephales promelas*	Ammonium chloride	4 d	S,M	7.48	19.85	43.49	76.61			EA Engineering 1985
Fathead minnow (4-6 d old), *Pimephales promelas*	Ammonium chloride	4 d	R,M	8.01	25	14.4	63.00			Buhl 2002
Fathead minnow, *Pimephales promelas*	Ammonium chloride	4 d	R,M	8	20	5.389	23.13			Diamond et al. 1993
Fathead minnow, *Pimephales promelas*	Ammonium chloride	4 d	R,M	8	20	6.1	26.19			Diamond et al. 1993
Fathead minnow (1.9 g), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.9	3.4	229.7	**818.4**			West 1985; Arthur et al. 1987
Fathead minnow (1.8 g), *Pimephales promelas*	Ammonium chloride	4 d	F,M	8.1	12.1	56.07	**291.3**			West 1985; Arthur et al. 1987
Fathead minnow (1.6 g), *Pimephales promelas*	Ammonium chloride	4 d	F,M	8	17.1	52.22	**224.2**			West 1985; Arthur et al. 1987
Fathead minnow (1.7 g), *Pimephales promelas*	Ammonium chloride	4 d	F,M	8.1	26.1	29.23	**151.8**			West 1985; Arthur et al. 1987
Fathead minnow, *Pimephales promelas*	Ammonium chloride	4 d	F,M	8.05	14	47.29	**223.2**			DeGraeve et al. 1980
Fathead minnow (4-5 mo), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.46	6	97.27	**166.4**			DeGraeve et al. 1987
Fathead minnow (4-5 mo), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.46	10	101.7	**174.0**			DeGraeve et al. 1987
Fathead minnow (4-5 mo), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.41	15	76.58	**122.0**			DeGraeve et al. 1987
Fathead minnow (4-5 mo), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.41	20	78.22	**124.6**			DeGraeve et al. 1987
Fathead minnow (4-5 mo), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.45	20	66.94	**112.9**			DeGraeve et al. 1987
Fathead minnow (4-5 mo), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.4	25	81.81	**128.5**			DeGraeve et al. 1987
Fathead minnow (4-5 mo), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.41	25	91.4	**145.6**			DeGraeve et al. 1987
Fathead minnow (4-5 mo), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.44	30	64.12	**106.6**			DeGraeve et al. 1987

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	Chemical Name	Duration	Methods[a]	pH	Temp. (˚C)	Total Ammonia (mg TAN/L)	Total Ammonia[b] (mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)	SMAV (mg TAN/L)	GMAV (mg TAN/L)	Reference
Fathead minnow (0.28 g, 26.6 mm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	8.14	22	25.16	**141.2**			Mayes et al. 1986
Fathead minnow (10 mm length), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.9	20.6	28.9	**103.0**			Nimmo et al. 1989
Fathead minnow (10 mm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	8.2	6.2	7.322	**46.15**			Nimmo et al. 1989
Fathead minnow (10 mm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.8	20.1	18.73	**55.68**			Nimmo et al. 1989
Fathead minnow (10 mm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.8	19.8	32.12	**95.49**			Nimmo et al. 1989
Fathead minnow (25 mm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	8.1	19.6	24.89	**129.3**			Nimmo et al. 1989
Fathead minnow (25 mm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	8.2	6.2	11.56	**72.86**			Nimmo et al. 1989
Fathead minnow (25 mm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	8.1	5.8	19.94	**103.6**			Nimmo et al. 1989
Fathead minnow (25 mm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	8.1	5.8	21.44	**111.4**			Nimmo et al. 1989
Fathead minnow (25 mm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.7	20.1	32.25	**80.61**			Nimmo et al. 1989
Fathead minnow (15 mm, 0.0301 g), *Pimephales promelas*	Ammonium chloride	4 d	F,M	8.46	4.1	18.54	**193.5**			Reinbold and Pescitelli 1982b
Fathead minnow (16 mm, 0.0315 g), *Pimephales promelas*	Ammonium chloride	4 d	F,M	8.02	23.9	19.55	**87.16**			Reinbold and Pescitelli 1982b
Fathead minnow (19 mm, 0.0629 g), *Pimephales promelas*	Ammonium chloride	4 d	F,M	8.26	4.6	30.57	**216.5**			Reinbold and Pescitelli 1982b
Fathead minnow (21 mm, 0.0662 g), *Pimephales promelas*	Ammonium chloride	4 d	F,M	8.16	25.2	17.65	**102.9**			Reinbold and Pescitelli 1982b

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	**Chemical Name**	**Duration**	**Methods**[a]	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia**[b] **(mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Fathead minnow (5.2 cm, 1.1 g), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.7	21.65	63.02	**157.5**			Sparks 1975
Fathead minnow (0.2 g), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.78	25.9	40.85	**117.3**			Swigert and Spacie 1983
Fathead minnow (0.5 g), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.8	25.6	42.65	**126.8**			Swigert and Spacie 1983
Fathead minnow (1.9 g, 5.2 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.83	11.8	45.71	**143.4**			Thurston et al. 1981c
Fathead minnow (1.9 g, 5.2 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.82	12	62.72	**193.3**			Thurston et al. 1981c
Fathead minnow (1.9 g, 5.2 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	6.51	13	260	**192.9**			Thurston et al. 1981c
Fathead minnow (1.9 g, 5.2 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	9.03	13.2	5.94	**169.6**			Thurston et al. 1981c
Fathead minnow (1.9 g, 5.2 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	8.51	13.5	18.88	**216.9**			Thurston et al. 1981c
Fathead minnow (1.9 g, 5.2 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.01	13.8	145.9	**147.2**			Thurston et al. 1981c
Fathead minnow (0.09 g, 2.0 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.91	16.3	51.55	**187.1**			Thurston et al. 1983
Fathead minnow (0.09 g, 2.1 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.89	13.1	50.2	**175.6**			Thurston et al. 1983
Fathead minnow (0.13 g, 2.3 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.64	13.6	58.4	**132.1**			Thurston et al. 1983

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals										
Species	**Chemical Name**	**Duration**	**Methods[a]**	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia[b] (mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Fathead minnow (0.19 g, 2.6 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.68	13.5	64.7	**156.3**			Thurston et al. 1983
Fathead minnow (0.22 g, 2.7 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	8.03	22.1	47.6	**216.3**			Thurston et al. 1983
Fathead minnow (0.22 g, 2.9 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	8.06	22	42.6	**205.0**			Thurston et al. 1983
Fathead minnow (0.26 g, 3.0 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.67	13.9	58.8	**139.7**			Thurston et al. 1983
Fathead minnow (0.31 g, 3.0 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	8.05	13	74.65	**352.4**			Thurston et al. 1983
Fathead minnow (0.31 g, 3.1 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	8.05	13.6	66.48	**313.8**			Thurston et al. 1983
Fathead minnow (0.35 g, 3.1 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.94	19.1	42.3	**162.3**			Thurston et al. 1983
Fathead minnow (0.42 g, 3.0 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.76	19	50.28	**139.3**			Thurston et al. 1983
Fathead minnow (0.42 g, 3.6 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.66	13.4	58.2	**136.0**			Thurston et al. 1983
Fathead minnow (0.47 g, 3.2 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.87	15.8	58.91	**198.7**			Thurston et al. 1983
Fathead minnow (0.47 g, 3.2 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.83	22	50.6	**158.7**			Thurston et al. 1983
Fathead minnow (0.5 g, 3.8 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.91	18.9	49.3	**178.9**			Thurston et al. 1983

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals										
Species	**Chemical Name**	**Duration**	**Methods**[a]	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia**[b] **(mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Fathead minnow (0.8 g, 4.2 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.77	14.3	66.7	**188.1**			Thurston et al. 1983
Fathead minnow (1.0 g, 4.6 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.77	14.1	72.71	**205.1**			Thurston et al. 1983
Fathead minnow (1.4 g, 4.9 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	8.04	22.4	36.59	**169.5**			Thurston et al. 1983
Fathead minnow (1.4 g, 5.0 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	8.08	21.4	44.8	**224.0**			Thurston et al. 1983
Fathead minnow (1.4 g, 5.0 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	8.16	21.4	47.39	**276.4**			Thurston et al. 1983
Fathead minnow (1.4 g, 5.1 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.88	21.7	50.9	**174.8**			Thurston et al. 1983
Fathead minnow (1.4 g, 5.4 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.68	12.9	91.8	**221.8**			Thurston et al. 1983
Fathead minnow (1.4 g, 5.5 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.63	13.2	89.85	**199.9**			Thurston et al. 1983
Fathead minnow (1.5 g, 5.6 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.76	12.9	107.5	**298.0**			Thurston et al. 1983
Fathead minnow (1.7 g, 5.2 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.84	21.7	55.43	**177.0**			Thurston et al. 1983
Fathead minnow (2.1 g, 6.1 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.76	13.1	66.73	**184.9**			Thurston et al. 1983
Fathead minnow (2.2 g, 6.2 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.74	12.8	52.2	**139.7**			Thurston et al. 1983

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	Chemical Name	Duration	Methods[a]	pH	Temp. (˚C)	Total Ammonia (mg TAN/L)	Total Ammonia[b] (mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)	SMAV (mg TAN/L)	GMAV (mg TAN/L)	Reference
Fathead minnow (2.3 g, 6.3 cm), *Pimephales promelas*	Ammonium chloride	4 d	F,M	7.91	15.9	47.43	**172.1**	159.2	159.2	Thurston et al. 1983
Brook trout (3.12 g, 7.2 cm), *Salvelinus fontinalis*	Ammonium chloride	4 d	F,U	7.86	13.6	45.21	**149.7**			Thurston and Meyn 1984
Brook trout (3.40 g, 7.4 cm), *Salvelinus fontinalis*	Ammonium chloride	4 d	F,U	7.83	13.8	52.03	**163.2**	156.3		Thurston and Meyn 1984
Lake trout, siscowet (0.9 g), *Salvelinus namaycush*	Ammonium chloride	4 d	S,M	7.45	8.5	90.43	**152.5**			Soderberg and Meade 1992
Lake trout, siscowet (0.9 g), *Salvelinus namaycush*	Ammonium chloride	4 d	S,M	7.45	8.5	110.2	**185.9**			Soderberg and Meade 1992
Lake trout, siscowet (8 g), *Salvelinus namaycush*	Ammonium chloride	4 d	S,M	7.45	8.5	96.25	**162.3**			Soderberg and Meade 1992
Lake trout, siscowet (8 g), *Salvelinus namaycush*	Ammonium chloride	4 d	S,M	7.45	8.5	83.11	**140.1**	159.3	157.8	Soderberg and Meade 1992
Shortnose sturgeon (fingerling), *Acipenser brevirostrum*	Ammonium chloride	4 d	S,M	7.05	18	149.8	**156.7**	156.7	156.7	Fontenot et al. 1998
White sucker (5.6 g), *Catostomus commersonii*	Ammonium chloride	4 d	F,M	7.8	3.6	89.57	**266.3**			West 1985; Arthur et al. 1987
White sucker (5.2 g), *Catostomus commersonii*	Ammonium chloride	4 d	F,M	8.1	11.3	60.86	**316.1**			West 1985; Arthur et al. 1987
White sucker (12.6 g), *Catostomus commersonii*	Ammonium chloride	4 d	F,M	8.2	12.6	40.85	**257.4**			West 1985; Arthur et al. 1987
White sucker (9.6 g), *Catostomus commersonii*	Ammonium chloride	4 d	F,M	8.2	15.3	43.01	**271.0**			West 1985; Arthur et al. 1987
White sucker (110 mm), *Catostomus commersonii*	Ammonium chloride	4 d	F,M	7.8	20.2	31.21	**92.80**			Nimmo et al. 1989
White sucker (110 mm), *Catostomus commersonii*	Ammonium chloride	4 d	F,M	7.8	20.2	18.93	**56.28**			Nimmo et al. 1989
White sucker (92 mm, 6.3 g), *Catostomus commersonii*	Ammonium chloride	4 d	F,M	8.16	15	30.28	**176.6**			Reinbold and Pescitelli 1982c

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	Chemical Name	Duration	Methods[a]	pH	Temp. (˚C)	Total Ammonia (mg TAN/L)	Total Ammonia[b] (mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)	SMAV (mg TAN/L)	GMAV (mg TAN/L)	Reference
White sucker (92 mm, 6.3 g), *Catostomus commersonii*	Ammonium chloride	4 d	F,M	8.14	15.4	29.65	**166.3**			Reinbold and Pescitelli 1982c
White sucker (11.4 g), *Catostomus commersonii*	Ammonium chloride	4 d	F,M	7.8	22.5	22.3	**66.32**	157.5		Swigert and Spacie 1983
Mountain sucker (63.3 g, 18.2 cm), *Catostomus platyrhynchus*	Ammonium chloride	4 d	F,U	7.67	12	66.91	**159.0**			Thurston and Meyn 1984
Mountain sucker (45.3 g, 16.2 cm), *Catostomus platyrhynchus*	Ammonium chloride	4 d	F,U	7.69	13.2	47.59	**117.0**			Thurston and Meyn 1984
Mountain sucker (47.8 g, 15.9 cm), *Catostomus platyrhynchus*	Ammonium chloride	4 d	F,U	7.73	11.7	51.62	**135.8**	136.2	146.5	Thurston and Meyn 1984
Water flea, *Ceriodaphnia acanthina*	Ammonium chloride	2 d	F,M	7.06	24	104.8	**154.3**	154.3		Mount 1982
Water flea (<24 hr), *Ceriodaphnia dubia*	Ammonium chloride	2 d	S,M	8.02	24.8	21.26	**141.1**			Andersen and Buckley 1998
Water flea (<24 hr), *Ceriodaphnia dubia*	Ammonium chloride	2 d	S,M	7.5	25	47.05	**129.2**			Bailey et al. 2001
Water flea (<24 hr), *Ceriodaphnia dubia*	Ammonium chloride	2 d	S,M	7.5	25	56.84	**156.1**			Bailey et al. 2001
Water flea (<24 hr), *Ceriodaphnia dubia*	Ammonium chloride	2 d	S,M	8.16	22	24.77	**170.5**			Black 2001
Water flea (<24 hr), *Ceriodaphnia dubia*	Ammonium chloride	2 d	S,M	8.4	23	28.06	**334.5**			Black 2001
Water flea (<24 hr), *Ceriodaphnia dubia*	Ammonium chloride	2 d	S,M	8.4	23	32.63	**389.0**			Black 2001
Water flea (<24 hr), *Ceriodaphnia dubia*	Ammonium chloride	2 d	S,M	8	25	14.52	**94.35**			Scheller 1997
Water flea, *Ceriodaphnia dubia*	Ammonium chloride	2 d	S,M	8.08	24.75	15.6	**114.5**			Andersen and Buckley 1998
Water flea, *Ceriodaphnia dubia*	Ammonium hydroxide	2 d	R,M	8.4	26.4	7.412	**117.1**			Cowgill and Milazzo 1991

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	**Chemical Name**	**Duration**	**Methods**[a]	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia**[b] **(mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Water flea, *Ceriodaphnia dubia*	Ammonium sulfate	2 d	R,NR	7.4	23	48.59	**97.89**			Manning et al. 1996
Water flea, *Ceriodaphnia dubia*	Ammonium chloride	2 d	R,M	7.8	25	33.98	**152.9**			Nimmo et al. 1989
Water flea, *Ceriodaphnia dubia*	Ammonium chloride	2 d	R,M	8.2	7	16.65	**35.72**			Nimmo et al. 1989
Water flea, *Ceriodaphnia dubia*	Ammonium chloride	2 d	S,M	7.85	23	28.65	**119.5**			Sarda 1994
Water flea, *Ceriodaphnia dubia*	Ammonium chloride	2 d	S,M	7.85	23	28.77	**120.0**	134.2	143.9	Sarda 1994
Water flea (adult), *Simocephalus vetulus*	Ammonium chloride	2 d	F,M	8.3	17	31.58	**188.5**			West 1985; Arthur et al. 1987
Water flea (adult), *Simocephalus vetulus*	Ammonium chloride	2 d	F,M	8.1	20.4	21.36	**114.7**			Arthur et al. 1987
Water flea, *Simocephalus vetulus*	Ammonium chloride	2 d	F,M	7.25	24.5	83.51	**157.0**			Mount 1982
Water flea, *Simocephalus vetulus*	Ammonium chloride	2 d	F,M	7.06	24	83.51	**122.9**	142.9	142.9	Mount 1982
Channel catfish, *Ictalurus punctatus*	Ammonium chloride	4 d	S,U	8.7	22	10.56	172.9			Colt and Tchobanoglous 1976
Channel catfish, *Ictalurus punctatus*	Ammonium chloride	4 d	S,U	8.7	26	10.19	166.9			Colt and Tchobanoglous 1976
Channel catfish, *Ictalurus punctatus*	Ammonium chloride	4 d	S,U	8.7	30	10.88	178.1			Colt and Tchobanoglous 1976
Channel catfish, *Ictalurus punctatus*	Ammonium chloride	4 d	S,M	7.49	19.7	131.5	235.0			EA Engineering 1985
Channel catfish, *Ictalurus punctatus*	Ammonium chloride	4 d	S,M	7.53	19.75	99.67	189.3			EA Engineering 1985
Channel catfish (larvae, 1 d), *Ictalurus punctatus*	Ammonium chloride	4 d	R,M	8.2	23.8	13.03	82.10			Bader and Grizzle 1992
Channel catfish (juvenile, 7 d), *Ictalurus punctatus*	Ammonium chloride	4 d	R,M	8.2	23.9	17.22	108.5			Bader and Grizzle 1992
Channel catfish (3.5 g), *Ictalurus punctatus*	Ammonium chloride	4 d	F,M	7.8	19.6	44.71	**132.9**			West 1985; Arthur et al. 1987

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	Chemical Name	Duration	Methods[a]	pH	Temp. (˚C)	Total Ammonia (mg TAN/L)	Total Ammonia[b] (mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)	SMAV (mg TAN/L)	GMAV (mg TAN/L)	Reference
Channel catfish (5.8 g), *Ictalurus punctatus*	Ammonium chloride	4 d	F,M	8	3.5	37.64	**161.6**			West 1985; Arthur et al. 1987
Channel catfish (6.4 g), *Ictalurus punctatus*	Ammonium chloride	4 d	F,M	8.1	14.6	24.94	**129.5**			West 1985; Arthur et al. 1987
Channel catfish, *Ictalurus punctatus*	Ammonium chloride	4 d	F,M	8.4	28	10.71	**99.59**			Colt and Tchobanoglous 1978
Channel catfish (3-11 mo), *Ictalurus punctatus*	Ammonium chloride	4 d	F,M	7.46	10	124.8	**213.5**			DeGraeve et al. 1987
Channel catfish (3-11 mo), *Ictalurus punctatus*	Ammonium chloride	4 d	F,M	7.41	15	113.1	**180.2**			DeGraeve et al. 1987
Channel catfish (3-11 mo), *Ictalurus punctatus*	Ammonium chloride	4 d	F,M	7.41	20	89.63	**142.8**			DeGraeve et al. 1987
Channel catfish (3-11 mo), *Ictalurus punctatus*	Ammonium chloride	4 d	F,M	7.45	20	72.15	**121.7**			DeGraeve et al. 1987
Channel catfish (3-11 mo), *Ictalurus punctatus*	Ammonium chloride	4 d	F,M	7.4	25	89.41	**140.5**			DeGraeve et al. 1987
Channel catfish (3-11 mo), *Ictalurus punctatus*	Ammonium chloride	4 d	F,M	7.41	25	85.69	**136.5**			DeGraeve et al. 1987
Channel catfish (3-11 mo), *Ictalurus punctatus*	Ammonium chloride	4 d	F,M	7.44	30	65.25	**108.5**			DeGraeve et al. 1987
Channel catfish (<110 d), *Ictalurus punctatus*	Ammonium chloride	4 d	F,M	8	20	15.09	**64.77**			Diamond et al. 1993
Channel catfish, *Ictalurus punctatus*	Ammonium chloride	4 d	F,M	7.94	23.8	33.1	**127.0**			Reinbold and Pescitelli 1982d
Channel catfish, *Ictalurus punctatus*	Ammonium chloride	4 d	F,M	7.98	23.8	30.49	**126.1**			Reinbold and Pescitelli 1982d
Channel catfish (4.5-10.8 g), *Ictalurus punctatus*	Ammonium chloride	4 d	F,M	8.08	28	44.44	**222.2**			Roseboom and Richey 1977
Channel catfish (7.1-12.7 g), *Ictalurus punctatus*	Ammonium chloride	4 d	F,M	8.09	22	32.33	**164.8**			Roseboom and Richey 1977
Channel catfish (14.3 mm, 19.0 g), *Ictalurus punctatus*	Ammonium chloride	4 d	F,M	7.93	20	74.35	**277.4**			Sparks 1975
Channel catfish (0.5 g), *Ictalurus punctatus*	Ammonium chloride	4 d	F,M	7.8	25.7	32.85	**97.67**			Swigert and Spacie 1983

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals										
Species	**Chemical Name**	**Duration**	**Methods**[a]	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia**[b] **(mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Channel catfish, *Ictalurus punctatus*	Ammonium chloride	4 d	F,M	8	26	32.34	**138.8**			West 1985
Channel catfish, *Ictalurus punctatus*	Ammonium chloride	4 d	F,M	8.1	17	40.83	**212.1**	142.4	142.4	West 1985
Red swamp crayfish (2.1 cm), *Procambarus clarkii*	Ammonium chloride	4 d	F,M	8	20	26.08	**112.0**			Diamond et al. 1993
Red swamp crayfish (<2.5 cm), *Procambarus clarkii*	Ammonium chloride	4 d	F,M	8	12	76.92	**170.1**	138.0	138.0	Diamond et al. 1993
Atlantic salmon (4.8-9.2 cm), *Salmo salar*	Ammonium sulfate	4 d	S,M	6.4	1.8	123	**87.86**			Knoph 1992
Atlantic salmon (4.8-9.2 cm), *Salmo salar*	Ammonium sulfate	4 d	S,M	6.4	1.8	133.9	**95.64**			Knoph 1992
Atlantic salmon (4.8-9.2 cm), *Salmo salar*	Ammonium sulfate	4 d	S,M	6	2.1	297.2	**195.1**			Knoph 1992
Atlantic salmon (4.8-9.2 cm), *Salmo salar*	Ammonium sulfate	4 d	S,M	6	2.1	341.1	**223.9**			Knoph 1992
Atlantic salmon (4.8-9.2 cm), *Salmo salar*	Ammonium sulfate	4 d	S,M	6.05	2.5	400	**264.4**			Knoph 1992
Atlantic salmon (4.8-9.2 cm), *Salmo salar*	Ammonium sulfate	4 d	S,M	6.05	2.5	491.7	**325.0**			Knoph 1992
Atlantic salmon (4.8-9.2 cm), *Salmo salar*	Ammonium sulfate	4 d	S,M	6	7.3	581.5	**381.7**			Knoph 1992
Atlantic salmon (4.8-9.2 cm), *Salmo salar*	Ammonium sulfate	4 d	S,M	6	7.3	587.6	**385.7**			Knoph 1992
Atlantic salmon (4.8-9.2 cm), *Salmo salar*	Ammonium sulfate	4 d	S,M	6.45	7.4	171.3	**124.4**			Knoph 1992
Atlantic salmon (4.8-9.2 cm), *Salmo salar*	Ammonium sulfate	4 d	S,M	6.45	7.4	214.4	**155.7**			Knoph 1992
Atlantic salmon (4.8-9.2 cm), *Salmo salar*	Ammonium sulfate	4 d	S,M	6.45	12.5	230.6	**167.4**			Knoph 1992
Atlantic salmon (4.8-9.2 cm), *Salmo salar*	Ammonium sulfate	4 d	S,M	6.45	12.5	248.3	**180.3**			Knoph 1992
Atlantic salmon (4.8-9.2 cm), *Salmo salar*	Ammonium sulfate	4 d	S,M	6.05	12.5	403.5	**266.7**			Knoph 1992

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	Chemical Name	Duration	Methods[a]	pH	Temp. (˚C)	Total Ammonia (mg TAN/L)	Total Ammonia[b] (mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)	SMAV (mg TAN/L)	GMAV (mg TAN/L)	Reference
Atlantic salmon (4.8-9.2 cm), *Salmo salar*	Ammonium sulfate	4 d	S,M	6.05	12.5	451.5	**298.5**			Knoph 1992
Atlantic salmon (4.8-9.2 cm), *Salmo salar*	Ammonium sulfate	4 d	S,M	6.05	17.1	356.1	**235.4**			Knoph 1992
Atlantic salmon (4.8-9.2 cm), *Salmo salar*	Ammonium sulfate	4 d	S,M	6.05	17.1	373	**246.6**			Knoph 1992
Atlantic salmon (1.5 g), *Salmo salar*	Ammonium chloride	4 d	S,M	7.45	8.5	60.29	**101.7**			Soderberg and Meade 1992
Atlantic salmon (1.5 g), *Salmo salar*	Ammonium chloride	4 d	S,M	7.45	8.5	35.74	**60.26**			Soderberg and Meade 1992
Atlantic salmon (36 g), *Salmo salar*	Ammonium chloride	4 d	S,M	7.45	8.5	118.2	**199.3**			Soderberg and Meade 1992
Atlantic salmon (36 g), *Salmo salar*	Ammonium chloride	4 d	S,M	7.45	8.5	70.62	**119.1**	183.3		Soderberg and Meade 1992
Brown trout (1.20 g, 5.4 cm), *Salmo trutta*	Ammonium chloride	4 d	F,U	7.85	13.2	29.58	**96.20**			Thurston and Meyn 1984
Brown trout (1.17 g, 5.3 cm), *Salmo trutta*	Ammonium chloride	4 d.	F,U	7.86	13.8	32.46	**107.5**			Thurston and Meyn 1984
Brown trout (0.91 g, 4.9 cm), *Salmo trutta*	Ammonium chloride	4 d	F,U	7.82	14.2	33.3	**102.6**	102.0	136.7	Thurston and Meyn 1984
White perch (76 mm), *Morone americana*	Ammonium chloride	4 d	S,M	8	16	14.93	**64.09**			Stevenson 1977
White perch (76 mm), *Morone americana*	Ammonium chloride	4 d	S,M	6	16	418.4	**274.7**	132.7		Stevenson 1977
White bass (4.4 g), *Morone chrysops*	Ammonium chloride	4 d	S,M	7.09	19.7	132.4	**144.0**	144.0		Ashe et al. 1996
Striped bass (20-93 mm), *Morone saxatilis*	Ammonium chloride	4 d	S,M	7.4	23.3	92.17	**144.8**			Hazel et al. 1971
Striped bass (20-93 mm), *Morone saxatilis*	Ammonium chloride	4 d	S,M	7.5	23.3	73.45	**133.3**			Hazel et al. 1971
Striped bass (20-93 mm), *Morone saxatilis*	Ammonium chloride	4 d	S,M	7.35	15	259.7	**378.9**			Hazel et al. 1971

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	Chemical Name	Duration	Methods[a]	pH	Temp. (˚C)	Total Ammonia (mg TAN/L)	Total Ammonia[b] (mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)	SMAV (mg TAN/L)	GMAV (mg TAN/L)	Reference
Striped bass (20-93 mm), *Morone saxatilis*	Ammonium chloride	4 d	S,M	7.5	15	182.3	**330.7**			Hazel et al. 1971
Striped bass (20-93 mm), *Morone saxatilis*	Ammonium chloride	4 d	S,M	7.93	23.3	48.03	**180.8**			Hazel et al. 1971
Striped bass (20-93 mm), *Morone saxatilis*	Ammonium chloride	4 d	S,M	7.5	23.3	125.9	**228.5**			Hazel et al. 1971
Striped bass (20-93 mm), *Morone saxatilis*	Ammonium chloride	4 d	S,M	7.84	15	165.7	**524.6**			Hazel et al. 1971
Striped bass (20-93 mm), *Morone saxatilis*	Ammonium chloride	4 d	S,M	7.5	15	354.9	**644.0**			Hazel et al. 1971
Striped bass (126.6 g), *Morone saxatilis*	Ammonium chloride	4 d	S,M	8.3	21	12.86	**98.43**	246.2		Oppenborn and Goudie 1993
Sunshine bass (larvae, 12 h), *Morone saxatilis x chrysops*	Ammonium chloride	4 d	S,M	8.5	18.7	3.903	**43.99**			Harcke and Daniels 1999
Sunshine bass (367.2 g), *Morone saxatilis x chrysops*	Ammonium chloride	4 d	S,M	8.3	21	8.147	**62.37**			Oppenborn and Goudie 1993
Sunshine bass (42.7 g), *Morone saxatilis x chrysops*	Ammonium chloride	4 d	S,M	7	25	63.62	**63.62**			Weirich et al. 1993
Sunshine bass (42.7 g), *Morone saxatilis x chrysops*	Ammonium chloride	4 d	S,M	7	25	83.06	**83.06**			Weirich et al. 1993
Sunshine bass (42.7 g), *Morone saxatilis x chrysops*	Ammonium chloride	4 d	S,M	7	25	56.55	**56.55**			Weirich et al. 1993
Sunshine bass (42.7 g), *Morone saxatilis x chrysops*	Ammonium chloride	4 d	S,M	7	25	65.39	**65.39**			Weirich et al. 1993
Sunshine bass (42.7 g), *Morone saxatilis x chrysops*	Ammonium chloride	4 d	S,M	7	25	60.09	**60.09**			Weirich et al. 1993
Sunshine bass (42.7 g), *Morone saxatilis x chrysops*	Ammonium chloride	4 d	S,M	7	25	64.51	**64.51**			Weirich et al. 1993
Sunshine bass (42.7 g), *Morone saxatilis x chrysops*	Ammonium chloride	4 d	S,M	7	25	79.53	**79.53**			Weirich et al. 1993
Sunshine bass (42.7 g), *Morone saxatilis x chrysops*	Ammonium chloride	4 d	S,M	7	25	86.6	**86.60**			Weirich et al. 1993
Sunshine bass (42.7 g), *Morone saxatilis x chrysops*	Ammonium chloride	4 d	S,M	7	25	95.43	**95.43**			Weirich et al. 1993

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	Chemical Name	Duration	Methods[a]	pH	Temp. (˚C)	Total Ammonia (mg TAN/L)	Total Ammonia[b] (mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)	SMAV (mg TAN/L)	GMAV (mg TAN/L)	Reference
Sunshine bass (42.7 g), *Morone saxatilis x chrysops*	Ammonium chloride	4 d	S,M	7	25	105.2	**105.2**	70.22	134.8	Weirich et al. 1993
Water flea, *Daphnia magna*	Ammonium chloride	2 d	S,M	8.5	20	26.34	**296.9**			Gersich and Hopkins 1986
Water flea, *Daphnia magna*	Ammonium chloride	2 d	S,M	7.92	21	9.463	**37.66**			Gulyas and Fleit 1990
Water flea, *Daphnia magna*	Ammonium chloride	2 d	S,M	8.2	25	20.71	**197.5**			Parkhurst et al. 1979, 1981
Water flea, *Daphnia magna*	Ammonium chloride	2 d	R,U	8.34	19.7	51.92	**419.1**			Reinbold and Pescitelli 1982a
Water flea, *Daphnia magna*	Ammonium chloride	2 d	S,M	8.07	19.6	51.09	**242.4**			Russo et al. 1985
Water flea, *Daphnia magna*	Ammonium chloride	2 d	S,M	7.51	20.1	48.32	**89.74**			Russo et al. 1985
Water flea, *Daphnia magna*	Ammonium chloride	2 d	S,M	7.53	20.1	55.41	**106.1**			Russo et al. 1985
Water flea, *Daphnia magna*	Ammonium chloride	2 d	S,M	7.5	20.3	43.52	**80.98**			Russo et al. 1985
Water flea, *Daphnia magna*	Ammonium chloride	2 d	S,M	7.4	20.6	42.31	**69.88**			Russo et al. 1985
Water flea, *Daphnia magna*	Ammonium chloride	2 d	S,M	8.09	20.9	41.51	**227.9**			Russo et al. 1985
Water flea, *Daphnia magna*	Ammonium chloride	2 d	S,M	7.95	22	51.3	**236.7**			Russo et al. 1985
Water flea, *Daphnia magna*	Ammonium chloride	2 d	S,M	8.15	22	37.44	**252.8**			Russo et al. 1985
Water flea, *Daphnia magna*	Ammonium chloride	2 d	S,M	8.04	22.8	38.7	**226.1**	157.7		Russo et al. 1985
Water flea, *Daphnia pulicaria*	Ammonium chloride	2 d	F,M	8.05	14	34.5	**99.03**	99.03	125.0	DeGraeve et al. 1980
Clawed toad (embryo), *Xenopus laevis*	Ammonium sulfate	4 d	R,M	7.2	22	38.59	**40.91**			Schuytema and Nebeker 1999a

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	Chemical Name	Duration	Methods[a]	pH	Temp. (˚C)	Total Ammonia (mg TAN/L)	Total Ammonia[b] (mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)	SMAV (mg TAN/L)	GMAV (mg TAN/L)	Reference
Clawed toad (embryo), *Xenopus laevis*	Ammonium sulfate	4 d	R,M	7.2	22	119.6	**126.8**			Schuytema and Nebeker 1999a
Clawed toad (embryo), *Xenopus laevis*	Ammonium nitrate	4 d	R,M	7.2	24	32.37	**39.55**			Schuytema and Nebeker 1999a
Clawed toad (embryo), *Xenopus laevis*	Ammonium sulfate	4 d	R,M	7.2	24	60.71	**74.17**			Schuytema and Nebeker 1999a
Clawed toad (17 mg, Gosner Stage 26-27), *Xenopus laevis*	Nitric acid ammonium salt	4 d	R,M	7.15	22	101.4	**117.2**			Schuytema and Nebeker 1999b
Clawed toad (17 mg, Gosner Stage 26-27), *Xenopus laevis*	Ammonium sulfate	4 d	R,M	7.15	22	135.9	**157.2**			Schuytema and Nebeker 1999b
Clawed toad (21 mg, Gosner Stage 26-27), *Xenopus laevis*	Ammonium chloride	4 d	R,M	7.15	22	128.3	**148.4**			Schuytema and Nebeker 1999b
Clawed toad (embryo), *Xenopus laevis*	Ammonium phosphate	4 d	R,M	8.43	25	37.3	**367.4**			Tietge et al. 2000
Clawed toad (embryo), *Xenopus laevis*	Ammonium phosphate	4 d	R,M	8.62	25	28.7	**405.6**	122.5	122.5	Tietge et al. 2000
Flatworm, *Dendrocoelum lacteum*	Ammonium chloride	4 d	S,U	8.2	18	22.37	**119.5**	119.5	119.5	Stammer 1953
Walleye, *Sander vitreus*	Ammonium chloride	4 d	F,U	8.08	18.2	17.43	87.13			Reinbold and Pescitelli 1982a
Walleye (22.6 g), *Sander vitreus*	Ammonium chloride	4 d	F,M	7.9	3.7	48.37	**172.3**			West 1985; Arthur et al. 1987
Walleye (19.4 g), *Sander vitreus*	Ammonium chloride	4 d	F,M	7.7	11.1	89.93	**224.8**			West 1985; Arthur et al. 1987
Walleye (13.4 g), *Sander vitreus*	Ammonium chloride	4 d	F,M	8.3	19	6.123	**46.87**			West 1985; Arthur et al. 1987
Walleye (3.0 g, 65.6 mm), *Sander vitreus*	Ammonium chloride	4 d	F,M	8.06	21.5	21.49	**103.4**	117.1	117.1	Mayes et al. 1986
Central stoneroller (2.1 g), *Campostoma anomalum*	Ammonium chloride	4 d	F,M	7.8	25.7	38.97	**115.9**	115.9	115.9	Swigert and Spacie 1983

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	Chemical Name	Duration	Methods[a]	pH	Temp. (˚C)	Total Ammonia (mg TAN/L)	Total Ammonia[b] (mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)	SMAV (mg TAN/L)	GMAV (mg TAN/L)	Reference
Rainbow dace, *Cyprinella lutrensis*	Ammonium chloride	4 d	F,M	8.3	24	24.37	**186.5**			Hazel et al. 1979
Rainbow dace, *Cyprinella lutrensis*	Ammonium chloride	4 d	F,M	9.1	24	6.502	**206.1**	196.1		Hazel et al. 1979
Spotfin shiner (31-85 mm), *Cyprinella spiloptera*	Ammonium chloride	4 d	F,M	7.95	26.5	18.52	**72.39**			Rosage et al. 1979
Spotfin shiner (41-78 mm), *Cyprinella spiloptera*	Ammonium chloride	4 d	F,M	8.15	26.5	16.27	**93.07**			Rosage et al. 1979
Spotfin shiner (0.5 g), *Cyprinella spiloptera*	Ammonium chloride	4 d	F,M	7.9	25.7	24.52	**87.36**	83.80		Swigert and Spacie 1983
Steelcolor shiner (0.5 g), *Cyprinella whipplei*	Ammonium chloride	4 d	F,M	7.9	25.7	22.72	**80.94**	80.94	110.0	Swigert and Spacie 1983
Dwarf wedgemussel (glochidia), *Alasmidonta heterodon*	Ammonium chloride	1 d	S,M	8.3	20	>14.24[c]	**>109.0**	>109.0	>109.0	Wang et al. 2007b
Pink papershell (glochidia), *Potamilus ohiensis*	Ammonium chloride	1 d	S,M	8.3	20	>14.24[c]	**>109.0**	>109.0	>109.0	Wang et al. 2007b
Green sunfish (larvae, 9 d swim up fry), *Lepomis cyanellus*	Ammonium chloride	4 d	F,U	8.28	26.2	8.43	62.07			Reinbold and Pescitelli 1982a
Green sunfish, *Lepomis cyanellus*	Ammonium chloride	4 d	F,M	7.84	12.3	33.09	**105.7**			Jude 1973
Green sunfish (62.5 mg), *Lepomis cyanellus*	Ammonium chloride	4 d	F,M	7.2	22.4	142.9	**174.5**			McCormick et al. 1984
Green sunfish (62.5 mg), *Lepomis cyanellus*	Ammonium chloride	4 d	F,M	6.61	22.4	254.5	**197.0**			McCormick et al. 1984
Green sunfish (62.5 mg), *Lepomis cyanellus*	Ammonium chloride	4 d	F,M	7.72	22.4	55.79	**144.3**			McCormick et al. 1984
Green sunfish (62.5 mg), *Lepomis cyanellus*	Ammonium chloride	4 d	F,M	8.69	22.4	9.24	**148.6**	150.8		McCormick et al. 1984

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals										
Species	**Chemical Name**	**Duration**	**Methods**[a]	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia**[b] **(mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Pumpkinseed (4.13-9.22 g), *Lepomis gibbosus*	Ammonium chloride	4 d	F,M	7.77	12	9.11	**25.69**			Jude 1973
Pumpkinseed, *Lepomis gibbosus*	Ammonium chloride	4 d	F,M	7.77	14	48.09	**135.6**			Thurston 1981
Pumpkinseed, *Lepomis gibbosus*	Ammonium chloride	4 d	F,M	7.77	14.5	42.02	**118.5**			Thurston 1981
Pumpkinseed, *Lepomis gibbosus*	Ammonium chloride	4 d	F,M	7.71	15.7	48.54	**87.54**	77.53		Thurston 1981
Bluegill, *Lepomis macrochirus*	Ammonium chloride	4 d	S,M	7.51	20.35	40.41	73.88			EA Engineering 1985
Bluegill, *Lepomis macrochirus*	Ammonium chloride	4 d	S,M	7.51	20.35	41.96	76.72			EA Engineering 1985
Bluegill, *Lepomis macrochirus*	Ammonium chloride	4 d	S,M	7.52	20.65	41.9	78.36			EA Engineering 1985
Bluegill, *Lepomis macrochirus*	Ammonium chloride	4 d	S,M	7.51	20.35	44.3	80.98			EA Engineering 1985
Bluegill, *Lepomis macrochirus*	Ammonium chloride	4 d	S,M	7.52	20.65	42.63	79.73			EA Engineering 1985
Bluegill, *Lepomis macrochirus*	Ammonium chloride	4 d	S,M	7.52	20.65	44.1	82.48			EA Engineering 1985
Bluegill (1.7 cm), *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	8	20	21.56	**92.54**			Diamond et al. 1993
Bluegill, *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	8	12	25.12	**107.9**			Diamond et al. 1993
Bluegill (20.0-70.0 mm, young of year), *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	8.11	18.5	16.73	**88.57**			Emery and Welch 1969
Bluegill (20.0-70.0 mm, young of year), *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	8.24	18.5	42.01	**286.1**			Emery and Welch 1969
Bluegill (20.0-70.0 mm, young of year), *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	8.75	18.5	12.7	**227.4**			Emery and Welch 1969

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	**Chemical Name**	**Duration**	**Methods**[a]	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia**[b] **(mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Bluegill (20.0-70.0 mm, young of year), *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	9.05	18.5	6.581	**193.8**			Emery and Welch 1969
Bluegill (20.0-70.0 mm, young of year), *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	9.19	18.5	3.755	**135.0**			Emery and Welch 1969
Bluegill (20.0-70.0 mm, young of year), *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	9.62	18.5	0.7859	**44.84**			Emery and Welch 1969
Bluegill (20.0-70.0 mm, young of year), *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	9.85	18.5	1.346	**89.70**			Emery and Welch 1969
Bluegill, *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	8.6	24	5.509	**75.01**			Hazel et al. 1979
Bluegill (5.2 cm), *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	7.9	24.25	33.06	**117.8**			Lubinski et al. 1974
Bluegill (0.38 g, 26.3 mm), *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	8.1	22	19.39	**100.7**			Mayes et al. 1986
Bluegill (19 mm, 0.0781 g), *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	8.4	4	14.64	**136.1**			Reinbold and Pescitelli 1982b
Bluegill (22 mm, 0.1106 g), *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	8.12	25	23.37	**126.2**			Reinbold and Pescitelli 1982b
Bluegill (28 mm, 0.250 g), *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	8.16	4.5	12.55	**73.19**			Reinbold and Pescitelli 1982b
Bluegill (30 mm, 0.267 g), *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	8.09	24.8	17.22	**87.75**			Reinbold and Pescitelli 1982b
Bluegill (217 mg), *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	8	22	12.75	**54.74**			Roseboom and Richey 1977
Bluegill (342 mg), *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	8.2	28	14.81	**93.31**			Roseboom and Richey 1977
Bluegill (646 mg), *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	7.93	22	24.08	**90.66**			Roseboom and Richey 1977
Bluegill (72 mg), *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	8.07	22	8.846	**43.38**			Roseboom and Richey 1977
Bluegill, *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	7.6	21.7	44.03	**93.31**			Smith et al. 1984

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	**Chemical Name**	**Duration**	**Methods**[a]	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia**[b] **(mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Bluegill (4.8 cm, 1.1 g), *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	7.85	22.05	59.93	**194.9**			Sparks 1975
Bluegill (0.9 g), *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	7.8	24.2	33.88	**100.7**			Swigert and Spacie 1983
Bluegill (0.9 g), *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	7.6	26.5	58.69	**124.4**			Swigert and Spacie 1983
Bluegill (1.2 g), *Lepomis macrochirus*	Ammonium chloride	4 d	F,M	7.8	26.6	37.52	**111.6**	104.5	106.9	Swigert and Spacie 1983
Common carp (206 mg), *Cyprinus carpio*	Ammonium chloride	4 d	R,M	7.72	28	51.78	**133.9**			Hasan and MacIntosh 1986
Common carp (299 mg), *Cyprinus carpio*	Ammonium chloride	4 d	R,M	7.72	28	48.97	**126.6**			Hasan and MacIntosh 1986
Common carp (4-5 cm), *Cyprinus carpio*	Ammonium chloride	4 d	R,M	7.4	28	45.05	**70.78**	106.3	106.3	Rao et al. 1975
Golden trout (0.09 g, 24 cm), *Oncorhynchus aguabonita*	Ammonium chloride	4 d	F,M	8.06	13.2	23.3	**112.1**	112.1		Thurston and Russo 1981
Cutthroat trout (3.6 g), *Oncorhynchus clarkii*	Ammonium chloride	4 d	F,M	7.7	10	17.3	**43.24**			Thurston et al. 1981a
Cutthroat trout (3.6 g), *Oncorhynchus clarkii*	Ammonium chloride	4 d	F,M	7.7	10	29.1	**72.73**			Thurston et al. 1981a
Cutthroat trout (4.1 g), *Oncorhynchus clarkii*	Ammonium chloride	4 d	F,M	7.7	10	19.3	**48.24**			Thurston et al. 1981a
Cutthroat trout (4.1 g), *Oncorhynchus clarkii*	Ammonium chloride	4 d	F,M	7.7	10	26.3	**65.73**			Thurston et al. 1981a
Cutthroat trout (3.4 g), *Oncorhynchus clarkii*	Ammonium chloride	4 d	F,M	7.78	12.2	32.57	**93.49**			Thurston et al. 1978
Cutthroat trout (3.3 g), *Oncorhynchus clarkii*	Ammonium chloride	4 d	F,M	7.8	12.4	36.55	**108.7**			Thurston et al. 1978
Cutthroat trout (1.0 g), *Oncorhynchus clarkii*	Ammonium chloride	4 d	F,M	7.8	12.8	37.75	**112.2**			Thurston et al. 1978
Cutthroat trout (1.0 g), *Oncorhynchus clarkii*	Ammonium chloride	4 d	F,M	7.81	13.1	43.72	**132.3**	78.92		Thurston et al. 1978

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	**Chemical Name**	**Duration**	**Methods**[a]	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia**[b] **(mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Pink salmon (late alevins), *Oncorhynchus gorbuscha*	Ammonium sulfate	4 d	S,M	6.4	4.3	230.5	**164.6**			Rice and Bailey 1980
Pink salmon (fry), *Oncorhynchus gorbuscha*	Ammonium sulfate	4 d	S,M	6.4	4.3	277.7	**198.3**	180.7		Rice and Bailey 1980
Coho salmon (6 g), *Oncorhynchus kisutch*	Ammonium chloride	4 d	F,M	8.1	17.2	11.59	**60.20**			Buckley 1978
Coho salmon, *Oncorhynchus kisutch*	Ammonium chloride	4 d	F,M	7	15	82.02	**82.02**			Wilson 1974; Robinson-Wilson and Seim 1975
Coho salmon, *Oncorhynchus kisutch*	Ammonium chloride	4 d	F,M	7	15	84.43	**84.43**			Wilson 1974; Robinson-Wilson and Seim 1975
Coho salmon, *Oncorhynchus kisutch*	Ammonium chloride	4 d	F,M	7.5	15	50.65	**91.90**			Wilson 1974; Robinson-Wilson and Seim 1975
Coho salmon, *Oncorhynchus kisutch*	Ammonium chloride	4 d	F,M	7.5	15	52.76	**95.73**			Wilson 1974; Robinson-Wilson and Seim 1975
Coho salmon, *Oncorhynchus kisutch*	Ammonium chloride	4 d	F,M	8	15	21.63	**92.84**			Wilson 1974; Robinson-Wilson and Seim 1975
Coho salmon, *Oncorhynchus kisutch*	Ammonium chloride	4 d	F,M	8	15	22	**94.44**			Wilson 1974; Robinson-Wilson and Seim 1975
Coho salmon, *Oncorhynchus kisutch*	Ammonium chloride	4 d	F,M	8.5	15	9.093	**102.5**	87.05		Wilson 1974; Robinson-Wilson and Seim 1975
Rainbow trout (0.5-3.0 g), *Oncorhynchus mykiss*	Ammonium sulfate	4 d	S,U	7.95	15	51.06	199.6			Qureshi et al. 1982
Rainbow trout (McConaughy strain, 251 mg), *Oncorhynchus mykiss*	Ammonium chloride	4 d	S,M	6.84	12	112	98.86			Buhl and Hamilton 2000
Rainbow trout, *Oncorhynchus mykiss*	Ammonium chloride	4 d	S,M	7.55	15	34.23	67.04			Craig and Beggs 1979
Rainbow trout (0.80 g), *Oncorhynchus mykiss*	-	4 d	S,M	6.95	14.7	163.6	156.9			Environment Canada 2004
Rainbow trout (0.60 g), *Oncorhynchus mykiss*	-	4 d	S,M	6.97	14.5	144	140.3			Environment Canada 2004
Rainbow trout (0.63 g), *Oncorhynchus mykiss*	-	4 d	S,M	7.02	15.4	146.7	149.4			Environment Canada 2004

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals										
Species	**Chemical Name**	**Duration**	**Methods[a]**	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia[b] (mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Rainbow trout (0.80 g), *Oncorhynchus mykiss*	-	4 d	S,M	7.02	14.6	159	161.8			Environment Canada 2004
Rainbow trout (0.80 g), *Oncorhynchus mykiss*	-	4 d	S,M	7.03	15.1	156.6	160.9			Environment Canada 2004
Rainbow trout (0.90 g), *Oncorhynchus mykiss*	-	4 d	S,M	7.18	15.1	141.6	169.2			Environment Canada 2004
Rainbow trout (2.01 g), *Oncorhynchus mykiss*	-	4 d	S,M	7.45	15.1	104.4	176.0			Environment Canada 2004
Rainbow trout (1.30 g), *Oncorhynchus mykiss*	-	4 d	S,M	7.47	14.7	72.65	126.1			Environment Canada 2004
Rainbow trout (0.78 g), *Oncorhynchus mykiss*	-	4 d	S,M	7.47	14.5	79.67	138.3			Environment Canada 2004
Rainbow trout (0.40 g), *Oncorhynchus mykiss*	-	4 d	S,M	7.51	14.2	73.71	135.8			Environment Canada 2004
Rainbow trout (1.64 g), *Oncorhynchus mykiss*	-	4 d	S,M	7.54	14.6	75.3	145.2			Environment Canada 2004
Rainbow trout (1.13 g), *Oncorhynchus mykiss*	-	4 d	S,M	7.59	13.9	59.4	123.9			Environment Canada 2004
Rainbow trout (1.50 g), *Oncorhynchus mykiss*	-	4 d	S,M	7.87	15.1	42.9	144.7			Environment Canada 2004
Rainbow trout (1.38 g), *Oncorhynchus mykiss*	-	4 d	S,M	7.93	15.2	41.15	155.0			Environment Canada 2004
Rainbow trout (0.90 g), *Oncorhynchus mykiss*	-	4 d	S,M	7.97	15.2	36.17	145.4			Environment Canada 2004
Rainbow trout (1.00 g), *Oncorhynchus mykiss*	-	4 d	S,M	7.98	15.1	35.29	145.9			Environment Canada 2004
Rainbow trout (1.30 g), *Oncorhynchus mykiss*	-	4 d	S,M	8.03	14.9	23.03	104.6			Environment Canada 2004
Rainbow trout (1.26 g), *Oncorhynchus mykiss*	-	4 d	S,M	8.04	14.3	25.84	119.7			Environment Canada 2004
Rainbow trout (1.60 g), *Oncorhynchus mykiss*	-	4 d	S,M	8.34	15.3	19.15	158.5			Environment Canada 2004
Rainbow trout (1.30 g), *Oncorhynchus mykiss*	-	4 d	S,M	8.39	15.3	12.05	109.9			Environment Canada 2004
Rainbow trout (1.11 g), *Oncorhynchus mykiss*	-	4 d	S,M	8.4	14.9	12.84	119.4			Environment Canada 2004

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals										
Species	**Chemical Name**	**Duration**	**Methods**[a]	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia**[b] **(mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Rainbow trout (1.40 g), *Oncorhynchus mykiss*	-	4 d	S,M	8.44	14.7	14.41	144.7			Environment Canada 2004
Rainbow trout (0.90 g), *Oncorhynchus mykiss*	-	4 d	S,M	8.46	14.5	11.82	123.4			Environment Canada 2004
Rainbow trout (1.26 g), *Oncorhynchus mykiss*	-	4 d	S,M	8.47	14.3	17.2	183.0			Environment Canada 2004
Rainbow trout (1.01 g), *Oncorhynchus mykiss*	-	4 d	S,M	8.93	14.2	4.8	117.0			Environment Canada 2004
Rainbow trout (1.44 g), *Oncorhynchus mykiss*	-	4 d	S,M	8.93	15	5.4	131.6			Environment Canada 2004
Rainbow trout (1.42 g), *Oncorhynchus mykiss*	-	4 d	S,M	9.46	14.6	1.6	79.03			Environment Canada 2004
Rainbow trout, *Oncorhynchus mykiss*	-	4 d	S,M	7.5	15	38.37	69.63			Holt and Malcolm 1979
Rainbow trout (129 mm), *Oncorhynchus mykiss*	Phosphoric acid, Diammonium salt	4 d	F,U	7	15	207.5	207.5			Blahm 1978
Rainbow trout (1.7-1.9 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,U	7.4	14.5	20.03	31.47			Calamari et al. 1981
Rainbow trout, *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,U	7.4	14.5	46.31	72.77			Calamari et al. 1981
Rainbow trout (8-10 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,U	7.4	14.5	55.07	86.53			Calamari et al. 1981
Rainbow trout (129 mm), *Oncorhynchus mykiss*	Phosphoric acid, Diammonium salt	4 d	F,U	8	15	70	300.5			Blahm 1978
Rainbow trout (10.9 g), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.7	3.6	38.52	**96.27**			West 1985; Arthur et al. 1987
Rainbow trout (14.0 g), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.7	9.8	55.15	**137.8**			West 1985; Arthur et al. 1987
Rainbow trout (22.4 g), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.9	16.2	15.23	**54.24**			West 1985; Arthur et al. 1987
Rainbow trout (10.3 g), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.9	11.3	30.15	**107.4**			West 1985; Arthur et al. 1987
Rainbow trout (3.3 g), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	8.3	18.7	12.75	**97.57**			West 1985; Arthur et al. 1987

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals										
Species	**Chemical Name**	**Duration**	**Methods**[a]	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia**[b] **(mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Rainbow trout (53 mm, 1.48 g), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.95	10	35.14	**137.3**			Broderius and Smith Jr. 1979
Rainbow trout (stage 8), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.4	14.4	40.99	**64.40**			Calamari et al. 1977
Rainbow trout, *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	8.05	14	22.9	**108.1**			DeGraeve et al. 1980
Rainbow trout (45 mm, 0.86 g), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	8.16	14.2	23.39	**136.4**			Reinbold and Pescitelli 1982b
Rainbow trout (119 mm, 20.6 g), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	8.28	12.8	15.4	**113.4**			Reinbold and Pescitelli 1982b
Rainbow trout (115 mm, 18.1 g), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	8.34	5	17.32	**143.3**			Reinbold and Pescitelli 1982b
Rainbow trout (42 mm, 0.61 g), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	8.43	3	11.86	**116.8**			Reinbold and Pescitelli 1982b
Rainbow trout (52 mm, 1.47 g), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	8.5	14.9	10.09	**113.7**			Reinbold and Pescitelli 1982b
Rainbow trout (44 mm, 0.76 g), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	8.6	3.3	15.27	**207.9**			Reinbold and Pescitelli 1982b
Rainbow trout (6.3 g, 8.1 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.44	12.8	32.49	**54.00**			Thurston and Russo 1983
Rainbow trout (8.0 g, 8.9 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.5	14.5	24.2	**43.91**			Thurston and Russo 1983
Rainbow trout (29.8 g, 13.1 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.59	12.7	32.62	**68.03**			Thurston and Russo 1983
Rainbow trout (28.0 g, 13.1 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.6	13	23.8	**50.43**			Thurston and Russo 1983
Rainbow trout (24.5 g, 12.7 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.6	12.9	25.14	**53.27**			Thurston and Russo 1983
Rainbow trout (2596 g, 57.0 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.62	7.9	20.53	**44.93**[f]			Thurston and Russo 1983

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	**Chemical Name**	**Duration**	**Methods**[a]	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia**[b] **(mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Rainbow trout (15.1 g, 10.7 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.62	14.4	28.62	**62.64**			Thurston and Russo 1983
Rainbow trout (29.6 g, 13.3 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.63	12.9	25.65	**57.06**			Thurston and Russo 1983
Rainbow trout (1496 g, 48.5 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.64	9.8	25.82	**58.38**[f]			Thurston and Russo 1983
Rainbow trout (18.9 g, 11.6 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.64	13.1	29.28	**66.21**			Thurston and Russo 1983
Rainbow trout (558 g, 37.0 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.64	10	31.85	**72.02**			Thurston and Russo 1983
Rainbow trout (1698 g, 50.9 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.65	9.8	19.46	**44.73**[f]			Thurston and Russo 1983
Rainbow trout (22.8 g, 12.3 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.65	13.2	28.64	**65.84**			Thurston and Russo 1983
Rainbow trout (12.3 g, 10.2 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.65	14.3	29.02	**66.71**			Thurston and Russo 1983
Rainbow trout (513 g, 35.9 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.66	9.8	25.95	**60.65**			Thurston and Russo 1983
Rainbow trout (22.6 g, 12.3 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.66	13.6	28.27	**66.07**			Thurston and Russo 1983
Rainbow trout (26.0 g, 13.0 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.66	12.8	33.97	**79.39**			Thurston and Russo 1983
Rainbow trout (14.8 g, 10.5 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.67	14	27.3	**64.87**			Thurston and Russo 1983

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	Chemical Name	Duration	Methods[a]	pH	Temp. (˚C)	Total Ammonia (mg TAN/L)	Total Ammonia[b] (mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)	SMAV (mg TAN/L)	GMAV (mg TAN/L)	Reference
Rainbow trout (38.0 g, 14.4 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.68	13	33.15	**80.11**			Thurston and Russo 1983
Rainbow trout (1122 g, 45.6 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.69	10.4	17.75	**43.62**[f]			Thurston and Russo 1983
Rainbow trout (1140 g, 46.4 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.69	10.7	20.18	**49.59**[f]			Thurston and Russo 1983
Rainbow trout (152 g, 23.4 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.69	10.7	25.62	**62.96**			Thurston and Russo 1983
Rainbow trout (23.6 g, 13.2 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.69	13.4	27.51	**67.60**			Thurston and Russo 1983
Rainbow trout (9.5 g, 9.4 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.9	12.7	20.03	**71.36**			Thurston and Russo 1983
Rainbow trout (4.3 g, 7.1 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.71	11.5	30.22	**76.83**			Thurston and Russo 1983
Rainbow trout (4.0 g, 7.0 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.71	11.4	32.02	**81.40**			Thurston and Russo 1983
Rainbow trout (248 g, 25.2 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.74	10.4	25.76	**68.95**			Thurston and Russo 1983
Rainbow trout (25.8 g, 13.6 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.75	11.8	31.53	**85.87**			Thurston and Russo 1983
Rainbow trout (8.1 g, 9.3 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.75	12.3	33.94	**92.43**			Thurston and Russo 1983
Rainbow trout (380 g, 32.4 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.76	10	22.44	**62.19**			Thurston and Russo 1983
Rainbow trout (42.0 g, 16.0 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.77	13.6	31.81	**89.71**			Thurston and Russo 1983

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals										
Species	**Chemical Name**	**Duration**	**Methods[a]**	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia[b] (mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Rainbow trout (1.7 g, 5.7 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.79	12.4	41.97	**122.6**			Thurston and Russo 1983
Rainbow trout (11.2 g, 10.0 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.8	9.7	23.65	**70.32**			Thurston and Russo 1983
Rainbow trout (5.7 g, 8.4 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.8	13.3	42.02	**124.9**			Thurston and Russo 1983
Rainbow trout (2.3 g, 6.1 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.8	12.4	47.87	**142.3**			Thurston and Russo 1983
Rainbow trout (8.0 g, 9.5 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.82	13.2	33.67	**103.7**			Thurston and Russo 1983
Rainbow trout (4.6 g, 7.4 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.83	13.5	33.55	**105.2**			Thurston and Russo 1983
Rainbow trout (6.7 g, 8.6 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.84	12.2	24.54	**78.38**			Thurston and Russo 1983
Rainbow trout (9.0 g, 9.3 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.84	12.9	32.3	**103.2**			Thurston and Russo 1983
Rainbow trout (1.8 g, 5.7 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.84	13.8	33.09	**105.7**			Thurston and Russo 1983
Rainbow trout (4.3 g, 7.1 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.84	13	38.69	**123.6**			Thurston and Russo 1983
Rainbow trout (0.47 g, 4.0 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.85	12.5	29.77	**96.81**			Thurston and Russo 1983
Rainbow trout (2.5 g, 6.0 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.85	13.1	31.55	**102.6**			Thurston and Russo 1983
Rainbow trout (0.61 g, 4.3 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.85	13.1	33.59	**109.2**			Thurston and Russo 1983
Rainbow trout (1.02 g, 4.9 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.85	12.3	33.99	**110.5**			Thurston and Russo 1983
Rainbow trout (9.4 g, 9.6 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.85	16.1	34.17	**111.1**			Thurston and Russo 1983
Rainbow trout (0.33 g, 3.6 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.86	13	20.7	**68.55**			Thurston and Russo 1983
Rainbow trout (0.33 g, 3.6 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.86	13.4	23.71	**78.52**			Thurston and Russo 1983

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals										
Species	**Chemical Name**	**Duration**	**Methods**[a]	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia**[b] **(mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Rainbow trout (0.47 g, 4.0 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.86	12.7	28.77	**95.27**			Thurston and Russo 1983
Rainbow trout (1.7 g, 5.8 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.86	14.1	34.95	**115.7**			Thurston and Russo 1983
Rainbow trout (48.6 g, 15.2 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.86	10.2	35.31	**116.9**			Thurston and Russo 1983
Rainbow trout (0.15 g, 2.7 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.87	12.9	16.81	**56.69**			Thurston and Russo 1983
Rainbow trout (0.18 g, 2.9 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.87	12.9	18.99	**64.04**			Thurston and Russo 1983
Rainbow trout (0.23 g, 3.2 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.87	13.1	19.08	**64.34**			Thurston and Russo 1983
Rainbow trout (7.0 g, 8.8 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.87	12.2	20.02	**67.51**			Thurston and Russo 1983
Rainbow trout (0.18 g, 2.9 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.87	13	21.15	**71.32**			Thurston and Russo 1983
Rainbow trout (2.6 g, 6.2 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.87	12.1	31.8	**107.2**			Thurston and Russo 1983
Rainbow trout (11.1 g, 9.9 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.87	13	34.32	**115.7**			Thurston and Russo 1983
Rainbow trout (0.12 g, 2.4 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.88	12.8	11.07	**38.02**			Thurston and Russo 1983
Rainbow trout (0.14 g, 2.6 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.88	12.9	15.91	**54.64**			Thurston and Russo 1983
Rainbow trout (0.23 g, 3.2 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.88	13.4	19.43	**66.73**			Thurston and Russo 1983
Rainbow trout (52.1 g, 15.5 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.88	10	28.6	**98.22**			Thurston and Russo 1983
Rainbow trout (1.8 g, 5.9 cm), *Oncorhynchus mykiss*	Ammonium sulfate	4 d	F,M	7.89	12.4	36.73	**128.5**			Thurston and Russo 1983
Rainbow trout (0.06 g, 1.7 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.9	13.4	19.44	**69.26**			Thurston and Russo 1983
Rainbow trout (0.06 g, 1.7 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.7	13.9	28.54	**71.33**			Thurston and Russo 1983

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals										
Species	**Chemical Name**	**Duration**	**Methods**[a]	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia**[b] **(mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Rainbow trout (7.9 g, 9.2 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.9	11.9	22.65	**80.69**			Thurston and Russo 1983
Rainbow trout (9.7 g, 9.7 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.9	13	35.75	**127.4**			Thurston and Russo 1983
Rainbow trout (9.3 g, 9.0 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.9	13	37.41	**133.3**			Thurston and Russo 1983
Rainbow trout (0.08 g, 2.0 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.91	13.1	12.68	**46.01**			Thurston and Russo 1983
Rainbow trout (0.06 g, 1.7 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.91	13	20.99	**76.17**			Thurston and Russo 1983
Rainbow trout (7.1 g, 8.4 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.91	19	25.36	**92.03**			Thurston and Russo 1983
Rainbow trout (10.1 g, 9.8 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.91	19.1	26.44	**95.95**			Thurston and Russo 1983
Rainbow trout (1.7 g, 5.8 cm), *Oncorhynchus mykiss*	Phosphoric acid, Diammonium salt	4 d	F,M	7.94	12.8	26.49	**101.6**			Thurston and Russo 1983
Rainbow trout (2.1 g, 6.2 cm), *Oncorhynchus mykiss*	Ammonium sulfate	4 d	F,M	7.94	12.5	39.25	**150.6**			Thurston and Russo 1983
Rainbow trout (0.15 g, 2.7 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.95	12.5	19.75	**77.19**			Thurston and Russo 1983
Rainbow trout (8.6 g, 8.9 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.96	19.2	23.21	**92.42**			Thurston and Russo 1983
Rainbow trout (2.1 g, 6.2 cm), *Oncorhynchus mykiss*	Phosphoric acid, Diammonium salt	4 d	F,M	7.98	12.5	27.02	**111.7**			Thurston and Russo 1983
Rainbow trout (1.01 g, 4.6 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	8.06	13.2	33.64	**161.8**			Thurston and Russo 1983
Rainbow trout (0.36 g, 3.4 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	8.08	12.8	23.05	**115.2**			Thurston and Russo 1983
Rainbow trout (1.7 g, 5.9 cm), *Oncorhynchus mykiss*	Ammonium bicarbonate	4 d	F,M	8.1	13.9	18.14	**94.23**			Thurston and Russo 1983
Rainbow trout (1.8 g, 5.8 cm), *Oncorhynchus mykiss*	Ammonium bicarbonate	4 d	F,M	8.12	13.6	17.34	**93.61**			Thurston and Russo 1983
Rainbow trout (2596 g), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.62	7.9	21.6	**47.27**			Thurston et al. 1981a

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	Chemical Name	Duration	Methods[a]	pH	Temp. (˚C)	Total Ammonia (mg TAN/L)	Total Ammonia[b] (mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)	SMAV (mg TAN/L)	GMAV (mg TAN/L)	Reference
Rainbow trout (2080 g), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.67	7.7	17	**40.40**			Thurston et al. 1981a
Rainbow trout (293 g), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.71	8.5	20.7	**52.62**			Thurston et al. 1981a
Rainbow trout (230 g), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.72	8.2	10.5	**27.15**			Thurston et al. 1981a
Rainbow trout (244 g), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.72	8.1	19.8	**51.20**			Thurston et al. 1981a
Rainbow trout (230 g), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.74	8.3	22.3	**59.69**			Thurston et al. 1981a
Rainbow trout (247 g), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.74	8.1	28	**74.94**			Thurston et al. 1981a
Rainbow trout (18 g), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.86	9.6	19.3	**63.91**			Thurston et al. 1981a
Rainbow trout (21 g), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.86	9.7	31.6	**104.6**			Thurston et al. 1981a
Rainbow trout (4.6 g, 7.3 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.75	12.7	32.09	**87.39**			Thurston et al. 1981b
Rainbow trout (5.7 g, 8.0 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.75	12.5	36.97	**100.7**			Thurston et al. 1981b
Rainbow trout (5.0 g, 7.6 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.76	12.5	39.08	**108.3**			Thurston et al. 1981b
Rainbow trout (5.7 g, 8.0 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.79	12.9	40.88	**119.4**			Thurston et al. 1981b
Rainbow trout (4.0 g, 7.2 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.83	12.8	36.49	**114.5**			Thurston et al. 1981b
Rainbow trout (9.5 g, 9.4 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	6.51	14.1	157.4	**116.8**			Thurston et al. 1981c
Rainbow trout (9.5 g, 9.4 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	6.8	14.1	94.05	**80.83**			Thurston et al. 1981c
Rainbow trout (9.5 g, 9.4 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.3	14	74.2	**102.2**			Thurston et al. 1981c
Rainbow trout (9.5 g, 9.4 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	8.29	14.1	13.85	**104.0**			Thurston et al. 1981c
Rainbow trout (9.5 g, 9.4 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	8.82	13.9	3.95	**80.02**			Thurston et al. 1981c

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	**Chemical Name**	**Duration**	**Methods[a]**	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia[b] (mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Rainbow trout (9.5 g, 9.4 cm), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	9.01	14.5	2.51	**69.50**			Thurston et al. 1981c
Rainbow trout (juvenile), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	7.2	10	174	**212.6**			Wicks and Randall 2002
Rainbow trout (40.0 g; swimming fish), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	6.97	16.6	32.38	**31.56**			Wicks et al. 2002
Rainbow trout (40.0 g; resting fish), *Oncorhynchus mykiss*	Ammonium chloride	4 d	F,M	6.97	16.6	207	**201.7**	82.88		Wicks et al. 2002
Chinook salmon (1.0-7 g), *Oncorhynchus tshawytscha*	Ammonia	4 d	S,M	7.96	7	28.03	**111.6**			Servizi and Gordon 1990
Chinook salmon (14.4 g, 11.9 cm), *Oncorhynchus tshawytscha*	Ammonium chloride	4 d	F,U	7.87	13.5	18.47	**62.29**			Thurston and Meyn 1984
Chinook salmon (15.3 g, 12.1 cm), *Oncorhynchus tshawytscha*	Ammonium chloride	4 d	F,U	7.82	12.2	27.23	**83.90**			Thurston and Meyn 1984
Chinook salmon (18.1 g, 12.7 cm), *Oncorhynchus tshawytscha*	Ammonium chloride	4 d	F,U	7.84	12.3	24.74	**79.02**	82.39	99.15	Thurston and Meyn 1984
Topeka shiner (adult, 29 mo), *Notropis topeka*	Ammonium chloride	4 d	F,M	7.85	24.6	21.40	**69.59**			Adelman et al. 2009
Topeka shiner (juvenile, 16 mo), *Notropis topeka*	Ammonium chloride	4 d	F,M	8.05	25.0	18.70	**88.27**			Adelman et al. 2009
Topeka shiner (juvenile, 15 mo), *Notropis topeka*	Ammonium chloride	4 d	F,M	8.09	13.2	28.90	**147.3**	96.72	96.72	Adelman et al. 2009
Leopard frog (embryo), *Rana pipiens*	Ammonium chloride	4 d	F,M	8	20	31.04	**133.3**			Diamond et al. 1993
Leopard frog (8 d), *Rana pipiens*	Ammonium chloride	4 d	F,M	8	12	16.23	**69.69**	96.38	96.38	Diamond et al. 1993

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	Chemical Name	Duration	Methods[a]	pH	Temp. (˚C)	Total Ammonia (mg TAN/L)	Total Ammonia[b] (mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)	SMAV (mg TAN/L)	GMAV (mg TAN/L)	Reference
Long fingernailclam, *Musculium transversum*	Ammonium chloride	4 d	F,M	8.1	14.6	32.83	**109.0**			West 1985; Arthur et al. 1987
Long fingernailclam, *Musculium transversum*	Ammonium chloride	4 d	F,M	8.2	5.4	38.18	**71.74**			West 1985; Arthur et al. 1987
Long fingernailclam, *Musculium transversum*	Ammonium chloride	4 d	F,M	8.6	20.5	6.429	**91.24**	89.36	89.36	West 1985; Arthur et al. 1987
Smallmouth bass (26-29 mm, 264-267 mg), *Micropterus dolomieu*	Ammonium chloride	4 d	F,M	7.16	22.3	123.4	**144.3**			Broderius et al. 1985
Smallmouth bass (26-29 mm, 264-267 mg), *Micropterus dolomieu*	Ammonium chloride	4 d	F,M	6.53	22.3	359.9	**269.2**			Broderius et al. 1985
Smallmouth bass (26-29 mm, 264-267 mg), *Micropterus dolomieu*	Ammonium chloride	4 d	F,M	7.74	22.3	39.3	**105.2**			Broderius et al. 1985
Smallmouth bass (26-29 mm, 264-267 mg), *Micropterus dolomieu*	Ammonium chloride	4 d	F,M	8.71	22.3	7.56	**126.0**	150.6		Broderius et al. 1985
Largemouth bass (0.086-0.322 g), *Micropterus salmoides*	Ammonium chloride	4 d	F,M	8.04	28	19.59	**90.72**			Roseboom and Richey 1977
Largemouth bass (2.018-6.286 g), *Micropterus salmoides*	Ammonium chloride	4 d	F,M	7.96	22	20.48	**81.56**	86.02		Roseboom and Richey 1977
Guadalupe bass (6.5 g), *Micropterus treculii*	Ammonium chloride	4 d	S,M/	8	22	12.7	**54.52**	54.52	89.06	Tomasso and Carmichael 1986
Great pond snail (25-30 mm), *Lymnaea stagnalis*	-	4 d	F,M	7.9	11.5	50.33	**88.62**	88.62	88.62	Williams et al. 1986
Guppy (0.13 g, 2.03 cm), *Poecilia reticulata*	Ammonium chloride	4 d	S,U	7.5	27.55	5.929	**10.76**			Kumar and Krishnamoorthi 1983

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals										
Species	**Chemical Name**	**Duration**	**Methods[a]**	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia[b] (mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Guppy (8.0 mm), *Poecilia reticulata*	-	4 d	S,U	7.22	25	129.4	**161.8**			Rubin and Elmaraghy 1976
Guppy (8.25(6.3-11.0) mm), *Poecilia reticulata*	-	4 d	S,U	7.45	25	75.65	**127.6**			Rubin and Elmaraghy 1976
Guppy (8.70(6.8-10.6) mm), *Poecilia reticulata*	-	4 d	S,U	7.45	25	82.95	**139.9**	74.66	74.66	Rubin and Elmaraghy 1976
Johnny darter (38 mm), *Etheostoma nigrum*	Ammonium chloride	4 d	F,M	7.9	20.6	28.9	**103.0**			Nimmo et al. 1989
Johnny darter (38 mm), *Etheostoma nigrum*	Ammonium chloride	4 d	F,M	8	20.1	24.61	**105.7**			Nimmo et al. 1989
Johnny darter (38 mm), *Etheostoma nigrum*	Ammonium chloride	4 d	F,M	8.2	6.2	6.937	**43.72**			Nimmo et al. 1989
Johnny darter (38 mm), *Etheostoma nigrum*	Ammonium chloride	4 d	F,M	8.1	5.8	11.47	**59.57**			Nimmo et al. 1989
Johnny darter (38 mm), *Etheostoma nigrum*	Ammonium chloride	4 d	F,M	8.1	5.8	13.46	**69.93**			Nimmo et al. 1989
Johnny darter (38 mm), *Etheostoma nigrum*	Ammonium chloride	4 d	F,M	8	20.1	15.63	**67.08**	71.45		Nimmo et al. 1989
Orangethroat darter, *Etheostoma spectabile*	Ammonium chloride	4 d	F,M	8.1	22	16.12	**83.74**			Hazel et al. 1979
Orangethroat darter, *Etheostoma spectabile*	Ammonium chloride	4 d	F,M	8.4	21	7.65	**71.12**	77.17	74.25	Hazel et al. 1979
Rio Grande silvery minnow (3-5 d old), *Hybognathus amarus*	Ammonium chloride	4 d	R,M	8	25	16.9	**72.55**	72.55	72.55	Buhl 2002
Spring peeper (embryo), *Pseudacris crucifer*	Ammonium chloride	4 d	F,U	8	12	17.78	**76.33**			Diamond et al. 1993
Spring peeper, *Pseudacris crucifer*	Ammonium chloride	4 d	F,U	8	20	11.42	**49.04**	61.18		Diamond et al. 1993
Pacific tree frog (embryo), *Pseudacris regilla*	Ammonium nitrate	4 d	R,M	6.7	22	41.19	**33.36**			Schuytema and Nebeker 1999a

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	**Chemical Name**	**Duration**	**Methods**[a]	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia**[b] **(mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Pacific tree frog (embryo), *Pseudacris regilla*	Ammonium chloride	4 d	R,M	6.7	22	60.44	**48.95**			Schuytema and Nebeker 1999a
Pacific tree frog (embryo), *Pseudacris regilla*	Ammonium sulfate	4 d	R,M	6.7	22	103.1	**83.53**			Schuytema and Nebeker 1999a
Pacific tree frog (90 mg, Gosner Stage 26-27), *Pseudacris regilla*	Nitric acid ammonium salt	4 d	R,M	7.3	22	136.6	**188.1**			Schuytema and Nebeker 1999b
Pacific tree frog (60 mg, Gosner Stage 26-27), *Pseudacris regilla*	Ammonium sulfate	4 d	R,M	7.3	22	116.4	**160.2**	83.71	71.56	Schuytema and Nebeker 1999b
Mucket (glochidia), *Actinonaias ligamentina*	Ammonium chloride	1 d	S,M	8.6	20	6.141[c]	**83.61**			Wang et al. 2007b
Mucket (glochidia), *Actinonaias ligamentina*	Ammonium chloride	1 d	S,M	8.4	20	8.099[c]	**75.29**			Wang et al. 2007b
Mucket (glochidia), *Actinonaias ligamentina*	Ammonium chloride	1 d	S,M	8.3	20	5.073[c]	**38.84**			Wang et al. 2007b
Mucket (glochidia), *Actinonaias ligamentina*	Ammonium chloride	1 d	S,M	8.3	20	8.900[c]	**68.13**	63.89		Wang et al. 2007b
Pheasantshell (juvenile), *Actinonaias pectorosa*	Ammonium chloride	4 d	S,M	7.9	25	14.06	**75.80**			Keller 2000
Pheasantshell (juvenile), *Actinonaias pectorosa*	Ammonium chloride	4 d	S,M	7.95	25	14.08	**83.30**	79.46	71.25	Keller 2000
Giant floater mussel (adult), *Pyganodon grandis*	Ammonium chloride	4 d	S,M	7.71	25	18.84	**72.49**			Scheller 1997
Giant floater mussel (adult), *Pyganodon grandis*	Ammonium chloride	4 d	S,M	7.5	25	25.13	**69.02**	70.73	70.73	Scheller 1997
Shortnose sucker (0.53-2.00 g), *Chasmistes brevirostris*	Ammonium chloride	4 d	F,M	8	20	11.42	**49.04**			Saiki et al. 1999
Shortnose sucker, *Chasmistes brevirostris*	Ammonium chloride	4 d	F,M	8	20	22.85	**98.09**	69.36	69.36	Saiki et al. 1999

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

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Pagoda hornsnail (adult), *Pleurocera uncialis*	Ammonium chloride	4 d	R,M	8.1	22	11.18	**68.54**	68.54	68.54	Goudreau et al. 1993
Golden shiner, *Notemigonus crysoleucas*	Ammonium chloride	4 d	S,M	7.5	19.6	89.61	162.6			EA Engineering 1985
Golden shiner, *Notemigonus crysoleucas*	Ammonium chloride	4 d	S,M	7.55	19.5	73.85	144.6			EA Engineering 1985
Golden shiner (8.7 g), *Notemigonus crysoleucas*	Ammonium chloride	4 d	F,M	7.5	24.5	34.73	**63.02**	63.02	63.02	Swigert and Spacie 1983
Pebblesnail (1.8 mm), *Fluminicola* sp.	Ammonium chloride	4 d	F,M	8.25	20.2	>8.801	**>62.15**	>62.15	>62.15	Besser 2011
Lost River sucker (0.49-0.80 g), *Deltistes luxatus*	Ammonium chloride	4 d	F,M	8	20	16.81	**72.18**			Saiki et al. 1999
Lost River sucker (larvae), *Deltistes luxatus*	Ammonium chloride	4 d	F,M	8	20	10.35	**44.42**	56.62	56.62	Saiki et al. 1999
Mountain whitefish (177 g, 27.0 cm), *Prosopium williamsoni*	Ammonium chloride	4 d	F,U	7.68	12.1	11.3	**27.31**			Thurston and Meyn 1984
Mountain whitefish (56.9 g, 19.1 cm), *Prosopium williamsoni*	Ammonium chloride	4 d	F,U	7.84	12.4	25.47	**81.35**			Thurston and Meyn 1984
Mountain whitefish (63.0 g, 20.4 cm), *Prosopium williamsoni*	Ammonium chloride	4 d	F,U	7.8	12.3	21.2	**63.04**	51.93	51.93	Thurston and Meyn 1984
Atlantic pigtoe (glochidia), *Fusconaia masoni*	Ammonium chloride	6 h	S,M	7.6	24.9	15.9	**47.40**	47.40	47.40	Black 2001
Pondshell mussel (juvenile), *Utterbackia imbecillis*	Ammonium chloride	4 d	S,M	7.9	24	8.235	**40.87**			Keller 2000
Pondshell mussel (juvenile), *Utterbackia imbecillis*	Ammonium chloride	4 d	S,M	8.35	25	3.269	**41.75**			Keller 2000

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Species	**Chemical Name**	**Duration**	**Methods**[a]	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia**[b] **(mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Pondshell mussel (juvenile), *Utterbackia imbecillis*	Ammonium chloride	4 d	S,M	7.9	25	9.355	**50.45**			Keller 2000
Pondshell mussel (8 d old juvenile), *Utterbackia imbecillis*	Ammonium chloride	4 d	R,M	7.8	24	14.29	**59.19**			Wade et al. 1992
Pondshell mussel (<2 d old juvenile), *Utterbackia imbecillis*	Ammonium chloride	4 d	R,M	8.16	25	5.254	**46.38**			Black 2001
Pondshell mussel (<2 d old juvenile), *Utterbackia imbecillis*	Ammonium chloride	4 d	R,M	8.17	25	5.781	**52.03**			Black 2001
Pondshell mussel (<2 d old juvenile), *Utterbackia imbecillis*	Ammonium chloride	4 d	R,M	8.29	25	8.845	**100.5**			Black 2001
Pondshell mussel (<2 d old juvenile), *Utterbackia imbecillis*	Ammonium chloride	4 d	R,M	8	25.1	2.734	**17.91**			Black 2001
Pondshell mussel (glochidia), *Utterbackia imbecillis*	Ammonium chloride	1 d	S,M	8.02	25	7.395	**49.90**	46.93	46.93	Black 2001
Pink mucket (2 mo old juvenile), *Lampsilis abrupta*	Ammonium chloride	4 d	R,M	8.3	20	1.921[d]	14.71			Wang et al. 2007b
Pink mucket (2 mo old juvenile), *Lampsilis abrupta*	Ammonium chloride	4 d	F,M	8.4	20	2.8	**26.03**	26.03		Wang et al. 2007a
Plain pocketbook (3-5 d old juvenile), *Lampsilis cardium*	Ammonium chloride	4 d	S,M	8.2	20.5	23.50[e]	154.4			Newton et al. 2003
Plain pocketbook (3-5 d old juvenile), *Lampsilis cardium*	Ammonium chloride	4 d	S,M	8.2	21.2	23.70[e]	165.0			Newton et al. 2003
Plain pocketbook (1-2 d old juvenile), *Lampsilis cardium*	Ammonium chloride	4 d	F,M	7.6	21.2	23.1	**54.07**			Newton and Bartsch 2007

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	Chemical Name	Duration	Methods[a]	pH	Temp. (˚C)	Total Ammonia (mg TAN/L)	Total Ammonia[b] (mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)	SMAV (mg TAN/L)	GMAV (mg TAN/L)	Reference
Plain pocketbook (1-2 d old juvenile), *Lampsilis cardium*	Ammonium chloride	4 d	F,M	7.1	21.2	38.9	**47.19**	50.51		Newton and Bartsch 2007
Wavy-rayed lampmussel (2-5 d old juvenile), *Lampsilis fasciola*	Ammonium chloride	4 d	R,M	7.83	12.6	14.9	**25.31**			Mummert et al. 2003
Wavy-rayed lampmussel (<5 d old juvenile), *Lampsilis fasciola*	Ammonium chloride	4 d	R,M	8.5	20	6.179[d]	**69.63**			Wang et al. 2007b
Wavy-rayed lampmussel (glochidia), *Lampsilis fasciola*	Ammonium chloride	1 d	S,M	8.3	20	7.743 [c]	**59.28**			Wang et al. 2007b
Wavy-rayed lampmussel (glochidia), *Lampsilis fasciola*	Ammonium chloride	1 d	S,M	8.4	20	5.518 [c]	**51.30**	48.11		Wang et al. 2007b
Higgin's eye (1-2 d old juvenile), *Lampsilis higginsii*	Ammonium chloride	4 d	F,M	7.6	21.2	19.5	**45.64**			Newton and Bartsch 2007
Higgin's eye (1-2 d old juvenile), *Lampsilis higginsii*	Ammonium chloride	4 d	F,M	7.1	21.2	31.7	**38.46**	41.90		Newton and Bartsch 2007
Neosho mucket (<5 d old juvenile), *Lampsilis rafinesqueana*	Ammonium chloride	4 d	R,M	8.3	20	9.185[d]	**70.31**			Wang et al. 2007b
Neosho mucket (<5 d old juvenile), *Lampsilis rafinesqueana*	Ammonium chloride	4 d	R,M	8.4	20	9.269[d]	**86.17**			Wang et al. 2007b
Neosho mucket (glochidia), *Lampsilis rafinesqueana*	Ammonium chloride	1 d	S,M	8.3	20	7.387 [c]	**56.55**	69.97		Wang et al. 2007b
Fatmucket (juvenile), *Lampsilis siliquoidea*	Ammonium chloride	4 d	S,M	8.3	24	1.275	13.60			Myers-Kinzie 1998

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals										
Species	**Chemical Name**	**Duration**	**Methods**[a]	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia**[b] **(mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Fatmucket (3 mo old juvenile), *Lampsilis siliquoidea*	Ammonium chloride, ammonium hydroxide	4 d	F,M	8.35	20	8.80	**74.25**			Miao et al. 2010
Fatmucket (2 mo old juvenile), *Lampsilis siliquoidea*	Ammonium chloride	4 d	R,M	8.1	20	4.092[d]	21.26			Wang et al. 2007b
Fatmucket (2 mo old juvenile), *Lampsilis siliquoidea*	Ammonium chloride	4 d	F,M	8.2	20	4.6	**28.99**			Wang et al. 2007a
Fatmucket (7 d old juvenile), *Lampsilis siliquoidea*	Ammonium chloride, ammonium hydroxide	4 d	F,M	7.6	20.5	11	**24.30**			Wang et al. 2008
Fatmucket (7 d old juvenile), *Lampsilis siliquoidea*	Ammonium chloride, ammonium hydroxide	4 d	F,M	8.1	20.6	5.2	**28.39**			Wang et al. 2008
Fatmucket (7 d old juvenile), *Lampsilis siliquoidea*	Ammonium chloride, ammonium hydroxide	4 d	F,M	8.5	20.6	3.4	**40.27**			Wang et al. 2008
Fatmucket (7 d old juvenile), *Lampsilis siliquoidea*	Ammonium chloride, ammonium hydroxide	4 d	F,M	9	20.6	0.96	**27.51**			Wang et al. 2008
Fatmucket (7 d old juvenile), *Lampsilis siliquoidea*	Ammonium chloride, ammonium hydroxide	4 d	F,M	6.6	19.6	88	**65.59**			Wang et al. 2008
Fatmucket (7 d old juvenile), *Lampsilis siliquoidea*	Ammonium chloride, ammonium hydroxide	4 d	F,M	8.1	19.4	11	**54.37**			Wang et al. 2008
Fatmucket (<5 d old juvenile), *Lampsilis siliquoidea*	Ammonium chloride	4 d	R,M	8.5	20	8.350[d]	94.09			Wang et al. 2007b
Fatmucket (glochidia), *Lampsilis siliquoidea*	Ammonium chloride	1 d	S,M	8.4	20	9.790 [c]	**91.01**			Wang et al. 2007b

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	**Chemical Name**	**Duration**	**Methods**[a]	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Total Ammonia**[b] **(mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)**	**SMAV (mg TAN/L)**	**GMAV (mg TAN/L)**	**Reference**
Fatmucket (glochidia), *Lampsilis siliquoidea*	Ammonium chloride	1 d	S,M	8.2	20	13.35[c]	**84.14**			Wang et al. 2007b
Fatmucket (glochidia), *Lampsilis siliquoidea*	Ammonium chloride	1 d	S,M	8.4	20	11.57[c]	**107.6**			Wang et al. 2007b
Fatmucket (glochidia), *Lampsilis siliquoidea*	Ammonium chloride	1 d	S,M	8.5	20	>14.24[c]	**160.5**			Wang et al. 2007b
Fatmucket (glochidia), *Lampsilis siliquoidea*	Ammonium chloride	1 d	S,M	8.3	20	6.497[c]	**49.74**			Wang et al. 2007b
Fatmucket (glochidia), *Lampsilis siliquoidea*	Ammonium chloride	1 d	S,M	8.3	20	8.772[c]	**66.77**	55.42	46.63	Wang et al. 2007b
Rainbow mussel (2 mo old juvenile), *Villosa iris*	Ammonium chloride	4 d	R,M	8.4	20	2.505[d]	**23.29**			Wang et al. 2007b
Rainbow mussel (2 mo old juvenile), *Villosa iris*	Ammonium chloride	4 d	R,M	8.3	20	8.935[d]	**68.40**			Wang et al. 2007b
Rainbow mussel (5 d old juvenile), *Villosa iris*	Ammonium chloride	4 d	S,M	8.18	25	7.81	**71.66**			Scheller 1997
Rainbow mussel (<5 d old juvenile), *Villosa iris*	Ammonium chloride	4 d	R,M	8.1	20	5.261[d]	**27.33**			Wang et al. 2007b
Rainbow mussel (2-5 d old juvenile), *Villosa iris*	Ammonium chloride	4 d	R,M	7.29	12.6	20.6	**15.17**			Mummert et al. 2003
Rainbow mussel (<3 d old juvenile), *Villosa iris*	Ammonium chloride	4 d	S,M	8.18	25	7.07	**64.87**			Scheller 1997
Rainbow mussel (< 24 h old glochidia), *Villosa iris*	Ammonium chloride	1 d	S,M	7.94	20.0	3.290	**12.62**			Scheller 1997
Rainbow mussel (glochidia), *Villosa iris*	Ammonium chloride	1 d	S,M	8.4	20	10.68[c]	**99.28**			Wang et al. 2007b

Appendix A. Acute Toxicity of Ammonia to Aquatic Animals

Species	Chemical Name	Duration	Methods[a]	pH	Temp. (˚C)	Total Ammonia (mg TAN/L)	Total Ammonia[b] (mg TAN/L) adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*)	SMAV (mg TAN/L)	GMAV (mg TAN/L)	Reference
Rainbow mussel (<1 h old glochidia), *Villosa iris*	Ammonium chloride	1 d	R,M	8.1	22	3.570	**21.89**			Goudreau et al. 1993
Rainbow mussel (<1 h old glochidia), *Villosa iris*	Ammonium chloride	1 d	R,M	8.1	22	4.278	**26.23**	34.23	34.23	Goudreau et al. 1993
Oyster mussel (<5 d old juvenile), *Epioblasma capsaeformis*	Ammonium chloride	4 d	R,M	8.5	20	4.760[d]	**53.63**			Wang et al. 2007b
Oyster mussel (glochidia), *Epioblasma capsaeformis*	Ammonium chloride	6 h	R,M	8.5	20	5.0[c]	**17.81**			Wang et al. 2007b
Oyster mussel (glochidia), *Epioblasma capsaeformis*	Ammonium chloride	6 h	R,M	8.5	20	3.4[c]	**31.61**	31.14	31.14	Wang et al. 2007b
Green floater (<2 d old juvenile), *Lasmigona subviridis*	Ammonium chloride	4 d	R,M	7.73	24	6.613	**24.24**			Black 2001
Green floater (<2 d old juvenile), *Lasmigona subviridis*	Ammonium chloride	4 d	R,M	7.73	24	6.613	**24.24**			Black 2001
Green floater (<2 d old juvenile), *Lasmigona subviridis*	Ammonium chloride	4 d	R,M	7.92	24.8	3.969	**21.84**	23.41	23.41	Black 2001
Ellipse (glochidia), *Venustaconcha ellipsiformis*	Ammonium chloride	1 d	S,M	8.1	20	4.550[c]	**23.12**	23.12	23.12	Wang et al. 2007b

[a] S = static, R = renewal, F = flow-through, and NR= not reported (uncertain) exposure types; M = measured and U = unmeasured tests.

[b] Acute values are normalized to pH 7 (all organisms) and temperature 20°C (invertebrates) as per the equations provided in this document (see also 1999 AWQC document for the basis of the pH- and temperature-dependence of ammonia toxicity and Appendix D for an example calculation).

[c] The EC$_{50}$s reported in this study were based on nominal concentrations. Percent nominal concentrations of measured ammonia concentrations on exposure days 0 and 2 declined from 104 to 44. EC$_{50}$s based on measured concentrations were estimated from the reported EC$_{50}$s based on nominal concentrations by multiplying by 0.890 for the 24 hr test; this factor is the average of the percent nominal concentrations of measured concentrations from ammonia measurements made on exposure day 0 (i.e., 104) and estimated for day 1 (i.e, 74) of the study.

[d] The EC_{50}s reported in this study were based on nominal concentrations. Percent nominal concentrations of measured ammonia concentrations on exposure days 0 and 4 declined from 104 to 63. EC_{50}s based on measured concentrations were estimated from the reported EC_{50}s based on nominal concentrations by multiplying by 0.835 or the average of the percent nominal concentrations of measured concentrations from ammonia measurements made on exposure days 0 and 4 in the study.

[e] EC_{50} values based on sediment porewater concentrations. **Note**: these EC_{50}s were not used to calculate the SMAV for the species.

[f] This small subset of LC_{50}s for adult rainbow trout from Thurston and Russo (1983) was used as the basis for the FAV calculated in the 1999 AWQC document. The FAV in the 1999 AWQC document of 11.23 mg TAN/L at pH 8 was lowered to the geometric mean of these five LC_{50} values at the time in order to protect large rainbow trout, which were shown to be measurably more sensitive than other life stages. The FAV prior to adjusting it to protect the commercially and recreationally important adult rainbow trout was calculated to be 14.32 mg TAN/L (CMC = 7.2 mg TAN/L) at pH 8. This FAV based on protection of adult rainbow trout at pH 7 is 48.21 mg TAN/L (see Table 7 in this document). Because several equivalent LC_{50}s representing different ages and life-stages have been added to the current (updated) acute criteria dataset, it no longer seems appropriate to lower the SMAV for rainbow trout based on only these five LC_{50}s considering the several other additional acute values which now exist.

Note: Each SMAV was calculated from the associated bold-face number(s) in the preceding column.

Appendix B. Chronic Toxicity of Ammonia to Aquatic Animals.

Appendix B. Chronic Toxicity of Ammonia to Aquatic Animals

Species	Test and Effect	pH	Temp. (˚C)	Total Ammonia (mg TAN/L)	Chronic value[a] Adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*) (mg TAN/L)	SMCV (mg TAN/L)	GMCV (mg TAN/L)	Reference
Stonefly, *Pteronarcella badia*	30-d Juv Survival	8.04	12.1	133.8	**207.0**			Thurston et al. 1984b
Stonefly, *Pteronarcella badia*	24-d Juv Survival	7.81	13.2	21.66	**26.27**	73.74	73.74	Thurston et al. 1984b
Water flea, *Ceriodaphnia acanthina*	7-d LC Reproduction	7.15	24.5	44.90	**64.10**	64.10		Mount 1982
Water flea, *Ceriodaphnia dubia*	7-d LC Reproduction	7.80	25.0	15.20	**38.96**			Nimmo et al. 1989
Water flea, *Ceriodaphnia dubia*	7-d LC Reproduction	8.57	26.0	5.800	**52.15**	45.08	53.75	Willingham 1987
Water flea, *Daphnia magna*	21-d LC Reproduction	8.45	19.8	7.370	**36.27**			Gersich et al. 1985
Water flea, *Daphnia magna*	21-d LC Reproduction	7.92	20.1	21.70	**47.40**	41.46	41.46	Reinbold and Pescitelli 1982a
Amphipod, *Hyalella azteca*	28-d PLC Biomass	8.04	25.0	8.207	**29.17**	29.17	29.17	Borgmann 1994
Channel catfish, *Ictalurus punctatus*	30-d ELS Weight	7.80	25.8	12.20	**22.66**			Reinbold and Pescitelli 1982a
Channel catfish, *Ictalurus punctatus*	30-d Juv Survival	8.35	27.9	5.020	**21.15**			Colt and Tchobanoglous 1978
Channel catfish, *Ictalurus punctatus*	30-d ELS Biomass	7.76	26.9	11.50	**20.35**	21.36	21.36	Swigert and Spacie 1983
Northern pike (fertilized), *Esox lucius*	52-d ELS Biomass	7.62	8.70	13.44	**20.38**	20.38	20.38	Harrahy et al. 2004
Common carp (fertilized), *Cyprinus carpio*	28-d ELS Weight	7.85	23.0	8.360	**16.53**	16.53	16.53	Mallet and Sims 1994

Appendix B. Chronic Toxicity of Ammonia to Aquatic Animals

Species	Test and Effect	pH	Temp. (˚C)	Total Ammonia (mg TAN/L)	Chronic value[a] Adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*) (mg TAN/L)	SMCV (mg TAN/L)	GMCV (mg TAN/L)	Reference
Lahontan cutthroat trout (fertilized), *Oncorhynchus clarkii henshawi*	103-d ELS Survival	7.57	13.7	17.89	**25.83**	25.83		Koch et al. 1980
Rainbow trout (fertilized), *Oncorhynchus mykiss*	42-d ELS Survival	7.50	10.0	<33.6	**<45.5**			Burkhalter and Kaya 1977
Rainbow trout, *Oncorhynchus mykiss*	72-d ELS Survival	7.40	14.5	2.600	**3.246**			Calamari et al. 1977, 1981
Rainbow trout (fertilized), *Oncorhynchus mykiss*	73-d ELS Survival	7.52	14.9	<2.55	**<3.515**			Solbe and Shurben 1989
Rainbow trout, *Oncorhynchus mykiss*	5-year LC	7.70	7.5-10.5	>6.71	**>11.08**			Thurston et al. 1984a
Rainbow trout, *Oncorhynchus mykiss*	90-d ELS Survival	7.75	11.4	8.919	**15.60**	6.663		Brinkman et al. 2009
Sockeye salmon, *Oncorhynchus nerka*	62-d Embryos Hatchability	8.42	10.0	<2.13	**<10.09**	10.09	12.02	Rankin 1979
White sucker (3 d old embryo), *Catostomus commersonii*	30-d ELS Biomass	8.32	18.6	2.900	**>11.62**	11.62	11.62	Reinbold and Pescitelli 1982a
Smallmouth bass, *Micropterus dolomieu*	32-d ELS Biomass	6.60	22.3	9.610	**8.650**			Broderius et al. 1985
Smallmouth bass, *Micropterus dolomieu*	32-d ELS Biomass	7.25	22.3	8.620	**9.726**			Broderius et al. 1985
Smallmouth bass, *Micropterus dolomieu*	32-d ELS Biomass	7.83	22.3	8.180	**15.77**			Broderius et al. 1985
Smallmouth bass, *Micropterus dolomieu*	32-d ELS Biomass	8.68	22.3	1.540	**11.31**	11.07	11.07	Broderius et al. 1985
Fathead minnow (embryo-larvae), *Pimephales promelas*	28-d ELS Survival	8.00	24.8	5.120	**12.43**			Mayes et al. 1986
Fathead minnow (embryo-larvae), *Pimephales promelas*	32-d ELS Biomass	7.95	25.5	7.457	**16.87**			Adelman et al. 2009
Fathead minnow, *Pimephales promelas*	30-d ELS Biomass	7.82	25.1	3.730	**7.101**			Swigert and Spacie 1983

Appendix B. Chronic Toxicity of Ammonia to Aquatic Animals

Species	Test and Effect	pH	Temp. (˚C)	Total Ammonia (mg TAN/L)	Chronic value[a] Adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*) (mg TAN/L)	SMCV (mg TAN/L)	GMCV (mg TAN/L)	Reference
Fathead minnow, *Pimephales promelas*	LC Hatchability	8.00	24.2	1.970	**4.784**	9.187	9.187	Thurston et al. 1986
Pebblesnail (1.81 mm, juvenile), *Fluminicola* sp.	28-d Juv Change in Length	8.22	20.1	2.281	**7.828**	7.828	7.828	Besser 2011
Long fingernailclam, *Musculium transversum*	42-d Juv Survival	8.15	23.5	5.820	**22.21**			Anderson et al. 1978
Long fingernailclam, *Musculium transversum*	42-d Juv Survival	7.80	21.8	1.230	**2.565**	7.547	7.547	Sparks and Sandusky 1981
Green sunfish, *Lepomis cyanellus*	30-d ELS Biomass	7.90	22.0	5.610	**11.85**			McCormick et al. 1984
Green sunfish, *Lepomis cyanellus*	30-d ELS Survival	8.16	25.4	5.840	**18.06**	14.63		Reinbold and Pescitelli 1982a
Bluegill, *Lepomis macrochirus*	30-d ELS Biomass	7.76	22.5	1.850	**3.273**	3.273	6.920	Smith et al. 1984
Rainbow mussel (2 mo old juvenile), *Villosa iris*	28-d Juv Survival	8.20	20.0	1.063	**3.501**	3.501	3.501	Wang et al. 2007a
Fatmucket (2 mo old juvenile), *Lampsilis siliquoidea*	28-d Juv Survival	8.25	20.0	0.8988	**3.211**	3.211		Wang et al. 20011
Wavy-rayed lamp mussel (2 mo old juvenile), *Lampsilis fasciola*	28-d Juv Survival	8.20	20.0	0.4272	**1.408**	1.408	2.126	Wang et al. 2007a

[a] The chronic value is an EC_{20} value calculated using EPA's TRAP (Versions 1.0 or 1.21a). Note: all chronic values were normalized to pH 7 (all organisms) and 20°C (invertebrates) as per the equations provided in this document (see also 1999 AWQC document for the basis of the pH- and temperature-dependence of ammonia toxicity and Appendix E for an example calculation).

Note: Each SMCV was calculated from the associated bold-face number(s) in the preceding column.

Appendix C. Other Chronic Ammonia Toxicity Data.

Appendix C. Other Chronic Ammonia Toxicity Data							
Species	**Test and Effect**	**Method[a]**	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Chronic value Adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*) (mg TAN/L)**	**Reference**
FRESHWATER INVERTEBRATES							
Pulmonate pondsnail (<1 wk post hatch), *Lymnaea stagnalis*	28-d NOEC - Growth	F,M	8.25	20.1	>8.00	>28.76	Besser et al. 2009
Pulmonate pondsnail (<1 wk post-hatch), *Lymnaea stagnalis*	28-d NOEC - Survival	F,M	8.25	20.1	>8.00	>28.76	Besser et al. 2009
Idaho springsnail (7-9 and 11-13 wk post hatch juvenile), *Pyrgulopsis idahoensis*	28-d NOEC - Growth	F,M	8.25	20.1	>8.00	>28.76	Besser et al. 2009
Idaho springsnail (7-9 and 11-13 wk post hatch juvenile), *Pyrgulopsis idahoensis*	28-d EC_{20} - Survival	F,M	8.25	20.1	0.480	1.726	Besser et al. 2009
Idaho springsnail (mixed-aged, adults), *Pyrgulopsis idahoensis*	28-d EC_{20} - Survival	F,M	8.26	20.8	3.24	12.39[b]	Besser et al. 2009
Pebblesnail (mixed-aged, field collected), *Fluminicola* sp.	28-d EC_{20} - Survival	F,M	8.26	20.8	1.02	3.900[c]	Besser et al. 2009
Pebblesnail (small, field collected), *Fluminicola* sp.	28-d MATC - Survival	F,M	8.19	20.1	2.75	8.977[d]	Besser 2011
Ozark springsnail (mixed age, field collected), *Fontigens aldrichi*	28-d EC_{20} - Survival	F,M	8.26	20.8	0.61	2.332[b]	Besser et al. 2009
Bliss Rapids snail (mixed age, field collected), *Taylorconcha serpenticola*	28-d EC_{20} - Survival	F,M	8.26	20.8	3.42	13.08[b]	Besser et al. 2009

Appendix C. Other Chronic Ammonia Toxicity Data

Species	**Test and Effect**	**Method[a]**	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Chronic value Adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*) (mg TAN/L)**	**Reference**
Silty hornsnail (mixed age, mature and field collected), *Pleurocera canaliculata*	28-d EC_{20} - Survival; (Alt Effect Conc.)	F,M	8.15	24.7	0.45 (≤1.86)	1.845 (≤7.667)[b, e]	GLEC 2011
Wavy-rayed lamp mussel (2 mo old juvenile), *Lampsilis fasciola*	28-d IC_{25} - Growth	F,M	8.20	20.0	0.5700	1.878	Wang et al. 2007a
Fatmucket (2 mo old juvenile), *Lampsilis siliquoidea*	28-d IC_{25} - Growth	F,M	8.20	20.0	0.4400	1.450	Wang et al. 2007a
Rainbow mussel (2 mo old juvenile), *Villosa iris*	28-d IC_{25} - Growth	F,M	8.20	20.0	0.7300	2.406	Wang et al. 2007a
Water flea, (<24 hr), *Ceriodaphnia dubia*	7-d; 3 broods in control IC_{25} Reproduction	R,U	7.90	25.0	1.300	3.790	Dwyer et al. 2005
FRESHWATER VERTEBRATES							
Cutthroat trout (3.3 g), *Oncorhynchus clarkii*	29-d LC_{50}	F,M	7.80	12.4	21.60	40.11	Thurston et al. 1978
Cutthroat trout (3.4 g), *Oncorhynchus clarkii*	29-d LC_{50}	F,M	7.78	12.2	21.40	38.78	Thurston et al. 1978
Cutthroat trout (1.0 g), *Oncorhynchus clarkii*	36-d LC_{50}	F,M	7.81	13.1	30.80	57.91	Thurston et al. 1978
Cutthroat trout (1.0 g), *Oncorhynchus clarkii*	36-d LC_{50}	F,M	7.80	12.8	32.20	59.79	Thurston et al. 1978
Atlantic salmon, *Salmo salar*	105-d Juv NOEC - Survival	F,M	6.84	12.1	>32.29	>30.64	Kolarevic et al. 2012
Lake trout, siscowet, *Salvelinus namaycush*	60-d LOEC- Weight gain	F,M	8.02	11.6	6.440	16.10	Beamish and Tandler 1990
Brook trout (juvenile), *Salvelinus fontinalis*	4-d Juv LOEC - Swimming Perf	F,M	9.10	15.0	0.7765	10.86	Tudorache et al. 2010
Bonytail chub (2 and 7 d post hatch), *Gila elegans*	7-d IC_{25} - Growth	R,U	7.90	25.0	11.00	23.24	Dwyer et al. 2005

Appendix C. Other Chronic Ammonia Toxicity Data							
Species	**Test and Effect**	**Method[a]**	**pH**	**Temp. (˚C)**	**Total Ammonia (mg TAN/L)**	**Chronic value Adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*) (mg TAN/L)**	**Reference**
Spotfin chub (<24 hr), *Erimonax monachus*	7-d IC_{25} - Growth	R,U	7.90	25.0	15.80	33.37	Dwyer et al. 2005
Cape Fear shiner (<24 hr), *Notropis mekistocholas*	7-d IC_{25} - Growth	R,U	7.90	25.0	8.800	18.59	Dwyer et al. 2005
Topeka shiner (adult), *Notropis topeka*	30-d EC_{20} - Survival	F,M	7.94	23.9	10.85	24.21	Adelman et al. 2009
Topeka shiner (juvenile, 11 mo), *Notropis topeka*	30-d EC_{20} - SGR	F,M	8.07	12.4	6.483	17.45	Adelman et al. 2009
Gila topminnow (<24, 48 and 72 hr), *Poeciliopsis occidentalis*	7-d IC_{25} - Growth	R,U	7.90	25.0	24.10	50.91	Dwyer et al. 2005
Fathead minnow (<24 hr), *Pimephales promelas*	7-d IC_{25} - Growth	R,U	7.90	25.0	7.200	15.21	Dwyer et al. 2005
Fathead minnow (4 d post hatch), *Pimephales promelas*	28-d LOEC-Survival	R,M	8.25	19.9	9.160	32.71	Fairchild et al. 2005
Colorado pikeminnow (5 and 6-d post hatch), *Ptychocheilus lucius*	7-d IC_{25} - Growth	R,U	7.90	25.0	8.900	18.80	Dwyer et al. 2005
Colorado pikeminnow (juvenile, 8 d), *Ptychocheilus lucius*	28-d LOEC- Growth	R,M	8.23	19.9	8.600	29.75	Fairchild et al. 2005
Razorback sucker (7 d post hatch), *Xyrauchen texanus*	7-d IC_{25} - Growth	R,U	7.90	25.0	13.40	28.30	Dwyer et al. 2005
Razorback sucker (9 d), *Xyrauchen texanus*	28-d LOEC-Survival	R,M	8.24	19.9	13.25	46.58	Fairchild et al. 2005
Lost River sucker (late-stage larva), *Deltistes luxatus*	30-d LOEC-Survival	F,M	9.43	22.3	1.230	25.31	Meyer and Hansen 2002
Green frog (Stage 24-26), *Rana clamitans*	103-d NOEC-Growth	R,M	8.70	24.0	>2.20	>16.74	Jofre and Karasov 1999

[a] R = renewal and F = flow-through exposure types; M = measured and U = unmeasured tests.

[b] Not used in the calculation of the SMCV because of the uncertainty of the chronic value, but included here as weight of evidence supporting the sensitivity of non-pulmonate snail species in general as determined by 28-day toxicity tests (see Additional 28-day Toxicity test Data for Freshwater Snails in Appendix I for more detail).

[c] Not used in the calculation of the SMCV because of the uncertainty of the chronic value, but included here as weight of evidence supporting the sensitivity of non-pulmonate snail species in general as determined by 28-day toxicity tests (see Chronic Toxicity Test Data: 28-day Tests with Juvenile and Adult Pebblesnails in Appendix H for more detail).

[d] Not used in the calculation of the SMCV because of low control survival (75 percent) for this size class.

[e] Value represents a 28-day ammonia survival effects concentration used in place of the EC_{20} due to the high degree of temporal variability in measured total ammonia concentrations in the test, as well as the unequal response among test replicates near this concentration (see Additional 28-day Toxicity test Data for Freshwater Snails in Appendix I for more detail).

Appendix D. Conversion of Acute Results of Toxicity Tests.

All of the ammonia acute values (LC_{50}s and EC_{50}s) in Appendix A of this document were converted to TAN acute values using the reported temperatures and pHs, and using the pKa relationship from Emerson et al. (1975). Conversions were dependent on the form of ammonia the acute values were expressed, e.g., unionized ammonia (UIA), unionized ammonia expressed as nitrogen (UIA-N), total ammonia (TA) and total ammonia nitrogen (TAN). After acute values were converted to TAN they were then normalized to pH 7 using the pH relationship developed in the 1999 AWQC document. Following the adjustment to pH 7, the TAN acute values were further normalized to a temperature of 20°C for invertebrates only, following recommendations in the 1999 AWQC document. It is worth noting here that while the relationship between pH and ammonia toxicity was first addressed in the 1985 criteria document, it was not fully developed until the 1999 AWQC update document. Detailed information regarding the development and parameterization of the pH-ammonia toxicity equations (acute and chronic) can be found in the 1999 AWQC document (pH-Dependence of Ammonia Toxicity – U.S. EPA 1999). In contrast to the pH-toxicity relationship, which applies to both vertebrates and invertebrates, the temperature-ammonia toxicity relationship only applies to invertebrates. Based on the results of the 1999 reanalysis of this relationship, it was determined that ammonia toxicity for invertebrates decreases with decreasing temperature to a temperature of approximately 7°C, below which the relationship ends (U.S. EPA 1999).

The conversion procedure for acute toxicity values is illustrated here using the data for the flatworm, *Dendrocoelum lacteum*, which is the first species listed in Table 1 in the 1984/1985 criteria document and was the species chosen to illustrate the conversion procedure in Appendix 3 of the 1999 AWQC document:

Acute value (AV) = 1.40 mg unionized ammonia (UIA) or NH_3/L
Test pH = 8.20
Test Temperature = 18.0°C

Step 1.

Equation 3 in the 1999 criterion document, and the Emerson et al. (1975) equation from page 7 of this document, is used to calculate the pKa at 18 °C:

pKa = 9.464905

Step 2.

The AV in terms of total ammonia (TA) is calculated as:

$$[NH_3]/[NH^+_4] = 10^{(pH\text{-}pK)} = 0.0543369$$

Step 3.

The Wood (1993) equation from page 7 (Equation 2 in the 1999 AWQC document) is rearranged to obtain the acute value for TA:

$$TA = [NH_3] + [NH^+_4] = [NH_3] + [NH_3]/(10^{(pH\text{-}pKa)})$$

$$TA = [NH_3] + [NH^+_4] = [NH_3] + [NH_3]/0.0543369$$

$$= 27.1652 \text{ mg TA/L}$$

Step 4.

The AV for TA is converted to the AV for TAN (AV_t) as follows:

AV_t/AV = (14 mg TAN/mmol) / (17 mg TA/mmol) = 14/17

AV_t = (27.1652 mg TA/L) x (14 mg TAN/17 mg TA)

= 22.3713 mg TAN/L

Step 5.

The AV in terms of TAN, or AV_t, is converted from test pH 8.2 to pH 7 using the equation for describing the pH-dependence of acute values (modified from Equation 11 in the 1999 AWQC document for normalization to pH 7)[2]:

$$AV_{t,7} = \frac{AV_t}{\left(\frac{0.0114}{1+\ 10^{7.204-pH}} + \frac{1.6181}{1+10^{pH-7.204}}\right)}$$

$AV_{t,7} = (AV_t)/(0.158673) = 140.990$ mg N/L

Step 6. (temperature adjustment for invertebrates only)

The AV in terms of TAN at pH 7, or $AV_{t,7}$, is converted from this concentration at test temperature to a standard test temperature of 20°C using the equation shown below (Equation 5 in the 1999 AWQC document)[3]:

$\log(AV_{t,7,20}) = \log(AV_{t,7}) - [-0.036(18^\circ C - 20^\circ C)]$
$= 119.451$ mg N/L

Because this is the only species in this genus for which data are in Table 1 in the 1984/1985 criteria document, 119.5 mg TAN/L is the GMAV for the genus *Dendrocoelum* in Table 3 of this update document.

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[2] The equation provided here should be applicable from pH 6 to 9, although uncertainty might exist at the lower end of this range for certain species. Extrapolation below pH 6 is not advisable because of the increasing scatter of the data from the common regression line at lower pH, and extrapolation above pH 9 is not advisable because of inadequate knowledge about the effect of the inhibition of ammonia excretion at high pH on results of toxicity tests (Russo et al. 1988).

[3] Note: Based on the 1999 reanalysis of the relationship between temperature and ammonia toxicity, when test temperature is less than 7°C, T should be set equal to 7, to reflect the plateau of the temperature-toxicity relationship at these temperatures.

Appendix E. Conversion of Chronic Results of Toxicity Tests.

As in the previous appendix with the acute results of toxicity tests, all of the ammonia chronic values (EC_{20}s) in Appendix B of this document were first converted to TAN at test temperature and pH using the p*K*a relationship from Emerson et al. (1975). Once all the chronic values were converted to total ammonia nitrogen, these values were then adjusted to pH 7 using the pH relationship developed in the 1999 AWQC document. After the adjustment to pH 7, the TAN chronic values were further normalized to a temperature of 20°C for invertebrates only, as per the recommendations in the 1999 AWQC document. The conversion procedure is illustrated here using the data for the amphipod species *Hyalella azteca*.

Chronic value (CV) = EC_{20} of 8.207 mg TAN/L
Test pH = 8.04
Test Temperature = 25.0°C

Steps 1 through 4.

(Not required in this case as CV is already expressed in terms of TAN. For more details regarding these steps, see Appendix D).

Step 5.

The CV in terms of TAN, or CV_t, is converted from test pH 8.04 to pH 7 using the equation for describing the pH dependence of chronic values (modified from Equation 12 in the 1999 AWQC document for normalization to pH 7)[4]:

$$CV_{t,7} = \frac{CV_t}{\left(\frac{0.0278}{1+\ 10^{7.688-pH}} + \frac{1.1994}{1+10^{pH-7.688}}\right)}$$

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[4] See footnote 3 in Appendix D.

$$CV_{t,7} = (CV_t)/(0.38855) = 21.13 \text{ mg TAN/L}$$

Step 6. (Temperature adjustment for invertebrates only)

The CV in terms of TAN at pH 7, or $CV_{t,7}$, is converted from this concentration at test temperature to a standard test temperature of 20°C using the equation shown below (Equation 5 in the 1999 AWQC document)[5]:

$$\log(CV_{t,7,20}) = \log(CV_{t,7}) - [-0.028(25^{\circ}C - 20^{\circ}C)]$$
$$= 29.17 \text{ mg TAN/L}$$

Because this is the only species in this genus for which data in appendix B are available, 29.17 mg TAN/L is the GMCV for the genus *Hyalella* reported in Table 4 of this update document.

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[5] See footnote 4 in Appendix D.

Appendix F. Acute-Chronic Ratios (ACRs).

The CCC was calculated directly from chronic values (EC_{20}s) in Appendix B using the standard fifth percentile procedure provided in the 1985 Guidelines (Stephan et al. 1985). As a result, acute-chronic ratios (ACRs) are not necessary for the derivation of the new chronic criterion presented in this document. It is still worthwhile, however, for EPA to provide recommended ACRs for predicting chronic sensitivity of untested species using measured or estimated acute values for other related efforts (e.g., developing Biological Evaluations in support of National Endangered Species Act Consultations on EPA 304(a) criteria recommendations, or when an ACR(s) is allowed to derive site-specific criteria for ammonia in fresh water). Table F.1 below presents ACRs for all species with chronic values that were used in the derivation of a GMCV and for which comparable acute values were found, as well as for a few additional species of special interest, such as threatened and endangered species. All acute and chronic values were adjusted to pH 7 and to 20°C (in the case of invertebrates). For each species or genera where more than a single ACR was calculated, Species and Genus Mean Acute-Chronic Ratios (SMACRs and GMACRs, respectively) were also calculated as the geometric mean value of individual ACRs and SMACRs. (Note: in the case of a single ACR within a Genus, the ACR is the SMACR.) Additionally, taxon-specific ACRs (TSACRs) were calculated where practical and for purpose of comparison at the taxonomic level of Family and Class.

The ACRs for freshwater aquatic invertebrates range from 2.406 to 49.45 (a factor of 21; see Table F.1). Likewise, the ACRs for fish range from 3.437 to 36.53 (factor of 11). The broad range in values can probably be explained because of the different kinds of chronic tests (life-cycle, ELS, 28-d juvenile mussel or snail) and toxicological endpoints (survival, growth, or reproduction) upon which they are based. The ACR of 36.53 for fathead minnow, for example, was based on hatchability from the life-cycle test of Thurston et al. (1986), whereas the early life-stage tests with fathead minnow of Mayes et al. (1986) and Swigert and Spacie (1983) gave ACRs of 11.35 and 17.17. The range of ACRs based on chronic values from the two early life-stage tests is small, and it is perhaps not surprising that a life-cycle test gave a higher ACR than the early life-stage tests. As another example illustrating the variability among ACRs from different kinds of tests and using different toxicological endpoints, but this time comparing amongst different species of invertebrates, the ACR of 49.45 for *Lampsilis fasciola* was based on survival from a 28-day test involving two month-old juveniles (Wang et al. 2007a,b), whereas the life-cycle tests with the two species of cladocerans (*Ceriodaphnia acanthina* and *C. dubia*) are based on adverse effects on reproduction with ACRs of 2.406 (Mount 1982) and 3.924 (Nimmo et al. 1989), respectively (Table F.1).

The ACRs for bivalve mollusks in general are larger compared to other freshwater aquatic animal taxa and range from 9.028 to 49.45. The ACRs for other freshwater invertebrates range from 2.406 to 15.81. The ACRs for fishes, in contrast, are quite varied even within species or genera. For example, the ACRs for *Lepomis* sp. range from 3.437 to 28.51 despite having been based on ELS tests and using biomass or survival as the toxicological endpoint.

Figure F.1 depicts SMACRs in relation to SMAVs to determine whether there is a trend. Only the weak trend of decreasing SMACR with increasing SMAV is apparent; primarily due to the comparatively large SMACRs for freshwater bivalve mollusks.

In general TSACRs for most freshwater aquatic animals (excluding bivalve mollusks) are within the relatively small range of 5.113 to 15.81 at the Class level, and may be acceptable for use when certain taxon-specific chronic toxicity data are not available. Perhaps not surprisingly, the CCC (2.1 mg TAN/L) calculated as the quotient of the FAV of 32.99 mg TAN/L (at pH 7 and 20°C) and geometric mean ACR for the Family Unionidae (15.52) agrees well with the CCC calculated directly from available chronic data (see Appendix B and Figure 4).

Table F.1. Species, Genus and Taxon-Specific ACRs for Freshwater Aquatic Animals Exposed to Ammonia.

Table F.1. Species, Genus and Taxon-specific ACRs for Freshwater Aquatic Animals Exposed to Ammonia

Species Scientific Name	Acute and Chronic Test Endpoint	pH	Temp	Normalized Values	Reference	ACR	SMACR	GMACR	TSACR (Family)	TSACR (Class)
Class Gastropoda (Family: Lithoglyphidae)										
Fluminicola sp.	LC50	8.25	20.2	>62.15	Besser 2011	7.940	7.940	7.940	7.940	7.940
	EC20 - Change in Length	8.22	20.1	7.828						
Class Bivalvia (Families Unionidae and Pasidiidae)										
Lampsilis fasciola	EC50	8.50	20.0	69.63	Wang et al. 2007b	49.45	49.45	21.13	15.52	25.68
	EC20 - Survival	8.20	20.0	1.408	Wang et al. 2007a					
Lampsilis siliquoidea	EC50	8.20	20.0	28.99	Wang et al. 2007a	9.028	9.028			
	EC20 - Survival	8.25	20.0	3.211	Wang et al. 2011					
Villosa iris	EC50	8.40	20.0	23.29	Wang et al. 2007b	11.40	11.40	11.40		
	EC50	8.30	20.0	68.40	Wang et al. 2007b					
	EC20 - Survival	8.20	20.0	3.501	Wang et al. 2007a					
Musculium transversum	EC50	8.10	14.6	109.0	West 1985; Arthur et al. 1987	42.50	42.50	42.50	42.50	
	EC20 - Survival	7.80	21.8	2.565	Sparks and Sandusky 1981					
Class Branchiopda (Family: Daphniidae)										
Ceriodaphnia acanthina	EC50	7.06	24.0	154.3	Mount 1982	2.406	2.406	3.073	5.113	5.113
	EC20 - Reproduction	7.15	24.5	64.10						
Ceriodaphnia dubia	EC50	7.80	25.0	152.9	Nimmo et al. 1989	3.924	3.924			
	EC20 - Reproduction	7.80	25.0	38.96						
Daphnia magna	EC50	8.50	20.0	296.9	Gersich and Hopkins 1986	8.186	8.507	8.507		
	EC20 - Reproduction	8.45	19.8	36.27	Gersich et al. 1985					
	EC50	8.34	19.7	419.1	Reinbold and Pescitelli 1982a	8.841				
	EC20 - Reproduction	7.92	20.1	47.40						
Class Malacostraca (Family: Dogielinotidae)										
Hyalella azteca	EC50	8.30	25.0	461.2	Ankley et al. 1995	15.81	15.81	15.81	15.81	15.81
	EC20 - Biomass	8.04	25.0	29.17	Borgmann 1994					

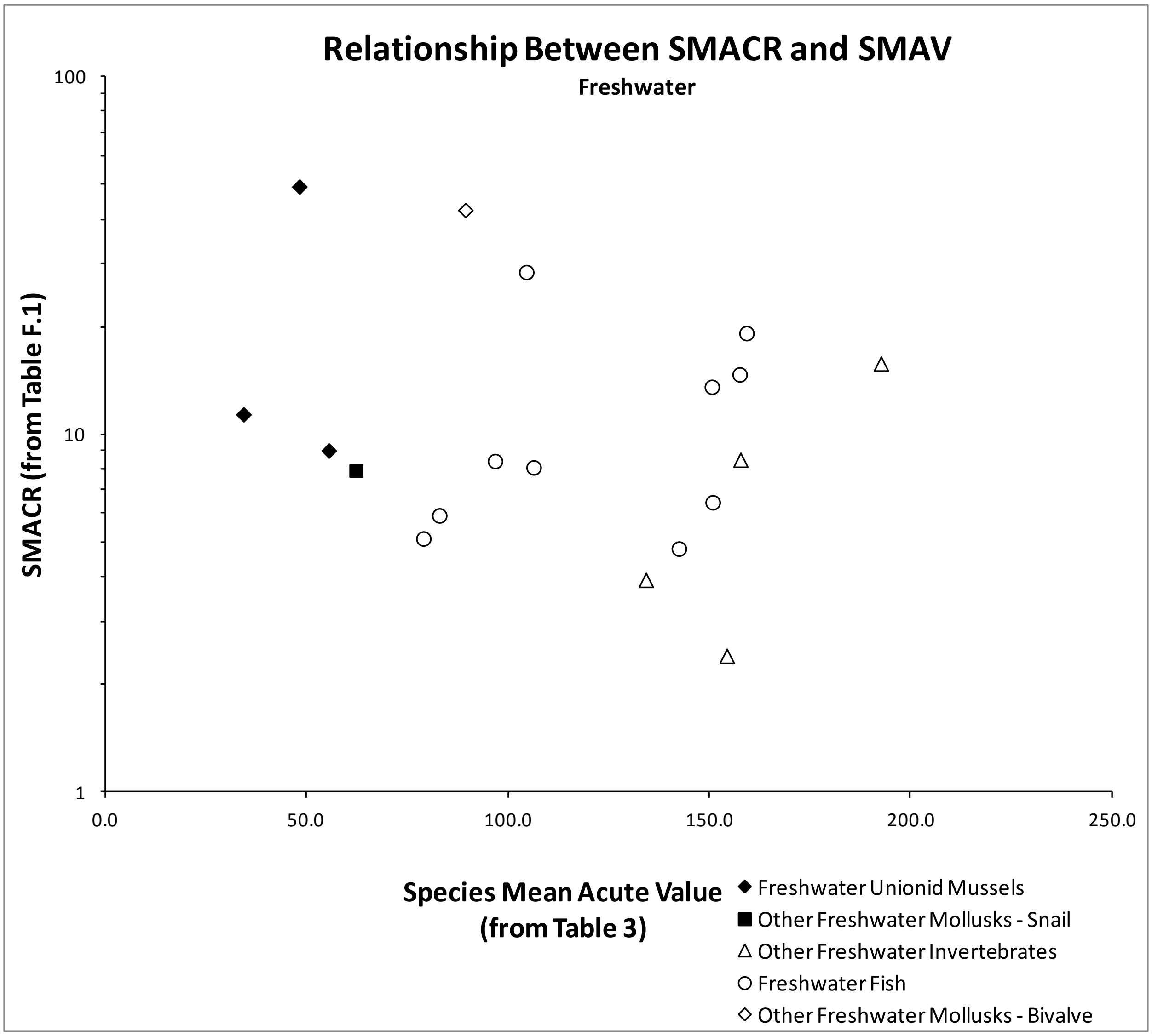
Table F.1. Species, Genus and Taxon-specific ACRs for Freshwater Aquatic Animals Exposed to Ammonia

Species Scientific Name	Acute and Chronic Test Endpoint	pH	Temp	Normalized Values	Reference	ACR	SMACR	GMACR	TSACR (Family)	TSACR (Class)
Class Actinopterygii (Families Salmonidae, Catostomidae, Cyprinidae, Ictaluridae and Centrarchidae)										
Oncorhynchus clarkii	LC50	7.81	13.1	132.3	Thurston et al. 1978	5.122	5.122	5.518	5.518	8.973
O. clarkii henshawi	EC20 - Survival	7.57	13.7	25.83	Koch et al. 1980					
Oncorhynchus mykiss	LC50	7.40	14.5	31.47	Calamari et al. 1981	9.696	5.945			
	EC20 - Survival	7.40	14.5	3.246	Calamari et al. 1977, 1981					
	LC50	7.67	7.7	40.40	Thurston et al. 1981a	3.646				
	EC20 - 5 yr Life Cycle	7.70	7.5-10.5	>11.08	Thurston et al. 1984a					
Catostomus commersoni	LC50	8.16	15.0	176.6	Reinbold and Pescitelli 1982c	14.75	14.75	14.75	14.75	
	LC50	8.14	15.4	166.3						
	EC20 - Biomass	8.32	18.6	11.62	Reinbold and Pescitelli 1982a					
Notropis topeka	LC50	8.09	13.2	147.3	Adelman et al. 2009 (EC$_{20}$ from Appendix C)	8.437	8.437	8.437	10.96	
	EC20 - Growth Rate	8.07	12.4	17.45						
Pimephales promelas	LC50	7.76	19.0	139.3	Thurston et al. 1983, 1986	36.53	19.24	19.24		
	LC50	7.83	22.0	158.7						
	LC50	7.91	18.9	178.9						
	LC50	7.94	19.1	162.3						
	LC50	8.06	22.0	205.0						
	LC50	8.03	22.1	216.3						
	EC20 - LC Hatchability	8.00	24.2	4.784						
	LC50	8.14	22.0	141.2	Mayes et al. 1986	11.35				
	EC20 - Survival	8.00	24.8	12.43						
	LC50	7.78	25.9	117.3	Swigert and Spacie 1983	17.17				
	LC50	7.80	25.6	126.8						
	EC20 - Biomass	7.82	25.1	7.101						
Cyprinus carpio	LC50	7.72	28.0	133.9	Hasan and MacIntosh 1986	8.100	8.100	8.100		
	EC20 - Growth: Weight	7.85	23.0	16.53	Mallet and Sims 1994					
Ictalurus punctatus	LC50	7.80	25.7	97.67	Swigert and Spacie 1983	4.800	4.800	4.800	4.800	
	EC20 - Biomass	7.76	26.9	20.35						
Lepomis cyanellus	LC50	7.72	22.4	144.3	McCormick et al. 1984	12.18	6.468	13.58	13.59	
	EC20 - Biomass	7.90	22.0	11.85						
	LC50	8.28	26.2	62.07	Reinbold and Pescitelli 1982a	3.437				

Table F.1. Species, Genus and Taxon-specific ACRs for Freshwater Aquatic Animals Exposed to Ammonia

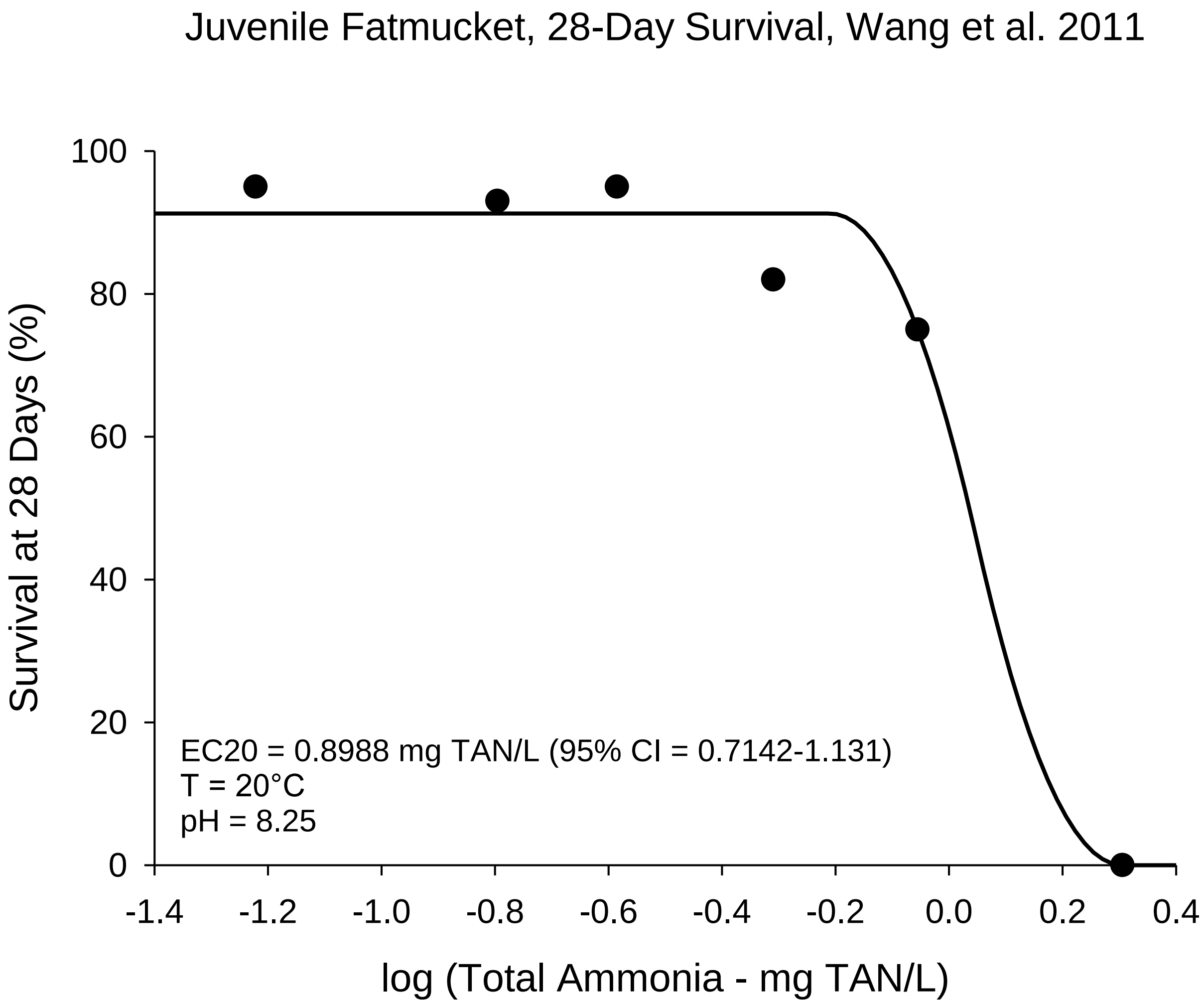
Species Scientific Name	Acute and Chronic Test Endpoint	pH	Temp	Normalized Values	Reference	ACR	SMACR	GMACR	TSACR (Family)	TSACR (Class)
	EC20 - Survival	8.16	25.4	18.06						
Lepomis macrochirus	LC50	7.60	21.7	93.31	Smith et al. 1984	28.51	28.51			
	EC20 - Biomass	7.76	22.5	3.273						
Micropterus dolomieui	LC50 (pH 6.5)	6.53	22.3	269.2	Broderius et al. 1985	31.12	13.61	13.61		
	EC20 (pH 6.5) - Biomass	6.60	22.3	8.650						
	LC50 (pH 7.0)	7.16	22.3	144.3		14.84				
	EC20 (pH 7.0) - Biomass	7.25	22.3	9.726						
	LC50 (pH 7.5)	7.74	22.3	105.2		6.670				
	EC20 (pH 7.5) - Biomass	7.83	22.3	15.77						
	LC50 (pH 8.5)	8.71	22.3	126.0		11.14				
	EC20 (pH 8.5) - Biomass	8.68	22.3	11.31						

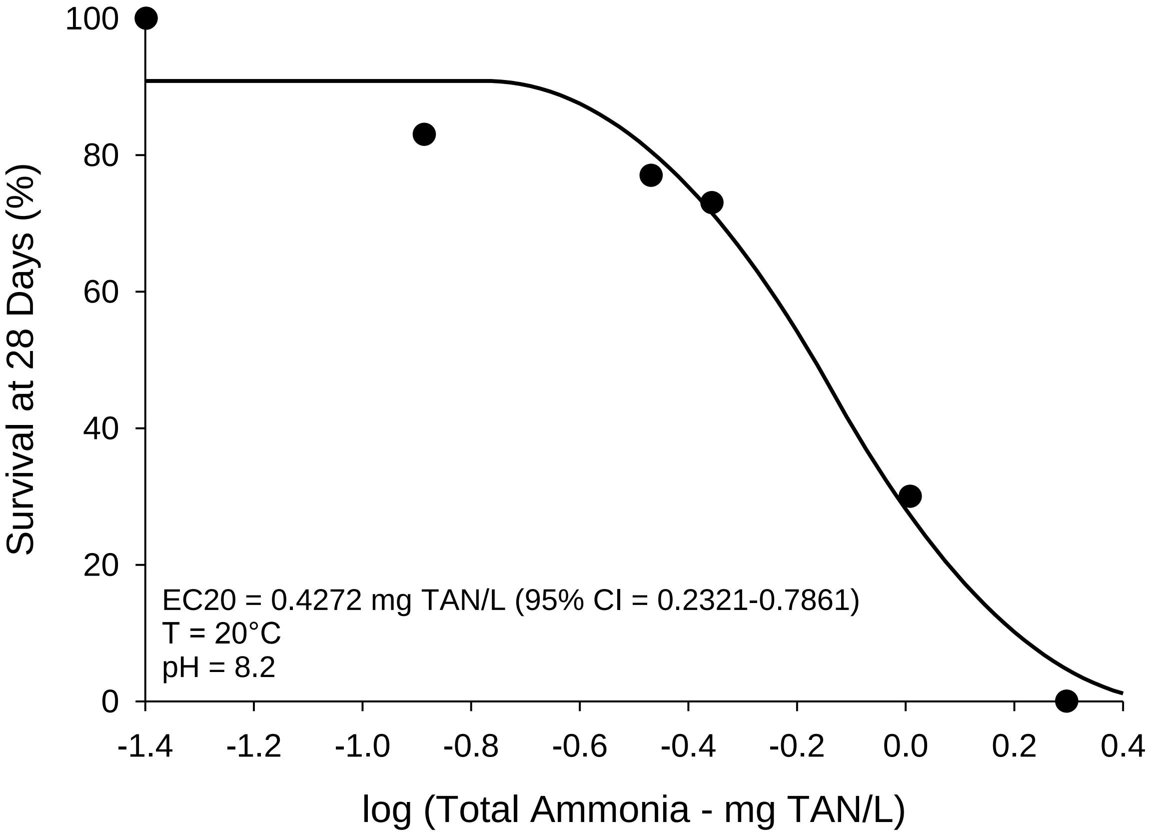
Figure F.1. SMACRs by SMAV Rank.



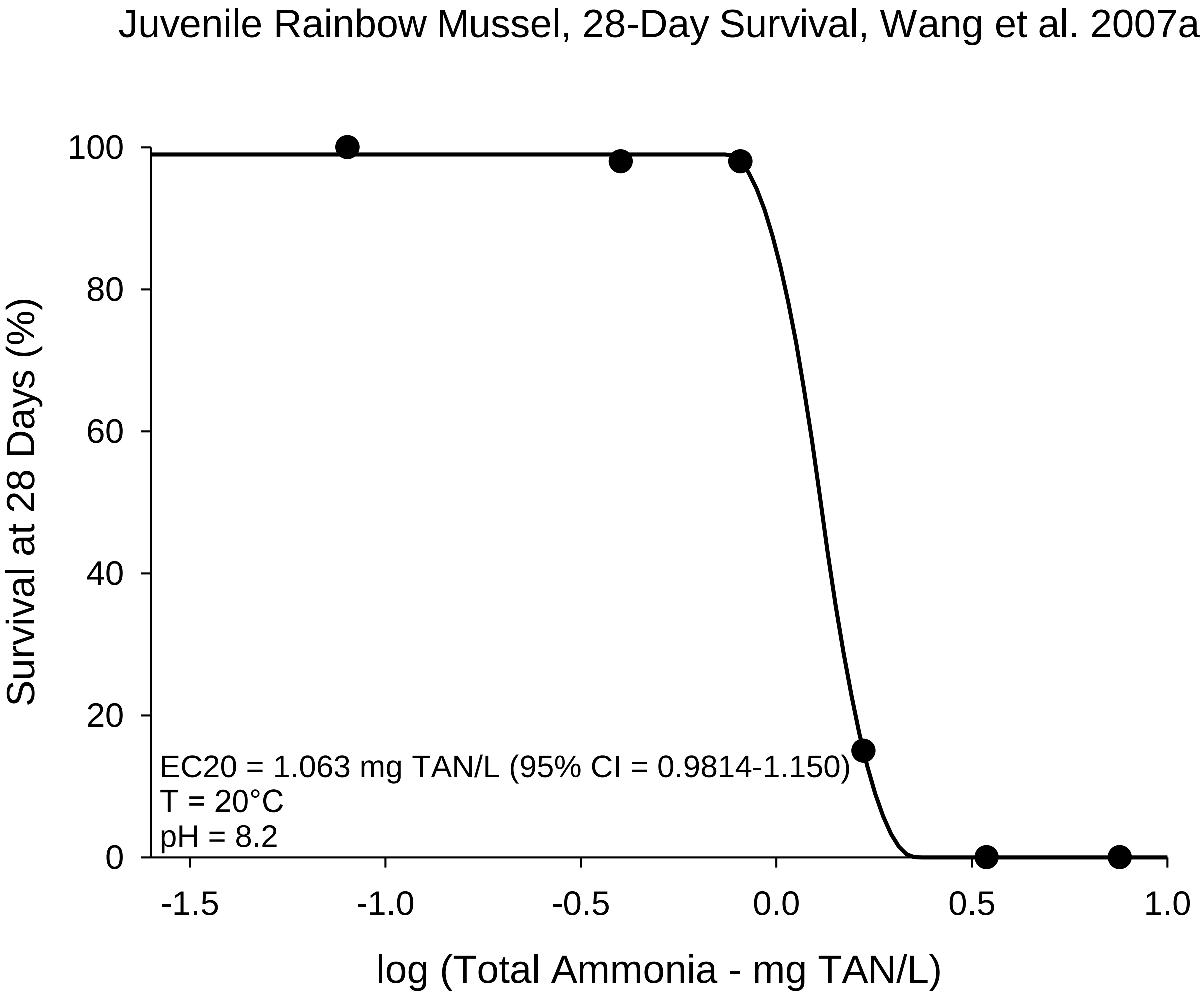
Appendix G. Results of the Regression Analyses of New Chronic Data for Unionid Mussels.

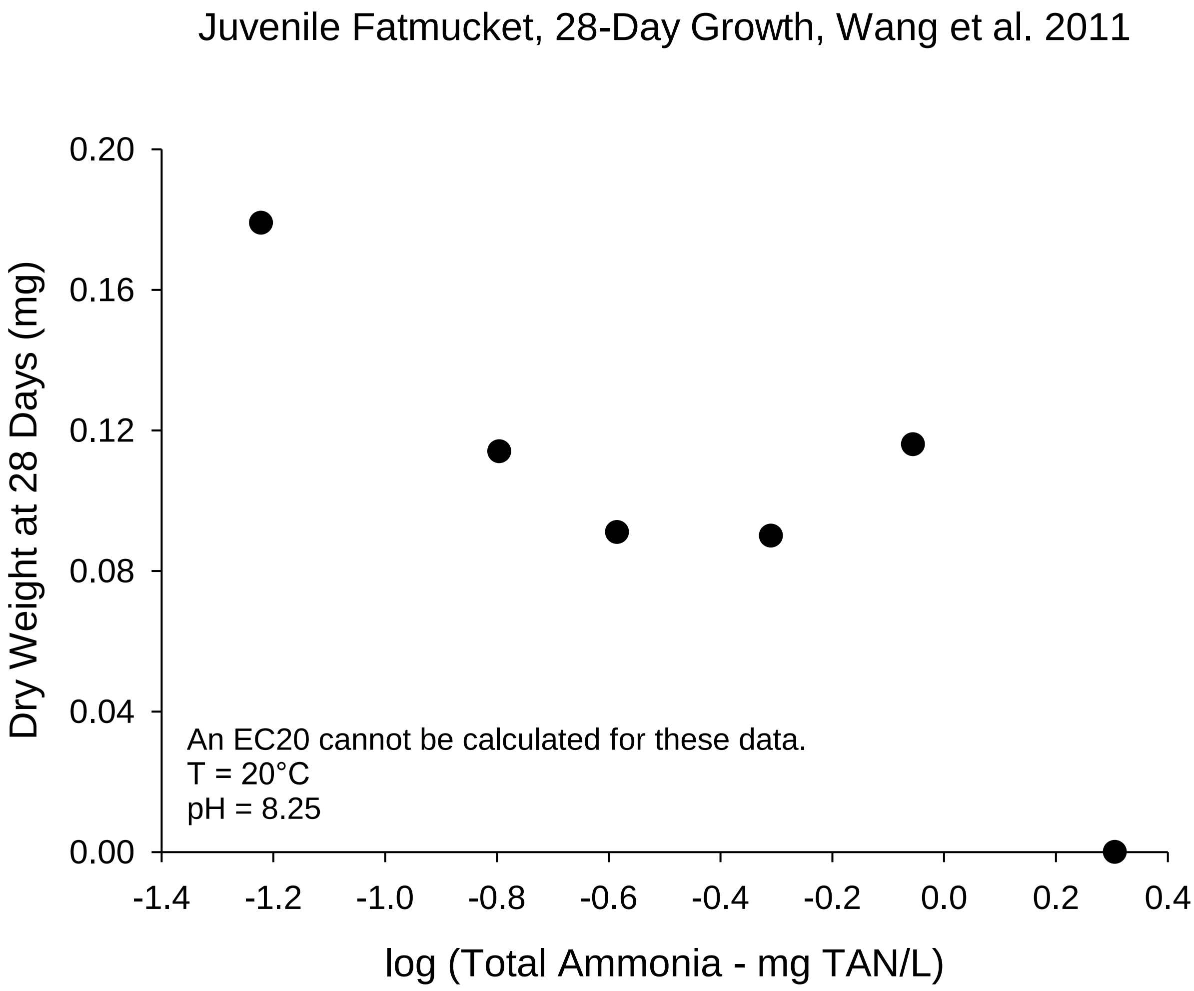
This appendix provides the figures generated using EPA's TRAP program that was used to calculate EC_{20}s for the new chronic ammonia toxicity studies conducted with unionid mussels. In the figures that follow, circles denote measured responses and solid lines denote estimated regression lines. The model-estimated EC_{20} values and corresponding 95% confidence limits are provided with each figure, as well as the pH and water temperature at which the test was conducted. Per the text on page 32 in *Chronic Toxicity to Freshwater Aquatic Animals* and as discussed in greater detail on page 56 in *Effects Characterization*, EPA decided that while 28-day survival EC_{20}s from these tests using juvenile freshwater mussels are acceptable for derivation of a chronic aquatic life criterion for ammonia, EC_{20}s based on growth responses from these tests are not. The decision not to use the growth data from these tests was based on the uncertainty in the test methods for assessing the growth endpoint and the need for additional research "to optimize feeding conditions, to conduct longer-term exposures (e.g., 90 d), and to compare growth effect to potential reproductive effect in partial life-cycle exposure" (Wang et al. 2011). Additionally, the growth response during these tests show a high degree of variability, and the test methods for assessing growth, based on substrate or water-only exposures, are currently being evaluated – see Figure below depicting the growth response of juvenile fatmucket in the 28-day tests reported in Wang et al. (2011).


Juvenile Fatmucket, 28-Day Survival, Wang et al. 2011
Survival at 28 Days (%)
100
80
60
40
20
0
EC20 = 0.8988 mg TAN/L (95% CI = 0.7142-1.131)
T = 20°C
pH = 8.25
-1.4
-1.2
-1.0
-0.8
-0.6
-0.4
-0.2
0.0
0.2
0.4
log (Total Ammonia - mg TAN/L)


Juvenile Wavy-rayed Lampmussel, 28-Day Survival, Wang et al. 2007a

EC20 = 0.4272 mg TAN/L (95% CI = 0.2321-0.7861)
T = 20°C
pH = 8.2


Juvenile Rainbow Mussel, 28-Day Survival, Wang et al. 2007a
Survival at 28 Days (%)
100
80
60
40
20
0
-1.5
-1.0
-0.5
0.0
0.5
1.0
log (Total Ammonia - mg TAN/L)
EC20 = 1.063 mg TAN/L (95% CI = 0.9814-1.150)
T = 20°C
pH = 8.2



Juvenile Fatmucket, 28-Day Growth, Wang et al. 2011

An EC20 cannot be calculated for these data.
T = 20°C
pH = 8.25

Appendix H. Detailed Descriptions of Select New Acute and Chronic Toxicity Test Data Used for Criteria Derivation.

Acute Toxicity Test Data

Venustaconcha ellipsiformis (ellipse)

As noted above, the ellipse test data was not directly used in the acute criterion calculation, but the data is described here as additional evidence supporting the determined acute criterion value. The GMAV for the ellipse is based on the 24-hr EC_{50} reported for an acute toxicity test initiated with 2-hr old glochidia of the species (Wang et al. 2007b). Glochidia were tested under static conditions at pH 8.1 and 20°C. Survival of control animals after 24 hours was 90 percent. The estimated measured EC_{50} at test temperature and pH was 4.450 mg N/L, after adjusting the reported nominal EC_{50} by multiplying by a factor of 0.89 (i.e., measured total ammonia concentrations were 89 percent of nominal concentrations for 24 hour glochidia exposures). The GMAV for this species is 23.12 mg TAN/L when adjusted to pH 7 and 20°C (Appendix A), and represents the lowest in the acute dataset (Table 3). The acute criterion of 17 mg TAN/L is considered protective of this species because the GMAV/2, a value used to estimate an effect level un-differentiable from controls (Federal Register on May 18, 1978 (43 FR 21506-18), is approximately 12 mg TAN/L for the ellipse, which is close to the current criterion value, given the variability and uncertainty in such toxicity tests.

Utterbackia imbecillis (pondshell mussel)

The GMAV for pondshell mussel of 46.93 mg TAN/L is the sixth lowest in the acute dataset (Table 3). Although this GMAV is not one of the four used in calculating the FAV, the value is composed of individual EC_{50} values ranging from a comparatively low acute value of 17.91 to 100.5 mg TAN/L (expressed as TAN and normalized to pH 7 and 20°C, Appendix A). This GMAV is based on several EC_{50}s (numbering nine in total) from three different studies (Wade et al. 1992; Keller 2000; Black 2001). This particular GMAV is based on tests with predominantly juvenile mussels of various ages, but also including a single test which employed glochidia (Appendix A). The pH and test temperature for all nine tests was relatively uniform and ranged from 7.80 to 8.35 and 24.0 to 25.1°C, respectively. Control survival exceeded 90 percent in all tests regardless of life-stage tested.

Fusconaia masoni (Atlantic pigtoe)

The GMAV for the Atlantic pigtoe represents the seventh lowest in the acute dataset, and lies just below the lowest GMAV for the most sensitive fish species, the mountain whitefish (Table 3). This GMAV is based on the 6-hr EC_{50} reported for an acute toxicity test initiated with 2-hr old glochidia of the species (Black 2001). Glochidia were tested under static conditions at pH 7.6 and 24.9°C. Survival of control animals after 6 hours was 93 percent, falling to 87 percent after 12 hours. The EC_{50} at test temperature and pH was 15.90 mg TAN/L, or 47.40 mg TAN/L when adjusted to pH 7 and 20°C (Appendix A).

Fluminicola sp. (pebblesnail)

The GMAV of 62.15 mg TAN/L for *Fluminicola* is the tenth most sensitive in the acute dataset (Table 3). As part of the study to evaluate the chronic sensitivity of pebblesnails (Gastropoda: Hydrobiidae) to ammonia via 28-day water only toxicity tests (see additional details below under Chronic Toxicity Test Data: 28-day Tests with Juvenile and Adult Pebblesnails (*Fluminicola* species), Besser (2011) reported survival of ‘large’ snails (i.e., mean starting shell length of 1.81 mm) after 96 hours of exposure. No mortality was observed in controls through the highest test concentration of 8.801 mg TAN/L where 32 of 40 snails (80 percent) survived. The mean pH and test temperature at this highest ammonia treatment level were 8.25 and 20.2°C, respectively. Because only 20 percent mortality occurred at this test concentration, the EC_{50} at test temperature and pH is recorded in this document as > 8.801 mg TAN/L, or >62.15 mg TAN/L when adjusted to pH 7 and 20°C (Appendix A).

Pleurocera uncialis (pagoda hornsnail)

Another non-pulmonate snail species (pagoda hornsnail) was determined to be nearly as sensitive to ammonia as pebblesnail, the pagoda hornsnail, which was ranked 12th in acute sensitivity. Goudreau et al. (1993) collected and acclimated (for six days) adult snails from Clinch River, Virginia prior to conducting a static renewal bioassay to determine a 96-hr LC_{50} for this species. The test was conducted in a walk-in experimental chamber set to a temperature of 22°C and using chlorine free laboratory dilution water at pH 8.1. Survival of adult snails in the control treatment was 100 percent. The reported LC_{50} at test temperature and pH was 11.18

mg TAN/L when expressed as total ammonia. The LC_{50} normalized to pH=7 and 20°C is 68.54 mg TAN/L (Appendix A).

Deltistes luxatis (Lost River sucker)

The endangered Lost River sucker is a freshwater fish species endemic to the Klamath Basin of northern California and southern Oregon (Appendix A). The acute toxicity of ammonia was determined for larval and juvenile Lost River sucker as reported in Saiki et al. (1999). Larval tests were initiated when fish reared from spawned eggs were 35 days old, whereas the juvenile tests were initiated after the fish reached 3-7 months old. All fish were exposed for 96 hours under flow-through conditions at pH 8.0 and 20°C. The reported LC_{50}s at test temperature and pH were 10.35 and 16.81 mg/L for larval and juvenile fish, expressed as total ammonia nitrogen (Appendix A). The LC_{50}s normalized to pH 7 and 20°C are 44.42 and 72.18 mg TAN/L, respectively (Appendix A). The GMAV for Lost River sucker is calculated as the geometric mean of the two normalized LC_{50}s, or 56.62 mg TAN/L (Table 3). Lost River sucker represents the ninth most sensitive genus in the acute dataset, and second most sensitive fish species (following mountain whitefish which was the most sensitive GMAV) and is expected to be protected by the CMC of 17 mg TAN/L.

Chronic Toxicity Test Data

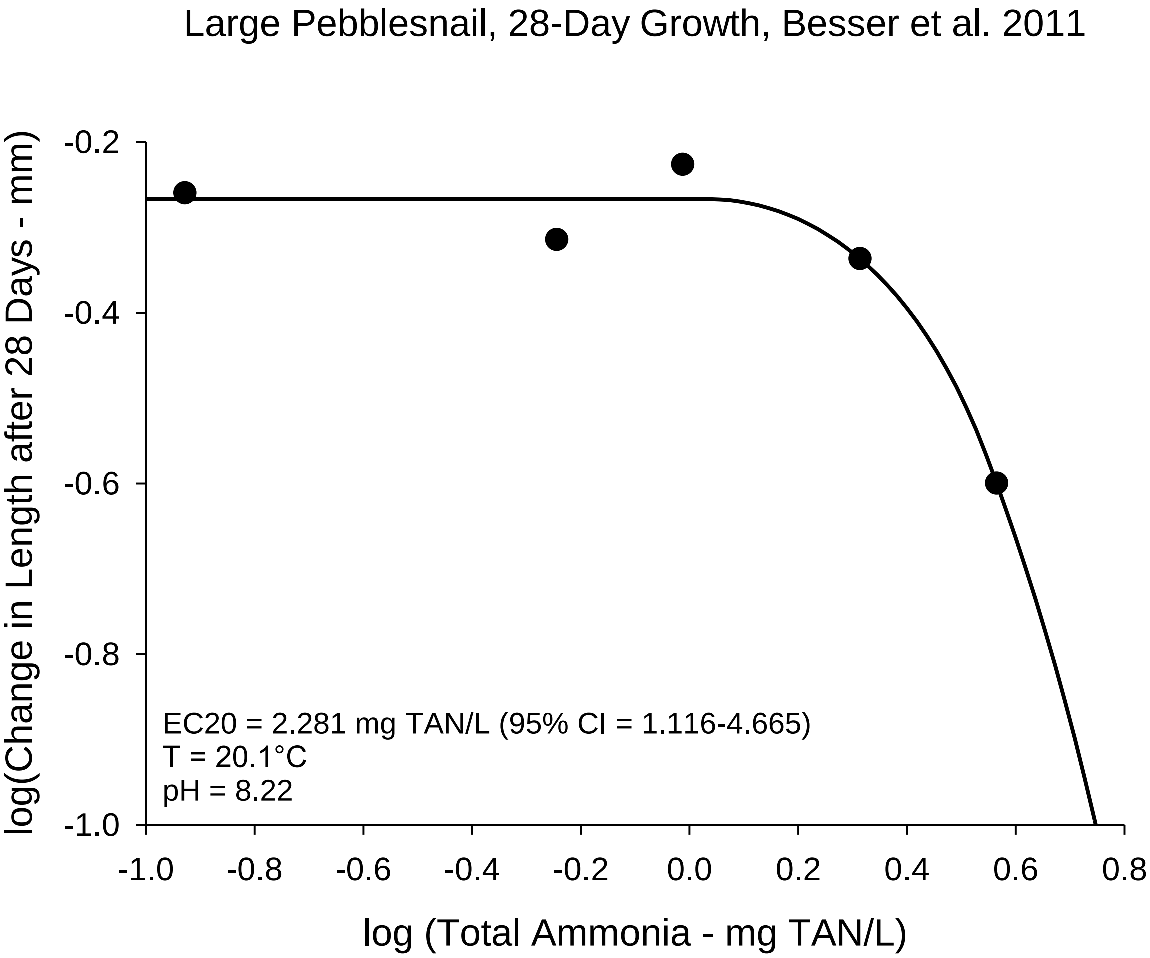
28-day Tests with Juvenile and Adult Pebblesnails (Fluminicola species)

The summary for 28-day tests recently conducted with *Fluminicola* sp. includes the results from repeat tests performed by Besser et al. in 2009 and 2010, the details of the latter of which are summarized in a memorandum to EPA in 2011 (this study referred to in this document as Besser 2011).

Test organisms used in the Besser et al. (2009) 28-day survival tests with wild-caught (Snake River, Idaho) *Fluminicola* sp. included mixed-aged adult and young-adult organisms (from 6 to 12 months). Mixed-age classes were used because the acclimation cultures produced only approximately 200 neonates for testing that were collected over a period of about four months. Despite the fact that snails in the control treatment exhibited 100 percent survival, while snails exposed to the highest ammonia concentration (7.9 mg TAN/L) exhibited 0 percent survival, extreme variation between replicates at the highest test concentrations was observed during the test, i.e., snails in replicates were either all alive or all dead in the 1.7 and 3.6 mg TAN/L treatments. Based on the mean survivals for *Fluminicola* sp., the reported survival EC_{20} for the species was estimated to be 1.02 mg TAN/L at test temperature (20.8°C) and pH (8.26), or 3.900 mg TAN/L when adjusted to pH 7 and 20°C (see Appendix C). The EC_{20} reported for the test is not considered reliable, however, due to the variability in survival among replicates in the 1.7 and 3.6 mg TAN/L test concentrations; therefore, this data was not used in the derivation of the final ammonia CCC (i.e., the all-or-none response in the replicates of these two treatments, which, when averaged and used as means instead of analyzing the replicates separately in the regression, allows estimation of an EC_{20} that would otherwise be incalculable because of the variability between treatment replicates). Thus, the upper limit CV for the test is uncertain. The value clearly is a concentration below 7.9 mg TAN/L (at test temperature and pH), but the exact concentration could not be determined at the time.

In an attempt to further define the 28-day ammonia survival effects threshold for *Fluminicola* sp., pebblesnails cultured in the laboratory at the USGS Columbia Environmental Research Center were tested in April 2010 via a similar 28-day test protocol (see Besser 2011). This 2010 test was conducted with two size classes of juvenile pebblesnails: small (mean shell length of 1.34 mm at the start of the test) and large (mean starting shell length of 1.81 mm). Both size groups were exposed in the same flow-through exposure system consisting of five ammonia concentrations (ranging from a nominal concentration of 0.5 to 8 mg N/L in 50 percent dilution series), plus a control, with four replicates of ten snails per replicate (or 40 small and 40 large snails per treatment). Mean measured TAN concentrations, pH, and temperature were maintained very close to target values throughout the test (i.e., mean measured ammonia concentrations were within 14 percent of nominal, mean treatment pH ranged from 8.18 to 8.26, and mean treatment water temperature ranged from 20.1 to 20.2°C). Survival was measured after 4 and 28 days. Survival of snails after 28 days in the small size group was lower overall (75 percent in the control and 60-68 percent in the nominal 0.5 to 2 mg TAN/L test concentration range) in relation to that of the large size group (93-100 percent in both the control and low ammonia test concentration). For both size groups, snail survival differed among test concentrations and was substantially lower than controls in the two highest ammonia concentrations (4.0 and 8.0 mg TAN/L nominal), however, due to the lower control survival of the small size group (<80 percent), the data for this group is not used quantitatively in the derivation of the final ammonia CCC and is instead presented in Appendix C as other chronic data.

Because the survival of the large size group of snails was acceptable in controls and snail length different among concentrations according to concentration-response, change in length for the large size group was analyzed further for inclusion in the derivation of the CCC. (Note: attempts to model concentration-response curves for survival in the large size group using TRAP software were not as informative because partial mortality was limited to only one treatment (i.e., 28-day survival ranged from 98 to 100 percent in the nominal 0.5, 1 and 2 mg TAN/L test concentrations, only 10 percent in the 4 mg TAN/L nominal test concentration, and zero percent at the highest nominal test concentration of 8 mg TAN/L). The growth EC_{20} for this freshwater non-pulmonate snail species calculated using EPA's TRAP (threshold sigmoid model with full convergence) is 2.281 mg TAN/L at test pH (8.22) and temperature (20.1°C), or; 7.828 mg TAN/L after adjustment to pH 7 and 20°C (see Appendix B). The TRAP output for this test is provided below to support the use of the growth-based EC_{20} for this particular species and test.



Chronic Toxicity Tests with Juvenile Hyalella azteca

Borgmann (1994) conducted four sets of experiments on *H. azteca* using different dilution water types and life-stages of test organisms. One set of experiments consisted of tests that began with <1-week-old organisms, all of which utilized weekly renewals and dechlorinated tap water originating from Lake Ontario. Of the three tests, one lasted four weeks and the other two lasted 10 weeks, the latter of which produced data on both survival and reproduction, as described in detail in the 1999 AWQC document (U.S. EPA 1999). At the time, the results of the two 10-week tests were deemed sufficiently similar such that the results were analyzed together and subsequently used as the basis for the pH and temperature adjusted EC_{20} of <1.45 mg TAN/L (at pH 8 and temperature 25°C) reported in Table 5 of the 1999 AWQC document (U.S. EPA 1999). Since then, however, EPA has re-evaluated the results of the three tests in light of the recent extensive research that has been undertaken to elucidate the specific water ionic composition and feeding requirements necessary to ensure the health of this particular freshwater aquatic test organism for use in long-term toxicity testing. During the EPA's re-evaluation of these tests, it was concluded that while the ionic composition of the water used for testing (dechlorinated city tap water originating from Lake Ontario) was acceptable, the results of the two 10-week chronic tests should not be used for deriving AWQC for the following reasons:

- Low control survival observed after 10 weeks of exposure (only 66.3%), possibly linked to inadequate food and feeding level that was employed, particularly after the first four weeks of testing;
- Poor control reproduction observed after 10 weeks of exposure; and
- The fact that the ammonia concentrations increased substantially in critical test treatments (e.g., the 0.1 mM ammonia treatment) during the final 3 weeks of testing (weeks 7 – 10).

However, four week data for these two tests, in combination with data from the third four-week test with the same life stage, were not affected by these limitations. The measured total ammonia concentrations and mean pH (8.04) reported for the "Tap water (young)" tests in Table 1 of Borgmann (1994) reflect the analytical measurements combined from all three tests conducted with this life stage (i.e., <1 wk old *H. azteca*). Likewise, the pooled results for survival (from Figure 1a) and wet weight (from Table 4) reflect the observations (weekly for survival and after four weeks for wet weight) from the three respective tests, and thus, represent observations stemming from six test replicates per treatment when combined. Using these data up through the first four weeks of exposure, as well as the water temperature of 25°C (maintained via an incubator) at which all sets of experiments in the study were run, a 28-day EC_{20} of 29.17 mg N/L (based on biomass and normalized to pH 7 and 20°C) was calculated for *H. azteca* for the study (Appendix B). These data were deemed sufficient to derive an SMCV for the species (as an upper limit), which is subsequently used here for chronic criterion development. This decision was largely predicated on the fact that:

- The ion composition of the water used in this test was acceptable;
- The control survival for the tests up through the first four weeks was good (88.4%); and

- The feeding level during the first four weeks of testing was acceptable (as judged via the growth performance of the test organisms during this timeframe.

New Chronic Data for Non-salmonid Fish Species

Cyprinus carpio (common carp)

Mallet and Sims (1994) conducted a 28-day early life-stage test starting with eggs approximately 6 hours post-fertilization. Mean pH and temperature for the test were 7.85 and 23°C, respectively. The measured DO concentrations reported for the test ranged from 79 to 94 percent of saturation. Ammonia had no effect on hatching success at the highest concentration tested (19.6 mg TAN/L); although survival of the post-hatch stages was significantly reduced at this level compared to controls (average fry survival in the control treatment was 86 percent). Growth of fry was the most sensitive endpoint, and mean fry wet weights were inhibited at concentrations ≥10.4 mg TAN/L. Even though the number of larvae in each replicate vessel was not made uniform on hatching, at least one vessel per concentration contained an equivalent stocking density (23 to 29 carp), so the mean wet weight of carp in the one selected replicate per concentration was analyzed using regression analysis. The resulting EC_{20} value was 8.360 mg TAN/L at 23°C and pH 7.85, which is calculated to be 16.53 mg TAN/L at pH 7, with a GMCV sensitivity rank of ten (see Appendix B and Table 4).

Esox lucius (northern pike)

Harrahy et al. (2004) conducted a 52-day early life-stage test starting with newly-fertilized northern pike embryos. The mean dissolved oxygen concentration in test water ranged from 8.7 to 9.1 mg/L during the test. There was no effect of ammonia on hatching success up to 62.7 mg TAN/L, and larval survival of control fish was 100 percent. A significant reduction in larval survival and growth was observed at concentrations of total ammonia ≥30.4 and 15.1 mg TAN/L, respectively, at pH 7.62 and 8.7°C. The estimated EC_{20} value reported for biomass was 13.44 mg TAN/L, which, normalized to pH 7 to support criteria development in this document, is 20.38 mg TAN/L (Appendix B). The GMCV of 20.38 mg TAN/L for northern pike is included in Table 4 as the GMCV ranked 11th in sensitivity.

New Chronic Toxicity Data for Salmonid Species

Chronic values for two additional studies with Oncorhynchus species are included in this AWQC document. Koch et al. (1980) exposed Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*) for 103 days in an ELS test. The measured dissolved oxygen concentrations for the entire study ranged from 7.0 to 8.9 mg/L, with an overall average of 7.9 mg/L. Survival of embryos in the control treatment was 80 percent, with approximately 95 percent surviving through the fry stage, and 80 percent surviving as fingerlings up to day 94 of the test. There were no successful hatches at exposure levels of 148 mg TAN/L or higher and no significant mortality at exposure levels below 32.9 mg TAN/L. Regression analysis of the survival data using an arcsine transformation resulted in a calculated EC_{20} value of 17.89 mg TAN/L at 13.7°C and pH 7.57. The EC_{20} value is 25.83 mg N/L when adjusted to pH 7 (Appendix B).

The recent results of a 90-day ELS test using a wild strain of rainbow trout exposed to ammonia were reported by Brinkman et al. (2009). The test was initiated with newly fertilized embryos (<24 h) exposed under flow-through conditions through hatch (28 days), swim-up (15 days) and early fry development (52 days) to five concentrations of total ammonia with a control. Each treatment consisted for four replicates containing 20 embryos each (N = 100 embryos per treatment). Mean pH and temperature of test water measured among treatments was 7.75 and 11.4°C, respectively. Hatch success and survival of sac fry were similar to controls for all ammonia concentrations, resulting in an unadjusted NOEC of >16.8 mg TAN/L. Survival, growth and biomass of swim-up fry were significantly reduced at 16.8 mg TAN/L compared to controls, but unaffected at 7.44 mg N/L, resulting in a chronic value (MATC) of 11.2 mg TAN/L. The EC_{20} calculated for biomass using TRAP and normalized to pH 7 is 15.60 mg TAN/L (Appendix B).

Appendix I. Qualitative Weight-of-Evidence Test Data.

Additional 28-day Toxicity Test data for Freshwater Mussels

As part of the same study summarized above in the *Effects Analyses to Freshwater Aquatic Organisms* under *Summaries of Studies Used in Chronic Criterion Determination* (page 34), Wang et al. (2007a) also attempted to determine the effect of ammonia on growth of 2-month old juvenile rainbow mussel, fatmucket, and wavy-rayed lampmussel. The 28-day tests were conducted following the same methods (see ASTM 2006). The mean length of juvenile rainbow mussel and fatmucket exposed to the lowest ammonia concentrations tested was reduced by 13 and 12 percent compared to mean length of control animals, respectively, but increased by 7 percent for the wavy-rayed lampmussel. There was no consistent effect of ammonia, however, on either length at 28 days or change in length after 28 days for fatmucket and wavy-rayed lampmussel at test concentrations where survival was unaffected; only the 28-day test with rainbow mussel exhibited such a concentration- response for length and change in length. For the reasons explained above under the section referenced, the growth endpoint was not used from these tests to derive the chronic criterion, and instead, the reported IC_{25} (inhibition concentration) estimated for these tests are included in Appendix C. The reported growth IC_{25} for juvenile rainbow mussel, fatmucket, and wavy-rayed lampmussel from their respective 28-day tests were 0.73, 0.44, and 0.57 mg TAN/L at test pH of 8.2 and temperature 20°C. These values, when adjusted to pH 7 and 20°C, are 2.406, 1.450 and 1.878 mg TAN/L, respectively (see Appendix C).

Additional 28-day Toxicity test Data for Freshwater Snails

Besser et al. (2009), in a USGS study report completed for EPA, conducted 28-day flow-through survival and growth tests with five species of snails, including four gill-bearing (non-pulmonate) species and an air-breathing (pulmonate) species. All tests were conducted in ASTM hard water (mean hardness and alkalinity of approximately 170 and 120 mg/L as $CaCO_3$, respectively) with a pH range of 8.20-8.29 and a temperature range of 19-21°C during testing. Total ammonia nitrogen (mg TAN/L) concentrations in tests were measured weekly with the percent of nominal concentrations ranging from 83 to 101 percent. Test results were based upon the mean of the measured concentrations. For all snail exposures, the effect of ammonia on growth was not determined for test species that were of mixed ages at test initiation (as explained further below); growth, however, was not as sensitive of an endpoint as survival for at least one (*Pyrgulopsis idahoensis*) of the two snail species (*Lymnaea stagnalis* and *P. idahoensis*) where both growth and survival were measured (see Appendix C).

Fontigens aldrichi (Ozark springsnail)

As part of the original study described above, Besser et al. (2009) also determined the effect of ammonia on survival of the non-pulmonate snail *F. aldrichi*. Because *F. aldrichi* did not reproduce during culturing and acclimation, field-collected organisms of "older" (adult) mixed-ages were used for ammonia exposures. *F. aldrichi* exposed to ammonia in the 28-day test exhibited approximately 94 percent survival at 0.45 mg TAN/L, but only 50 percent at 0.83 mg TAN/L. Similar to the 2009 adult pebblesnail study, the replicates associated with the latter 0.83 mg TAN/L treatment in particular were characterized by high variability, and therefore, these data were not used quantitatively in the derivation of the final ammonia CCC. In addition, field-collected *F. aldrichi* did not reproduce in captivity and animals in the control group did not grow during testing. The reported EC_{20} for *F. aldrichi* was 0.61 mg TAN/L, or 2.332 mg TAN/L when adjusted to pH 7.0 and 20°C, and is presented as other chronic data in Appendix C.

Pyrgulopsis idahoensis (Idaho springsnail)

Two separate 28-day tests with the de-listed (from the Federal threatened and endangered species list) non-pulmonate snail species, *P. idahoensis,* were conducted which included exposing juvenile organisms that were 7-9 and 11-13 weeks post-hatch (organisms in each cohort tested as separate replicates in the same test; test identified as test #3 in the 2009 Besser et al. report), as well as a cohort of mixed-age adults for all subsequent tests (test identified as test #5 in Besser et al. 2009). The older life stages were chosen for testing because of the high control mortality demonstrated in preliminary tests using 2-3 week post-hatch *P. idahoensis.*

In the 28-day test with juveniles, snails in four of the five test concentrations exhibited ≤44.4 percent survival, whereas control survival was 100 percent; the single exception being the snails in the middle test concentration of 1.8 mg TAN/L, which demonstrated only 62.5 percent survival. The survival EC_{20} reported for the test was 0.48 mg TAN/L at 20.1°C and pH 8.25, or

1.726 mg TAN/L when adjusted to pH 7 and 20°C, however, due to the poor concentration-response relationship exhibited in this test, this EC_{20} is highly uncertain, and therefore, the data are included in Appendix C as "other data" and are not used in the derivation of the CCC.

The 28-day chronic test initiated with mixed-aged adult *P. idahoensis* (4 to 8 months of age), on the other hand, resulted in an EC_{20} reported for the test of 3.24 mg TAN/L, or 12.39 mg TAN/L when adjusted to pH 7 and 20°C (Appendix C). Comparison of the juvenile and adult *P. idahoensis* survival results indicates that juveniles are possibly the more sensitive of the two life stages; however, due to the unreliability of the juvenile data, specifically the irregular survival concentration-response relationship, such an assertion is uncertain at this time and the CVs are not used quantitatively in the derivation of the CCC.

Taylorconcha serpenticola (Bliss Rapids snail)

A non-pulmonate snail species listed under the Endangered Species Act, *Taylorconcha serpenticola*, was exposed to ammonia in 28-day flow-through toxicity tests as described above. Because *T. serpenticola* did not reproduce or grow well during culturing and acclimation, field-collected organisms of "older" (adult) mixed-ages were used. Survival of snails in the control treatment was 100 percent, whereas survival of snails exposed to concentrations up to 3.6 mg TAN/L exceeded 80 percent. Survival of snails exposed to the highest concentration tested (7.9 mg TAN/L) was reduced to only 30 percent. The survival EC_{20} reported for *T. serpenticola* in the test was 3.42 mg TAN/L at 20.8°C and pH 8.26, or 13.08 mg TAN/L at pH 7.0 and 20°C, but because these snails did not grow well preceding the test, the data are also considered "other data" and placed in Appendix C.

Pleurocera canaliculata (silty hornsnail)

EPA sponsored a study (GLEC 2011) to independently confirm the results of the 28-day juvenile and adult tests performed by the USGS, Columbia, MO laboratory (i.e., Besser et al. 2009 and Besser 2011) with non-pulmonate snails. The USGS test results indicated that specialized and Federally-listed non-pulmonate gill-bearing snails, such as the Idaho springsnail, Bliss Rapids snail and pebblesnail, are potentially: 1) sensitive to prolonged, 28-day ammonia exposure, and 2) as sensitive as ammonia-sensitive freshwater unionid mussel species to such exposure. The EPA-sponsored study involved a 28-day flow-through toxicity test using a more widely-distributed non-pulmonate snail species, *P. canaliculata.* Two other non-pulmonate snail species were also selected based on distribution and generalized habitat preference; however, *P. canaliculata* was the only one of the three wild-caught snail species that were successfully held and maintained in the laboratory for subsequent testing. Following a protocol similar to that used in the USGS studies, a 28-day toxicity test of mature, mixed-age *P. canaliculata* was conducted. The test design consisted of five ammonia test concentrations (0.9, 1.9, 3.8, 7.5, and 15 mg TAN/L, nominal) and one control, with four replicate chambers containing six snails each per test concentration (N=24 snails per treatment). Test concentrations were based on the results of a 96-hr range finding test with the species, which provided a 96-hr EC_{50} of 9.66 mg TAN/L, or approximately 88 mg N/L at 20°C and pH 7.0. The endpoint for the 28-day toxicity test was mortality or immobilization, measured daily, the results of which were used to calculate an EC_{20} (at pH 7 and 20°C) of 1.845 mg TAN/L (Appendix C). However, due to the high degree of temporal variability in the measured total ammonia concentrations, as well as the unequal response amongst replicates at the 1.9 mg TAN/L nominal test concentration, these data were not used quantitatively in the derivation of the final ammonia CCC; a 28-day ammonia survival effect concentration of <7.667 mg TAN/L was recommended as the CV for the species which supports the recent findings for the pebblesnails (1.8 mm) which were re-tested and reported to EPA via Besser (2011).

(**Note**: The calculated EC_{20} values using TRAP for *P. idahoensis*, *F. aldrichi*, and *T. serptenticola*, and the recommended 28-day ammonia survival effects concentration of <7.667 mg TAN/L for *P. canaliculata*, are deemed representative of non-pulmonate snail sensitivity in general and are included in Appendix C for the purpose of comparison.)

Lymnaea stagnalis (pulmonate pondsnail)

The effect of ammonia in a 28-day test on survival and growth of a third freshwater snail species, the air-breathing *L. stagnalis,* was also reported in Besser et al. (2009). The tests with *L. stagnalis* utilized organisms that were <1 week post-hatch due to the abundance of young produced during culturing. *L. stagnalis* exposed to ammonia in a 28-day flow-through test exhibited approximately 98 percent survival at the highest concentration tested (8.0 mg TAN/L). Because of the apparent negligible effect of ammonia on growth (i.e., the magnitude of the growth reduction was so small, 6 percent at 1.8 mg TAN/L and only 16 percent at 8 mg TAN/L), only the CV of >8.0 mg TAN/L (for survival and growth) is reported in this document for the test, or >28.76 mg TAN/L when adjusted to pH 7 and 20°C. Note: For the purposes of this document, the CV for this test species is included in Appendix C and was not used in the derivation of the CCC because of the uncertainty of this value (> 28.76 mg TAN/L) as an upper limit SMCV for the species.

Chronic Toxicity Data for Other Salmonids

A few other chronic toxicity tests produced applicable data for salmonid species that were excluded from Appendix B and subsequent SMCV and GMCV calculation because either the exposure did not include the appropriate life stage for the species, or the tests did not meet other general 1985 Guidelines requirements for use in calculating the CCC. These tests are summarized below and shown in Appendix C.

The effects of water temperature and ammonia on the swimming characteristics of brook charr (*Salvelinus fontinalis*) were investigated by Tudorache et al. (2010). Juvenile brook charr were exposed to four ammonia concentrations in de-chlorinated tap water for 96 hours at pH 9.10 and 15°C. The following swimming characteristics were measured in a 4.5 m long raceway following this exposure: gait transition speed, maximum swimming speed, tail-beat amplitude, tail-beat frequency, maximum acceleration of bursts, number of bursts, distance of bursts, and total swimming distance. The most sensitive swimming parameters (maximum swimming speed and maximum acceleration) had a reported LOEC of 0.7765 mg TAN/L, or 10.86 mg TAN/L when normalized to pH 7.

The effects of long-term exposure of ammonia on the molecular response of Atlantic salmon (*Salmo salar*) parr were investigated by Kolarevic et al. (2012). The juvenile fish were exposed for 105 days to three concentrations of total ammonia nitrogen (TAN) in a flow-through apparatus with two different feeding regimes: full and restricted. Average water temperature during the exposure was 12.1°C with a pH of 6.84. There was no effect of ammonia exposure on survival, resulting in a NOEC of 32.29 mg N/L (highest concentration tested) in the full feeding regime. When normalized to pH 7, the CV for this test is >30.64 mg TAN/L.

Beamish and Tandler (1990) exposed juvenile lake trout (*Salvelinus namaycush*) for 60 days on two different diets and observed a significant reduction in rate of weight gain when total ammonia was 6.44 mg TAN/L at pH 8.02 and temperature was 11.6°C. Food intake by fish was initially decreased at this concentration of total ammonia, but was no different from controls by the end of the test. The growth LOEC for the study, when adjusted to pH 7, was calculated to be 16.10 mg TAN/L. Note: this test was not included in the calculation of the CCC because it was not a true ELS having been initiated with juvenile fish.

Chronic Toxicity Data for Threatened and Endangered Fish Species

Meyer and Hansen (2002) conducted a 30-day toxicity test with late-stage larvae (0.059 g) of Lost River suckers (*Deltistes luxatus*) at pH 9.43. The exposure duration and pH were chosen to represent the period of combined elevated unionized ammonia concentrations and elevated pH that occur during cyanobacterial blooms in surface waters of Upper Klamath Lake, which have been shown to last for several weeks to a month. Survival decreased significantly at 1.23 and 2.27 mg TAN/L, whereas the highest NOEC for all endpoints (survival, growth, body ions, and swimming performance) was 0.64 mg TAN/L. Most deaths in the 2.27 mg TAN/L exposure occurred during the first three days of the test, while mortality of larvae in the 1.230 mg TAN/L treatment occurred gradually from days 2 to 24. The 29 percent average mortality in the 0.64 mg TAN/L treatment was all due to an unexplained complete loss of one replicate between days 5 and 7 of the exposure. Control survival was > 90 percent. The calculated LOEC of 1.230 mg TAN/L total ammonia normalized to pH 7 corresponds to a value of 25.31 mg TAN/L, substantially higher than the 2013 chronic criterion value (Appendix C).

Fairchild et al. (2005) conducted 28-day toxicity tests with early life stages of Colorado pikeminnow (*Ptychocheilus lucius*) and razorback sucker (*Xyrauchen texanus*), and compared the results of those tests with a test using a surrogate fish species, the fathead minnow (*Pimephales promelas*). Tests were initiated 2 days after swim-up when the larvae were feeding exogenously (or at 8-day post hatch for Colorado pikeminnow, 9-day post hatch for razorback sucker, and 4-day post-hatch for fathead minnow). Temperature, pH and dissolved oxygen over the 28-day test period averaged 19.9°C, 8.24, and 7.4 mg/L (80 percent saturation) over the course of the three studies. Control mortality was 7 percent (fathead minnows and Colorado pikeminnow) or less (3 percent, razorback sucker) on day 28. Effect concentrations based on the survival and growth endpoints of the fathead minnow and razorback sucker tests were not different; however, growth was the more sensitive endpoint for the Colorado pikeminnow test.

The 28-day growth LOEC for the Colorado pikeminnow was 8.60 mg N/L, or 29.75 mg TAN/L at pH 7, substantially greater than the 2013 chronic criterion. The 28-day survival LOEC for the razorback sucker was 13.25 mg TAN/L, or 46.58 mg TAN/L at pH 7. Both endangered fish species exhibited similar sensitivity to ammonia as the fathead minnow (LOEC of 32.71 mg TAN/L at pH=7; see Appendix C). The same can be said for the Lost River sucker, which indicates that these particular endangered fish species will be protected by the CCC value calculated in this 2013 AWQC Update.

Finally, Adelman et al. (2009) conducted both acute and chronic toxicity tests with ammonia on the endangered Topeka shiner (*Notropis topeka*) and compared those values to chronic studies with fathead minnows. All tests used a flow-through dosing apparatus and deep well water with a total hardness and alkalinity of 210-230 mg/L $CaCO_3$, and chloride concentration of 0.64-1.04 mg/L. Acute survival studies with Topeka shiner lasted 96 hours and were conducted on two different life-stages (juvenile and adult) and at two test temperatures, warm, 25°C (adult and juvenile), and cold, 13°C (juvenile only). LC_{50}s for total ammonia ranged from 18.7-21.4 mg TAN/L at 25°C and 28.9 mg TAN/L at 13°C; all acute studies were conducted at approximately pH 8. Normalized to pH 7, the 96-hr LC_{50}s were 69.59 – 88.27 mg TAN/L at 25°C and 147.3 mg TAN/L at 13°C, both substantially greater than the acute criterion value of 17 mg TAN/L, respectively (see Appendix A).

Chronic studies with Topeka shiners started with both adults and juveniles, since embryos were not available, and lasted 30 days. The results of the survival and growth studies with juvenile Topeka shiners were compared to a 30-day juvenile survival study and 32-day embryo-larval study conducted with fathead minnows in the same dilution water. The authors interpreted the results of the relationship between the comparative studies using Topeka shiners versus fathead minnows to infer what an expected result for an embryo-larval study with Topeka shiner would be. Reported MATC values (normalized to pH 8, according to USEPA 1999) were 16.95 mg TAN/L for the 30-day juvenile fathead growth test and 8.62 mg TAN/L for the 32-day embryo-larval survival and growth test. Using the relationship from the results obtained between juvenile Topeka shiners and juvenile (growth) and embryo-larval test using fathead minnows (growth and survival), a 32-day embryo-larval study with Topeka shiner might be expected to result in a chronic value that is approximately 51% more sensitive than the 30-day juvenile growth test with that species, or a chronic value of approximately 5.63 mg TAN/L (i.e., the reported 30-day MATC of 11.10 mg TAN/L at pH 8 based on growth of juvenile Topeka shiners multiplied by a factor of 0.507). Using EPA's TRAP (version 1.21a) the 32-day biomass EC_{20} for embryo-larval fathead minnow (measured from days 7-32), 30-day adult survival EC_{20} for Topeka shiner, and 30-day juvenile specific growth rate EC_{20} for Topeka shiner were 7.457, 10.85, and 6.483 mg TAN/L at test temperatures (25.5, 23.9, and 12.4°C) and pH (7.95, 7.94, and 8.07), respectively. When adjusted to pH 7, the EC_{20}s for the respective tests are 16.87 mg TAN/L for the fathead minnow (Appendix B), and 24.21 and 17.45 mg TAN/L for the Topeka shiner (Appendix C), much higher than the 2013 chronic criterion.

Chronic Toxicity Data for Amphibians

In a long term chronic study by Jofre and Karasov (1999), pre-metamorphic (Gosner stage 24-26) green frog (*Rana clamitans*) tadpoles were exposed to ammonia for 103 days under renewal conditions. Tadpoles were evaluated in two different experiments conducted in successive years. In the 1997 (repeat) experiment, survival and growth were not statistically different from controls at the highest concentration tested, or 2.2 mg TAN/L at pH 8.7 and 24°C, although only approximately 50 percent of the frogs survived at this concentration compared to the controls (98 percent survival). Survival was reduced to approximately 78 percent at 0.9416 mg TAN/L at test temperature and pH (or 7.149 mg TAN/L at pH 7). Growth, measured as total length, was no different between treatments. The frogs grew from an average total length of approximately 7.5 mm at test initiation to approximately 50 mm in all treatments. The NOEC for growth of green frog tadpoles in the study (which does not reflect an ELS or partial life cycle test) is >16.74 mg TAN/L at pH 7.

Appendix J. Unused Acute Studies Potentially Influential for Freshwater Ammonia Criteria Development.

Appendix J. Unused Acute Studies Potentially Influential for Freshwater Ammonia Criteria Development			
Reference:	**Organism:**	**Reported or Normalized Acute Value Expressed as Total Ammonia (mg TAN/L) at pH=7 and 20°C, Where Applicable**	**Rationale for Omission:**
Abdalla, A.A.F. and C.D. McNabb. 1999. Acute and sublethal growth effects of unionized ammonia to Nile tilapia *Oreochromis niloticus*. In: Nitrogen production and excretion in fish. Randall D.J. and D.D. Mackinlay (Eds.), Department of Fisheries and Oceans, Vancouver, BC, Canada and Towson University, Baltimore, MD. pp. 35-48.	*Oreochromis niloticus*	Normalized LC_{50} = 87.0	Species is a resident, non-North American "invasive" species known to cause or likely to cause economic or environmental harm (see ISAC 2006). Because the species is in the Family Centrarchidae which is well represented in the current acute criteria dataset, it has been intentionally excluded from further consideration and calculation of an acute criterion.
Alonso, A. and J.A. Camargo. 2011. The freshwater planarian *Polycelis felina* as a sensitive species to assess the long-term toxicity of ammonia. Chemosphere 84: 533-537.	*Polycelis felina*	Normalized 96 h LC_{50} = 25.72	Species not resident in North America.
Ankley, G.T., M.K. Schubauer-Berigan and P.D. Monson. 1995. Influence of pH and hardness on toxicity of ammonia to the amphipod *Hyalella azteca*. Can. J. Fish. Aquat. Sci. 52(10): 2078-2083.	*Hyalella azteca*	Normalized 96 h LC_{50}s: Softwater (Lake Superior) - **25.51 (pH 6.50)** **47.35 (pH 7.49)** 233.4 (pH 8.21) Hardwater (Reconstituted-ASTM) - 232.8 (pH 6.55) 337.6 (pH 7.41) 545.5 (pH 8.45)	Ankley et al. conducted several static-renewal acute tests with *H. azteca* to determine the effect of pH and hardness on the toxicity of ammonia. For the hardness evaluation, Ankley chose three waters for testing, soft water (SW; unaltered lake Superior water), moderately hard water (MW; hardened Lake Superior water), and hard water (HW; hard reconstituted water). At the time, Ankley et al. focused only on hardness in the test waters, but the ion ratios in these three waters were not consistent. Of the three water types, only the moderately hard water (MW) that Ankley used is suitable for testing and culturing amphipods (see Appendix A for results). The SW was not suitable for testing this species because the sodium concentration was too low. Similarly, the reconstituted HW was not suitable because the bromide was too low. Bold values indicate LC_{50}s below the cutoff of 93 mg TAN/L for unused, potentially influential acute values.

Appendix J. Unused Acute Studies Potentially Influential for Freshwater Ammonia Criteria Development

Reference:	**Organism:**	**Reported or Normalized Acute Value Expressed as Total Ammonia (mg TAN/L) at pH=7 and 20°C, Where Applicable**	**Rationale for Omission:**
Augspurger, T., A.E. Keller, M.C. Black, W.G. Cope and F.J. Dwyer. 2003. Water quality guidance for protection of freshwater mussels (Unionidae) from ammonia exposure. Environ. Toxicol. Chem. 22(11): 2569-2575.	*Medionidus conradicus*	Normalized 48 h LC$_{50}$ = 27.56	48-hr glochidia test. Secondary data from Keller 2000
Babu, T.R., P. Surendranath and K.V. Ramana Rao. 1987. Comparative evaluation of DDT and fenvalerate toxicity on *Penaeus indicus* (H. Milne Edwards). Mahasagar 20(4): 249-253.	*Daphnia magna*	Reported LC$_{50}$s: 60 (25 h), 32 (50 h), 20 (100 h)	pH not reported – LC$_{50}$s could not be normalized.
Belanger, S.E., D.S. Cherry, J.L. Farris, K.G. Sappington and J.J. Cairns. 1991. Sensitivity of the Asiatic clam to various biocidal control agents. J. Am. Water Works Assoc. 83(10): 79-87.	*Corbicula fluminea*	Normalized LC$_{50}$s: 23.55 (4.1 d) 64.99 (4.2 d)	Species is a resident, non-North American "invasive" species known to cause or likely to cause economic or environmental harm (see ISAC 2006). This species is the target of current eradication and control programs in various states, and because this Phylum (Mollusca) is well represented in the current acute criteria dataset, this species has been intentionally excluded from further consideration and calculation of an acute criterion.
Dehedin, A., C. Piscart and P. Marmonier. 2012. Seasonal variations of the effect of temperature on lethal and sublethal toxicities of ammonia for three common freshwater shredders. Chemopshere In press.	*Gammarus pulex*	Normalized 96h LC$_{50}$s: 36.98 49.31 49.31 69.40	Species not resident in North America. Control mortality less than 15%.
	Gammarus roeselii	Normalized 96 h LC$_{50}$s: 2.466 24.66 36.98 46.27 46.27 55.31 55.31 57.84	Species not resident in North America. Control mortality less than 15%.

Appendix J. Unused Acute Studies Potentially Influential for Freshwater Ammonia Criteria Development			
Reference:	**Organism:**	**Reported or Normalized Acute Value Expressed as Total Ammonia (mg TAN/L) at pH=7 and 20°C, Where Applicable**	**Rationale for Omission:**
Dowden, B.F. and H.J. Bennett. 1965. Toxicity of selected chemicals to certain animals. J. Water Pollut. Control Fed. 37(9): 1308-1316.	*Daphnia magna*	Reported LC_{50}s: 202 (24 h), 423 (25 h), 161 (48 h), 433 (50 h), 67 (72 h), 50 (96 h), 202, 139 (100 h)	pH not reported – LC_{50}s could not be normalized.
	Lymnaea sp.	Reported LC_{50}s: 241 (24 h), 173 (48 h), 73 (72 h), 70 (96 h)	pH not reported – LC_{50}s could not be normalized.
Ewell, W.S., J.W. Gorsuch, R.O. Kringle, K.A. Robillard and R.C. Spiegel. 1986. Simultaneous evaluation of the acute effects of chemicals on seven aquatic species. Environ. Toxicol. Chem. 5(9): 831-840.	*Daphnia magna*	Reported LC_{50} in paper = >100; Reported LC_{50} in ECOTOX = >20 Normalized LC_{50} = 36.29	Insufficient controls; pH that varied from 6.5-8.5 during the exposure. LC_{50} based on a 96 h (non-standard) test duration.
Fairchild, J.F., A. Allert, J. Mizzi, R. Reisenburg and B. Waddell. 1999. Determination of a safe level of ammonia that is protective of juvenile Colorado pikeminnow in the upper Colorado River, Utah. Final Report.1998 Quick Response Program. U.S. Fish and Wildlife Service, Region 2 (Salt Lake City Office).	*Pimephales promelas*	Normalized LC_{50} = 60.12	72-hour test in well water
Hazel, R.H., C.E. Burkhead and D.G. Huggins. 1982. Development of water quality criteria for ammonia and total residual chlorine for the protection of aquatic life in two Johnson County, Kansas Streams. In: J.G. Pearson, R.B. Foster, and W.E. Bishop (Eds.), Proc. Annu. Symp. Aq. Tox., ASTM STP 766, Philadelphia, PA: 381-388.	*Etheostoma spectabile*	Normalized 96 h LC_{50}s = 83.74, 71.12	Same data as in Hazel (1979) – see *E. spectabile* in Appendix A.
Hecnar, S.J. 1995. Acute and chronic toxicity of ammonium nitrate fertilizer to amphibians from Southern Ontario. Environ. Toxicol. Chem. 14(12): 2131-2137.	*Pseudacris triseriata*	Reported values: 4-d LC_{50} = 17 4-d NOEC = 5, 4-d LOEC = 45	Formulation - ammonium nitrate fertilizer

Appendix J. Unused Acute Studies Potentially Influential for Freshwater Ammonia Criteria Development

Reference:	Organism:	Reported or Normalized Acute Value Expressed as Total Ammonia (mg TAN/L) at pH=7 and 20°C, Where Applicable	Rationale for Omission:
Hickey, C.W. and M.L. Vickers. 1994. Toxicity of ammonia to nine native New Zealand freshwater invertebrate species. Arch. Environ. Contam. Toxicol. 26(3): 292-298.	*Potamopyrgus antipodarum*	Normalized 96 h LC_{50}s: 33.14 29.79 38.93 36.27	Species is a resident, non-North American "invasive" species known to cause or likely to cause economic or environmental harm (see ISAC 2006). This species is the target of current eradication and control programs in various states, and because this Phylum (Mollusca) is well represented in the current acute criteria dataset, this species has been intentionally excluded from further consideration and calculation of an acute criterion.
Horne, F.R. and S. McIntosh. 1979. Factors influencing distribution of mussels in the Blanco River of Central Texas. Nautilus 94(4): 119-133.	*Cyrtonaias tampicoensis*	Normalized LC_{50} = 26.75	LC_{50} based on a 7-d (non-standard) test duration.
	Toxolasma texasensis	Normalized LC_{50} = 26.75	LC_{50} based on a 7-d (non-standard) test duration.
	Corbicula manilensis	Normalized LC_{50} = 26.75	LC_{50} based on a 7-d (non-standard) test duration.
Jofre, M.B., and W.H. Karasov. 1999. Direct effect of ammonia on three species of North American anuran amphibians. Environ.Toxicol.Chem. 18(8): 1806-1812.	*Bufo americanus*	Normalized 96 h LC_{50} = 62.85	Non-standard acute endpoint based on hatch success/ deformity.UIA calculated using Thurston et al. (1979) EPA-600/3-79-091 from measured values
	Rana clamitans	Normalized 96 h LC_{50} = 40.80	Non-standard acute endpoint based on hatch success/ deformity.UIA calculated using Thurston et al. (1979) EPA-600/3-79-091 from measured values
Jofre, M.B., M.L. Rosenshield and W.H. Karasov. 2000. Effects of PCB 126 and ammonia, alone and in combination, on green frog (*Rana clamitans*) and leopard frog (*R. pipiens*) hatching success, development, and metamorphosis. J. Iowa Acad. Sci. 107(3): 113-122.	*Rana clamitans*	Normalized 96 h LC_{50} = 49.56	Non-standard acute endpoint based on hatch success/ deformity. pH not reported; assume same as Jofre and Karasov 1999.
Kaniewska-Prus, M. 1982. The Effect of ammonia, chlorine, and chloramine toxicity on the mortality of *Daphnia magna* Straus. Pol. Arch. Hydrobiol. 29(3/4): 607-624.	*Daphnia magna*	Normalized LC_{50} = 1.980	LC_{50} based on a 24-h (non-standard) test duration.
Meyer, J.S. and J.A. Hansen. 2002. Subchronic toxicity of low dissolved oxygen concentrations, elevated pH, and elevated ammonia concentrations to Lost River suckers. Trans. Amer. Fish. Soc. 131: 656-666.	*Deltistes luxatus*	Normalized 48 h LC_{50}: 78.23	The pH for this test was reported as 9.5, which is outside of the acceptable pH range of (6.0-9.0) these criteria were meant to apply.
Morgan, W.S.G. 1979. Fish locomotor behavior patterns as a monitoring tool. J. Water Pollut. Control. Fed. 51(3): 580-589.	*Micropterus salmoides*	Normalized EC_{50} = 5.010	Acute toxicity evaluated electronically based on activity. Exposure was only 24-h (non-standard) in test duration. Concentrations were nominal.
Morgan, W.S.G. 1976. Fishing for toxicity: Biological automonitor for continuous water quality control. Effl. Water Treat. J. 16(9): 471-475.	*Micropterus salmoides*	Normalized EC_{50} = 5.010	Added nominal concentrations equivalent to 48-h LC_{50} from previous literature values, then monitored opercular rhythm activity for 24 h.

Appendix J. Unused Acute Studies Potentially Influential for Freshwater Ammonia Criteria Development

Reference:	**Organism:**	**Reported or Normalized Acute Value Expressed as Total Ammonia (mg TAN/L) at pH=7 and 20°C, Where Applicable**	**Rationale for Omission:**
Morgan, W.S.G. and P.C. Kuhn. 1974. A method to monitor the effects of toxicants upon breathing rate of largemouth bass (*Micropterus salmoides* Lacepede). Water Res. 8(1): 67-77	*Micropterus salmoides* Lacepede	Normalized EC$_{50}$s: 110.3 (11 h), 31.32 (22 h), 110.3 (23 h), 1.556 (44 h)	Similar to Morgan (1976). This is not an actual toxicity test. Rather, it is a test of a monitoring system that relates nominal LC$_{50}$ concentrations (based on literature values), to breathing rate monitored over 24 h.
Morgan, W.S.G. 1978. The use of fish as a biological sensor for toxic comparison in potable water. Prog. Water Tech. 10: 395-398.	*Micropterus salmoides*	Normalized LC$_{50}$ = 9.091	Similar to other Morgan studies listed in this table where nominal ammonia concentrations based on literature LC$_{50}$ concentrations are added to tanks and breathing rate and activity level are monitored electronically for 24 h.
Passell, H.D., C.N. Dahm and E.J. Bedrick. 2007. Ammonia modeling for assessing potential toxicity to fish species in the Rio Grande, 1989-2002. Ecol. Appl. 17(7): 2087-2099.	*Hybognathus amarus*	Secondary data; reported LC$_{50}$ from Buhl 2002 = 1.01 mg/L unionized ammonia-N	In this study the frequency of acute ammonia exceedances were modeled by relating discharge, pH, temperature, and stream ammonia concentrations to literature LC$_{50}$ values.
Scheller, J.L. 1997. The effect of dieoffs of Asian clams (*Corbicula fluminea*) on native freshwater mussels (Unionidae). Virginia Polytechnic Institute and State University, Blacksburg, VA.	*Corbicula fluminea*	Normalized LC$_{50}$s: 6.498 (96 h) 11.57 (96 h) 14.62 (96 h)	Species is a resident, non-North American "invasive" species known to cause or likely to cause economic or environmental harm (see ISAC 2006). This species is the target of current eradication and control programs in various states, and because this Phylum (Mollusca) is well represented in the current acute criteria dataset, this species has been intentionally excluded from further consideration and calculation of an acute criterion.
	Pimephales promelas	Normalized LC$_{50}$ = 38.46	Non-standard (48 h) test duration.
Watton, A.J. and H.A. Hawkes. 1984. The acute toxicity of ammonia and copper to the gastropod *Potamopyrgus jenkinsi* (Smith). Environ. Pollut. Ser. A 36: 17-29.	*Potamopyrgus jenkinsi*	Normalized EC$_{50}$s: 40.31 and 42.06 (48 h), 27.60 and 27.17 (96 h)	Species not resident in North America.

Appendix J. Unused Acute Studies Potentially Influential for Freshwater Ammonia Criteria Development

Reference:	**Organism:**	**Reported or Normalized Acute Value Expressed as Total Ammonia (mg TAN/L) at pH=7 and 20°C, Where Applicable**	**Rationale for Omission:**
Whiteman, F.W., G.T. Ankley, M.D. Kahl, D.M. Rau and M.D. Balcer. 1996. Evaluation of interstitial water as a route of exposure for ammonia in sediment tests with benthic macroinvertebrates. Environ. Toxicol. Chem. 15(5): 794-801.	*Hyalella azteca*	Normalized 96 h LC_{50}s: 10.27 (Lake Superior water) 11.06 (sediment test) 72.67 (sediment test)	Tests were fed. The results from the two sediment tests were not used because sediment toxicity tests using pore water measurements likely underestimate the toxicity of ammonia in a water-only exposure, i.e., test animals could have been exposed to the higher interstitial ammonia concentrations during the exposure[a]. The 96 h LC_{50} for *H. azteca* from water-only exposure to Lake Superior water was not used from this study because the sodium concentration in this dilution water is too low for maintaining adequate animal health – see also the results in this appendix from Ankley et al. (1995) above.

[a] For the same reason the sediment tests reported by Whiteman et al. (1996) for *H. azteca* were unused for criteria derivation, results from the sediment tests from Besser et al. (1998) were also not used. The normalized 96 h LC_{50}s for *H. azteca* from the Besser et al. (1998) sediment tests were 120.5 and 321.4 mg TAN/L at pH 6.69 and 7.56, respectively. Two other LC_{50}s generated for *H. azteca* which are also not used for criteria derivation (due to the insufficient amount of detail provided) include values of 251.5 and 262.7 mg TAN/L from Sarda (1994). Because these latter values exceed 93 mg TAN/L, they are considered non-influential data for the purpose of criteria derivation.

Appendix K. Unused Chronic Studies Potentially Influential for Freshwater Ammonia Criteria Development.

Appendix K. Unused Chronic Studies Potentially Influential for Freshwater Ammonia Criteria Development

Reference:	**Organism:**	**Reported or Normalized Chronic Value Expressed as Total Ammonia (mg TAN/L) at pH=7 and 20°C, Where Applicable**	**Rationale for Omission:**
El-Shafai, S.A., F.A. El-Gohary, F.A. Nasr, N.P. Vander Steen and H.J. Gijzen. 2004. Chronic ammonia toxicity to duckweed-fed tilapia (*Oreochromis niloticus*). Aquacult. 232(1-4): 117-127.	*Oreochromis niloticus*	Normalized Chronic value = 6.881 (75 d)	Test was a 35-day juvenile test; not a true fish ELS test. Species is also a resident, non-North American "invasive" species known to cause or likely to cause economic or environmental harm (see ISAC 2006).
DeGraeve, G.M., W.D. Palmer, E.L. Moore, J.J. Coyle and P.L. Markham. 1987. The effect of temperature on the acute and chronic toxicity of unionized ammonia to fathead minnows and channel catfish. Battelle, Columbus, OH.	*Ictalurus punctatus*	Normalized 30-day NOEC = 0.5628	Per the 1999 update, this 30-day test with juvenile catfish encountered some problems that precluded effective use of these data. For example, some of the test organisms were treated with acriflavine up to two days prior to the beginning of the test. In addition, the mean measured DO concentration was below 5.5 mg/L and below 60 percent of saturation in some of the treatments.
Hecnar, S.J. 1995. Acute and chronic toxicity of ammonium nitrate fertilizer to amphibians from Southern Ontario. Environ. Toxicol. Chem. 14(12): 2131-2137.	*Pseudacris triseriata*	Reported values: 100-d NOEC = 2.5, 100-d LOEC = 10	Formulation - ammonium nitrate fertilizer.
Hermanutz, R.O., S.F. Hedtke, J.W. Arthur, R.W. Andrew and K.N. Allen. 1987. Ammonia effects on macroinvertebrates and fish in outdoor experimental streams. Environ. Pollut. 47: 249-283.	*Ictalurus punctatus*	Normalized NOEC = 4.369	Survival and growth of juvenile channel catfish were evaluated via exposure to ammonia in experimental streams. Three separate tests lasted from 36 to 177 days and were started with individuals whose average weights ranged from 6 to 19 g. Average temperatures in the three tests were 17 to 21°C. Both of the longer tests showed monotonic, substantial reductions in biomass; these results are in reasonable agreement with the results of the laboratory tests. However, juveniles might not be as sensitive to ammonia toxicity as early life stages are. These results are not included because they are from a field study where ammonia concentrations were highly variable.
	Sander vitreus	Normalized NOEC = 4.182	Omitted for the same reasons as was *Ictalurus punctatus*.

Appendix K. Unused Chronic Studies Potentially Influential for Freshwater Ammonia Criteria Development			
Reference:	**Organism:**	**Reported or Normalized Chronic Value Expressed as Total Ammonia (mg TAN/L) at pH=7 and 20°C, Where Applicable**	**Rationale for Omission:**
Hickey, C.W., L.A. Golding, M.I. Martin and G.F. Croker. 1999. Chronic toxicity of ammonia to New Zealand freshwater invertebrates: A mesocosm study. Arch. Environ. Contam. Toxicol. 37:338-351.	*Deleatidium sp.* (Ephemeroptera)	Normalized 29-day EC_{25} (survival) = 3.844	Species not resident in North America. These results are not included because they are from a field study where ammonia concentrations were highly variable.
Rice, S.D. and J.E. Bailey. 1980. Survival, size, and emergence of pink salmon, *Oncorhynchus gorbuscha*, alevins after short- and long-term exposures to ammonia. Fish. Bull. 78(3):641-648.	*Oncorhynchus gorbuscha*	Normalized 61-d NOEC = 5.859	Per the 1999 update, the only chronic test began sometime after hatch and ended when the alevins emerged (i.e., at the beginning of swim-up); therefore the test did not include effects of ammonia on the growth and survival of fry after feeding started. In addition, no information was given concerning survival to the end of the test in the control or any other treatment. This test did not provide data concerning survival and is not an ELS test because it began after hatch.
Schulter, M. and J. Groeneweg. 1985. The inhibition by ammonia of population growth of the rotifer, *Brachionus rubens*, in continuous culture. Aquaculture 46: 215-220.	*Brachionus rubens*	Normalized 7-d NOEC = 3.000	Species is not resident in North America. Generally a marine Rotifera. Undescribed culture medium. NOEC based on population growth of cultures.
Smith, C.E. 1972. Effects of metabolic products on the quality of rainbow trout. Am. Fish. Trout News 17:7-8.	*Oncorhynchus mykiss*	Normalized 84-d NOEC = 2.304	This test did not provide data concerning survival and is not an ELS test because it began after hatch. The authors reported that as long as the DO concentration was maintained at 5 mg/L or greater, growth of young rainbow trout was not significantly reduced until average total ammonia concentrations reached 1.6 mg TAN/L at test pH and temperature (7.75 and 10°C, respectively).
Zischke, J.A. and J.W. Arthur. 1987. Effects of elevated ammonia levels on the fingernail clam, *Musculium transversum*, in outdoor experimental streams. Arch. Environ. Contam. Toxicol. 16(2): 225-231.	*Musculium transversum*	Normalized LOEC = 6.933 (survival)	This was a flow-through, measured mesocosm experiment performed in the field. The test concentrations varied during the length of the experiment.

Appendix L. Unused (Non-Influential) Acute and Chronic Studies for Freshwater Ammonia Criteria Development – Screened Out Studies with Code List.
(appears separately at end of appendix)

Appendix L. Unused (Non-influential) Acute and Chronic Studies for Freshwater Ammonia Criteria Development			
Citation	**ECOTOX or Other Ref. No**	**Rejection Code(s)**	**Comment(s)**
Academy of Natural Sciences. 1960. The sensitivity of aquatic life to certain chemicals commonly found in industrial wastes. Final Report No. RG-3965 (C2R1). U.S. Public Health Service Grant, Academy of Natural Sciences, Philadelphia, PA.	5683	AF	
Alabaster, J.S., D.G. Shurben and G. Knowles. 1979. The effect of dissolved oxygen and salinity on the toxicity of ammonia to smolts of salmon, *Salmo salar* L. J. Fish Biol.15(6): 705-712 (Personal Communication Used).	406	Dur - 1d	
Alabaster, J.S., D.G. Shurben and M.J. Mallett. 1983. The acute lethal toxicity of mixtures of cyanide and ammonia to smolts of salmon, *Salmo salar* L. at low concentrations of dissolved oxygen. J. Fish Biol. 22: 215-222.	10252	Dur - 1d	
Alam, M., T.L. Frankel and M. Alam. 2006. Gill ATPase activities of silver perch, *Bidyanus bidyanus* (Mitchell), and golden perch, *Macquaria ambigua* (Richardson): Effects of environmental salt and ammonia. Aquaculture 251(1): 118-133.	84839	NonRes	
Allan, I.R.H., D.W.M. Herbert and J.S. Alabaster. 1958. A field and laboratory investigation of fish in a sewage effluent. Minist. Agric. Fish. Food, Fish. Invest. Ser. 1. 6(2): 76.	10316	AF, Det	
Alonso, A. and J.A. Camargo. 2003. Short-term toxicity to ammonia, nitrite, and nitrate to the aquatic snail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca). Bull. Environ. Contam. Toxicol. 70: 1006-1012		INV	
Alonso, A. and J.A. Camargo. 2006. Ammonia toxicity to the freshwater invertebrates *Polycelis felina* (Planariidae, Turbellaria) and *Echinogammarus echinosetosus* (Gammaridae, Crustacea). Fresenius Environ. Bull. 15(12b): 1578-1583.		NonRes	
Arillo, A., B. Uva and M. Vallarino. 1981. Renin activity in rainbow trout (*Salmo gairdneri* Rich.) and effects of environmental ammonia. Comp. Biochem. Physiol. A 68(3): 307-311.	5704	Dur - 2d	
Armstrong, D.A. 1978. Toxicity and metabolism of nitrogen compounds: Effects on survival, growth and osmoregulation of the prawn, *Macrobrachium rosenbergii*. Ph.D. Thesis, University of California, Davis, CA. (Personal Communication Used).	5620	Dur - 1d	
Bailey, H.C., C. DiGiorgio, K. Kroll, J.L. Miller, D.E. Hinton and G. Starrett. 1996. Development of procedures for identifying pesticide toxicity in ambient waters: Carbofuran, diazinon, chlorpyrifos. Environ. Toxicol. Chem. 15(6): 837-845.	16844	AF	
Ball, I.R. 1967. The relative susceptibilities of some species of fresh-water fish to poisons - I. Ammonia. Water Res. 1(11/12): 767-775.	10000	Dur	
Banerjee, S. and S. Bhattacharya. 1994. Histopathology of kidney of *Channa punctatus* exposed to chronic nonlethal level of elsan, mercury, and ammonia. Ecotoxicol. Environ. Saf. 29(3): 265-275.	13750	NonRes, Eff, UEndp	

Appendix L. Unused (Non-influential) Acute and Chronic Studies for Freshwater Ammonia Criteria Development

Citation	ECOTOX or Other Ref. No	Rejection Code(s)	Comment(s)
Banerjee, S. and S. Bhattacharya. 1995. Histopathological changes induced by chronic nonlethal levels of elsan, mercury, and ammonia in the small intestine of *Channa punctatus* (Bloch). Ecotoxicol. Environ. Saf. 31(1): 62-68.	15256	NonRes, Eff, UEndp	
Banerjee, S. and S. Bhattacharya. 1997. Histopathological changes induced by chronic nonlethal levels of elsan, mercury and ammonia in the liver of *Channa punctatus* (Bloch). J. Environ. Biol. 18(2): 141-148.	18229	NonRes, Eff, UEndp	
Banerjee, T.K. and V.I. Paul. 1993. Estimation of acute toxicity of ammonium sulphate to the fresh water catfish, *Heteropneustes fossilis* II. A histopathological analysis of the epidermis. Biomed. Environ. Sci. 6(1): 45-58.	13480	NonRes, UEndp	
Batley, G.E. and S.L. Simpson. 2009. Development of guidelines for ammonia in estuarine and marine water systems. Mar. Pollut. Bull. 58(10): 1472-1476.		Dilut	Salt water
Bergerhouse, D.L. 1989. Lethal effects of elevated pH and ammonia on early life stages of several sportfish species. Ph.D. Thesis, Southern Illinois University, Carbondale, IL.	3822	UEndp, Dur - 8h	
Bergerhouse, D.L. 1992. Lethal effects of elevated pH and ammonia on early life stages of walleye. N. Am. J. Fish. Manage. 12(2): 356-366.	6903	UEndp, Dur - 8h	
Bergerhouse, D.L. 1993. Lethal effects of elevated pH and ammonia on early life stages of hybrid striped bass. J. Appl. Aquacult. 2(3/4): 81-100.	4290	UEndp, Dur - 8h	
Besser, J.M., W.G. Brumbaugh, A.L. Allert, B.C. Poulton, C.J. Schmitt and C.G. Ingersoll. 2009. Ecological impacts of lead mining on Ozark streams: Toxicity of sediment and pore water. Ecotoxicol. Environ. Saf. 72(2): 516-526.		Tox	
Bhattacharya, T., S. Bhattacharya, A.K. Ray and S. Dey. 1989. Influence of industrial pollutants on thyroid function in *Channa punctatus* (Bloch). Indian J. Exp. Biol. 27(1): 65-68.	3106	NonRes, AF, UEndp, Dur - 1d	
Biswas, J.K., D. Sarkar, P. Chakraborty, J.N. Bhakta and B.B. Jana. 2006. Density dependent ambient ammonium as the key factor for optimization of stocking density of common carp in small holding tanks. Aquaculture 261(3): 952-959.		No Dose, VarExp	Only 1 exposure concentration (naturally increased over time)
Blanco S., S. Romo, M. Fernandez-Alaez and E. Becares. 2008. Response of epiphytic algae to nutrient loading and fish density in a shallow lake: A mesocosm experiment. Hydrobiologia 600(1): 65-76.		Tox	Mesocosm; no ammonia
Boone, M.D., R.D. Semlitsch, E.E. Little and M.C. Doyle. 2007. Multiple stressors in amphibian communities: Effects of chemical contamination, bullfrogs, and fish. Ecol. Appl. 17(1): 291-301.		Tox	
Braun, M.H., S.L. Steele and S.F. Perry. 2009. The responses of zebrafish (*Danio rerio*) to high external ammonia and urea transporter inhibition: Nitrogen excretion and expression of rhesus glycoproteins and urea transporter proteins. J. Exp. Biol. 212(pt. 23): 3846-3856.		NonRes	
Brun, F.G., I. Olive, E.J. Malta, J.J. Vergara, I. Hernandez and J.L. Perez-Llorens. 2008. Increased vulnerability of *Zostera noltii* to stress caused by low light and elevated ammonium levels under phosphate deficiency. Mar. Ecol. Prog. Ser. 365: 67-75.		Tox	

Appendix L. Unused (Non-influential) Acute and Chronic Studies for Freshwater Ammonia Criteria Development

Citation	ECOTOX or Other Ref. No	Rejection Code(s)	Comment(s)
Buikema, A.L., Jr., J. Cairns, Jr. and G.W. Sullivan. 1974. Evaluation of *Philodina acuticornis* (Rotifera) as bioassay organisms for heavy metals. Water Resour. Bull. 10(4): 648-661.	2019	Dur	
Burrows, R.E. 1964. Effects of accumulated excretory products on hatchery-reared salmonids. Res. Rep. No. 66. U.S. Fish Wildl. Serv., Washington, DC.	10002	Uenpd	
Cairns, J., Jr. and A. Scheier. 1959. The relationship of bluegill sunfish body size to tolerance for some common chemicals. Proc. 13th Ind. Waste Conf., Purdue Univ. Eng. Bull. 96: 243-252.	930	AF	
Cairns, J., Jr., B.R. Niederlehner and J.R. Pratt. 1990. Evaluation of joint toxicity of chlorine and ammonia to aquatic communities. Aquat. Toxicol. 16(2): 87-100.	3207	Ace, No Org	
Camargo, J.A. and I. Alonso. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. Environ. Internat. 32(6): 831-849.		Sec	
Cao, T., P. Xie, L. Ni, M. Zhang and J. Xu. Carbon and nitrogen metabolism of an eutrophication tolerative macrophyte, *Potamogeton crispus*, under NH4+ stress and low light availability. Environ. Exper. Bot. In Press, Corrected Proof.		No Dose	Only 1 exposure concentration
Carey, R.O., K.W. Migliaccio and M.T. Brown. 2011. Nutrient discharges to Biscayne Bay, Florida: Trends, loads, and a pollutant index. Sci. Total. Environ. 409(3): 530-539.		No Dose	Fate
Carr, R.S., J.M. Biedenbach and M. Nipper. 2006. Influence of potentially confounding factors on sea urchin porewater toxicity tests. Arch. Environ. Contam. Toxicol. 51(4): 573-579.		Tox	
Centeno, M.D.F., G. Persoone and M.P. Goyvaerts. 1995. Cyst-based toxicity tests. IX. The potential of *Thamnocephalus platyurus* as test species in comparison with *Streptocephalus proboscideus* (Crustacea: Branchiopoda: Anostraca). Environ. Toxicol. Water Qual. 10(4): 275-282.	14017	AF, Dur - 1d	
Chetty, A.N. and K. Indira. 1994. Alterations in the tissue lipid profiles of *Lamellidens marginalis* under ambient ammonia stress. Bull. Environ. Contam. Toxicol. 53(5): 693-698.	13744	NonRes, Dur - 2d	Freshwater bivalve mollusk
Colt, J.E. 1978. The effects of ammonia on the growth of channel catfish, *Ictalurus punctatus*. Ph.D. Thesis, Univ. of California, Davis, CA.	59792	UChron, Sec	Data also published in Colt and Tchobanoglous (1978)
Corpron, K.E. and D.A. Armstrong. 1983. Removal of nitrogen by an aquatic plant, *Elodea densa*, in recirculating macrobrachium culture systems. Aquaculture 32(3/4): 347-360.	15323	UEndp, Con	Plant
Craig, G.R. 1983. Interlaboratory fish toxicity test comparison - Ammonia. Environ. Protection Service, Quality Protection Section, Water Resour. Branch, Canada.	10259	AF	
Cucchiari, E., F. Guerrini, A. Penna, C. Totti and R. Pistocchi. 2008. Effect of salinity, temperature, organic and inorganic nutrients on growth of cultured *Fibrocapsa japonica* (Raphidophyceae) from the northern Adriatic Sea. Harmful Algae 7(4): 405-414.		Tox	

Appendix L. Unused (Non-influential) Acute and Chronic Studies for Freshwater Ammonia Criteria Development			
Citation	**ECOTOX or Other Ref. No**	**Rejection Code(s)**	**Comment(s)**
Da Silva, J.M., J. Coimbra and J.M. Wilson. 2009. Ammonia sensitivity of the glass eel (*Anguilla anguilla* L.): Salinity dependence and the role of a branchial sodium/potassium adenosine triphosphatase. Environ. Toxicol. Chem. 28(1): 141-147.		NonRes	
Dabrowska, H. and H. Sikora. 1986. Acute toxicity of ammonia to common carp (*Cyprinus carpio* L.). Pol. Arch. Hydrobiol. 33(1): 121-128.	12711	Dur - 2d	
Danecker, E. 1964. The jauche poisoning of fish - An ammonia poisoning. Osterreichs Fischerei. 3/4: 55-68 (ENG TRANSL).	10305	AF, UEndp, Dur	
Daniels, S.M., M.G. Evans , C.T. Agnew and T.E.H. Allott. 2012. Ammonium release from a blanket peatland into headwater stream systems. Environ. Pollut. 163(0): 261-272.		No Dose	Fate
Daoust, P.Y. and H.W. Ferguson. 1984. The pathology of chronic ammonia toxicity in rainbow trout, *Salmo gairdneri* Richardson. J. Fish Dis. 7: 199-205.	10217	UEndp, Eff	
Dayeh, V.R., K. Schirmer and N.C. Bols. 2009. Ammonia-containing industrial effluents, lethal to rainbow trout, induce vacuolization and neutral red uptake in the rainbow trout gill cell line, RTgill-W1. Altern. Lab. Anim. 37(1): 77-87.		In Vit	
De Moor, I.J. 1984. The toxic concentration of free ammonia to *Brachionus calyciflorus* Pallas, a rotifer pest species found in high rate algal ponds (HRAP'S). J. Limnol. Soc. South Afr. 10(2): 33-36.	5433	UEndp	
Dendene, M.A., T. Rolland, M. Tremolieres and R. Carbiener. 1993. Effect of ammonium ions on the net photosynthesis of three species of elodea. Aquat. Bot. 46(3/4): 301-315.	4268	UEndp	Plant
Dey, S. and S. Bhattacharya. 1989. Ovarian damage to *Channa punctatus* after chronic exposure to low concentrations of elsan, mercury, and ammonia. Ecotoxicol. Environ. Saf. 17(2): 247-257.	446	AF, Dur - 2d	
DeYoe H.R., E.J. Buskey and F.J. Jochem. 2007. Physiological responses of *Aureoumbra lagunensis* and *Synechococcus* sp. to nitrogen addition in a mesocosm experiment. Harmful Algae 6(1): 48-55.		No Dose	Only one exposure concentration
Dhanasiri, A.K., V. Kiron, J.M. Fernandes, O. Bergh and M.D. Powell. Novel application of nitrifying bacterial consortia to ease ammonia toxicity in ornamental fish transport units: Trials with zebrafish. J. Appl. Microbiol. 111(2): 278-292.		UEndp	
Diamond, J.M., S.J. Klaine and J.B. Butcher. 2006. Implications of pulsed chemical exposures for aquatic life criteria and wastewater permit limits. Environ. Sci. Technol. 40(16): 5132-5138.	102216	No Dose, Dur, VarExp	Only 2 exposure concentrations
dos Miron, D., B. Moraes, A.G. Becker, M. Crestani, R. Spanevello, V.L. Loro and B. Baldisserotto. 2008. Ammonia and pH effects on some metabolic parameters and gill histology of silver catfish, *Rhamdia quelen* (Heptapteridae). Aquaculture 277(3-4): 192-196.		NonRes	
Dowden, B.F. and H.J. Bennett. 1965. Toxicity of selected chemicals to certain animals. J. Water Pollut. Control Fed. 37(9): 1308-1316.	915	AF	

Appendix L. Unused (Non-influential) Acute and Chronic Studies for Freshwater Ammonia Criteria Development			
Citation	**ECOTOX or Other Ref. No**	**Rejection Code(s)**	**Comment(s)**
Dowden, B.F. 1961. Cumulative toxicities of some inorganic salts to *Daphnia magna* as determined by median tolerance limits. Proc. LA. Acad. Sci. 23: 77-85.	2465	AF	
Drath, M., N. Kloft, A. Batschauer, K. Marin, J. Novak and K. Forchhammer. 2008. Ammonia triggers photodamage of photosystem II in the cyanobacterium *Synechocystis* sp. strain Pcc 6803. Plant Physiol. 147(1): 206-215.		No Dose	Only 1 or 2 exposure concentrations at a specific pH
D'Silva, C. and X.N. Verlencar. 1976. Relative toxicity of two ammonium compounds found in the waste of fertilizer plants. Mahasagar 9(1/2): 41-44.	6084	Dur - 2d	
Egea-Serrano, A., M. Tejedo and M. Torralva. 2008. Analysis of the avoidance of nitrogen fertilizers in the water column by juvenile Iberian water frog, *Pelophylax perezi* (Seoane, 1885), in laboratory conditions. Bull. Environ. Contam. Toxicol. 80(2): 178-183.	103070	NonRes, Tox, No Dose	Only one exposure concentration
Fairchild II, E.J. 1954. Effects of lowered oxygen tension on the susceptibility of *Daphnia magna* to certain inorganic salts. Ph.D. Thesis, Louisiana State Univ., LA. 134 p.		Dilut, Dur, AF	
Fairchild II, E.J. 1955. Low dissolved oxygen: Effect upon the toxicity of certain inorganic salts to the aquatic invertebrate *Daphnia magna*. In: Proc. 4th Ann. Water Symp., March 1955, Baton Rouge, LA, Eng. Expt. Stat. Bull. 51: 95-102.	115940	Dilut, Dur, AF	
Fang, J.K.H., R.S.S. Wu, A.K.Y. Chan, C.K.M. Yip and P.K.S. Shin. 2008. Influences of ammonia-nitrogen and dissolved oxygen on lysosomal integrity in green-lipped mussel *Perna viridis*: Laboratory evaluation and field validation in Victoria Harbour, Hong Kong. Mar. Pollut. Bull. 56(12): 2052-2058.		No Dose	Only one exposure concentration
Fedorov, K.Y. and Z.V. Smirnova. 1978. Dynamics of ammonia accumulation and its effect on the development of the pink salmon, *Oncorhynchus gorbuscha*, in closed circuit incubation systems. Vopr. Ikhtiol. 19(2): 320-328.	5478	UEndp	
Flagg, R.M. and L.W. Hinck. 1978. Influence of ammonia on aeromonad susceptibility in channel catfish. Proc. Annu. Conf. Southeast. Assoc. Fish Wildl. Agencies 32: 415-419.	10317	UEndp	
Flis, J. 1963. Anatomicohistopathological changes induced in carp (*Cyprinus carpio* L.) by ammonia water. Part 1. Effects of toxic concentrations. Zmiany. Acta Hydrobiol. 10(1/2): 205-224.	10005	UEndp, Dur - 1d	
Foss, A., A.K. Imsland, B. Roth, E. Schram and S.O. Stefansson. 2007. Interactive effects of oxygen saturation and ammonia on growth and blood physiology in juvenile turbot. Aquaculture 271(1-4): 244-251.		No Dose	Only 2 exposure concentrations
Foss, A., A.K. Imsland, B. Roth, E. Schram and S.O. Stefansson. 2009. Effects of chronic and periodic exposure to ammonia on growth and blood physiology in juvenile turbot (*Scophthalmus maximus*). Aquaculture 296(1/2): 45-50.		NonRes	
Ge, F., Y. Xu, R. Zhu, F. Yu, M. Zhu and M. Wong. 2010. Joint action of binary mixtures of cetyltrimethyl ammonium chloride and aromatic hydrocarbons on *Chlorella vulgaris*. Ecotoxicol. Environ. Saf. 73(7): 1689-1695.		Tox	
Gohar, H.A.F. and H. El-Gindy. 1961. Tolerance of vector snails of bilharziasis and fascioliasis to some chemicals. Proc. Egypt. Acad. Sci. 16: 37-48.	115940	NonRes	

Appendix L. Unused (Non-influential) Acute and Chronic Studies for Freshwater Ammonia Criteria Development			
Citation	**ECOTOX or Other Ref. No**	**Rejection Code(s)**	**Comment(s)**
Golding, C., R. Krassoi and E. Baker. 2006. The development and application of a marine Toxicity Identification Evaluation (TIE) protocol for use with an Australian bivalve. Australas. J. Ecotoxicol. 12(1): 37-44.	108468	Tox, No Dose	Only one exposure concentration
Goncalves, A.F., I. Pascoa, J.V. Neves, J. Coimbra, M.M. Vijayan, P. Rodrigues and J.M Wilson. 2012. The inhibitory effect of environmental ammonia on *Danio rerio* LPS induced acute phase response. Dev. Comp. Immunol. 36(2): 279-288.		NonRes	
Griffis-Kyle, K.L. and M.E. Ritchie. 2007. Amphibian survival, growth and development in response to mineral nitrogen exposure and predator cues in the field: An experimental approach. Oecologia 152(4): 633-42.		Tox	
Gyore, K. and J. Olah. 1980. Ammonia tolerance of *Moina rectirostris* Leydig (Cladocera). Aquacult. Hung. (Szarvas) 2: 50-54.	5708	Dur - 1d	
Hanna, T.D. 1992. The effect of oxygen supplementation on the toxicity of ammonia (NH3) in rainbow trout *Oncorhynchus mykiss* (Richardson). M.S. Thesis, Montana State Univ., Bozeman, MT.	7823	UEndp, Dur	
Harader, R.R.J. and G.H. Allen. 1983. Ammonia toxicity to Chinook salmon Parr: Reduction in saline water. Trans. Am. Fish. Soc. 112(6): 834-837.	10510	Dur - 1d	
Healey, F.P. 1977. Ammonium and urea uptake by some freshwater algae. Can. J. Bot. 55(1): 61-69.	7486	AF, Uendp	Plant
Hedtke, J.L. and L.A. Norris. 1980. Effect of ammonium chloride on predatory consumption rates of brook trout (*Salvelinus fontinalis*) on juvenile Chinook salmon (*Oncorhynchus tshawytscha*) I. Bull. Environ. Contam. Toxicol. 24(1): 81-89.	6216	UEndp, Eff	
Hemens, J. 1966. The toxicity of ammonia solutions to the mosquito fish (*Gambusia affinis* Baird & Girard). J. Proc. Inst. Sewage Purif. 3: 265-271.	10152	Dur - 17h	
Henderson, C., Q.H. Pickering and A.E. Lemke. 1961. The effect of some organic cyanides (nitriles) on fish. Proc. 15th Ind. Waste Conf., Eng. Bull. Purdue Univ., Ser. No.106, 65(2): 120-130.	923	Tox; AF	
Herbert, D.W.M. and D.S. Shurben. 1963. A preliminary study of the effect of physical activity on the resistance of rainbow trout (*Salmo gairdnerii* Richardson) to two poisons. Ann. Appl. Biol. 52: 321-326.	8005	Dur - 1d	
Herbert, D.W.M. and D.S. Shurben. 1964. The toxicity to fish of mixtures of poisons I. Salts of ammonia and zinc. Ann. Appl. Biol. 53: 33-41.	8006	Dur - 2d	
Herbert, D.W.M. and D.S. Shurben. 1965. The susceptibility of salmonid fish to poisons under estuarine conditions – II. Ammonium chloride. Int. J. Air Water Pollut. 9(1/2): 89-91.	10318	Dur - 1d	
Herbert, D.W.M. and J.M. Vandyke. 1964. The toxicity to fish of mixtures of poisons. II. Copper-ammonia and zinc-phenol mixtures. Ann. Appl. Biol. 53(3): 415-421.	10193	Tox; Dur - 2d	
Hernandez, C., M. Martin, G. Bodega, I. Suarez, J. Perez and B. Fernandez. 1999. Response of carp central nervous system to hyperammonemic conditions: An immunocytochemical study of glutamine synthetase (GS), glial fibrillary acidic protein (GFAP) and 70 kDa heat-shock protein (HSP70). Aquat. Toxicol. 45(2/3): 195-207.	19920	UEndp, Eff	

Appendix L. Unused (Non-influential) Acute and Chronic Studies for Freshwater Ammonia Criteria Development			
Citation	**ECOTOX or Other Ref. No**	**Rejection Code(s)**	**Comment(s)**
Hiatt, R.W., J.J. Naughton and D.C. Matthews. 1953. Effects of chemicals on a schooling fish, *Kulia sandvicensis*. Biol. Bull. 104: 28-44.		NonRes	
Holland, G.A., J.E. Lasater, E.D. Neumann and W.E. Eldridge. 1960. Toxic effects of organic and inorganic pollutants on young salmon and trout. Res. Bull. No. 5. State of Washington Dept. Fish., Seattle, WA.	14397	Dur - 3d	
Hong, M., L. Chen, X. Sun, S. Gu, L. Zhang and Y. Chen. 2007. Metabolic and immune responses in Chinese mitten-handed crab (*Eriocheir sinensis*) juveniles exposed to elevated ambient ammonia. Comp. Biochem. Physiol. C 145(3): 363-369.		INV, Det	Dilution water not described; Prior exposure?
Hued, A.C., M.N. Caruso, D.A. Wunderlin and M.A. Bistoni. 2006. Field and *in vitro* evaluation of ammonia toxicity on native fish species of the central region of Argentina. Bull. Environ. Contam. Toxicol. 76(6): 984-991.		NonRes	
Hurlimann, J. and F. Schanz. 1993. The effects of artificial ammonium enhancement on riverine periphytic diatom communities. Aquat. Sci. 55(1): 40-64.	4134	No Org	Periphytic community
Ingersoll, C. 2004. Memo summarizing ammonia toxicity data for freshwater mussels generated by the USGS Columbia Environmental Research Center in 2003 and 2004. Memorandum, USGS Columbia Environmental Research Center, Columbia, MO, 13 p.		Sec	
Ingersoll, C.G., N.E. Kemble, J.L. Kunz, W.G. Brumbaugh, D.D. MacDonald and D. Smorong. 2009. Toxicity of sediment cores collected from the Astabula River in Northeastern Ohio, USA, to the amphipod *Hyalella azteca*. Arch. Environ. Contam. Toxicol. 57(2): 315-329.		SedExp	
Inman, R.C. 1974. Acute toxicity of Phos-Check (trade name) 202 and diammonium phosphate to fathead minnows. U.S. NTIS AD/A-006122. Environ. Health Lab., Kelly Air Force Base, TX.	6010	Tox	
Ip, Y.K., A.S.L. Tay, K.H. Lee and S.F. Chew. 2004. Strategies for surviving high concentrations of environmental ammonia in the swamp eel *Monopterus albus*. Physiol. Biochem. Zool. 77: 390-405.		INV	
Ip, Y.K., S.M.L. Lee, W.P. Wong and S.F. Chew. 2008. Mechanisms of and defense against acute ammonia toxicity in the aquatic Chinese soft-shelled turtle, *Pelodiscus sinensis*. Aquat. Toxicol. 86(2): 185-196.		NonRes, RouExp	Injected
Ishio, S. 1965. Behavior of fish exposed to toxic substances. In: Advances in Water Pollution Research. Jaag, O. (Ed.). Pergamon Press, NY. pp.19-40.	14092	AF, Dur - 6h, UEndp, No Org	
James, R., K. Sampath and M. Narayanan. 1993. Effect of sublethal concentrations of ammonia on food intake and growth in *Mystus vittatus*. J. Environ. Biol. 14(3): 243-248.	8994	NonRes, AF, UEndp	
Jampeetong, A. and H. Brix. Effects of NH4+ concentration on growth, morphology and NH4+ uptake kinetics of *Salvinia natans*. Ecol. Engineer. In Press, Corrected Proof.		VarExp	Concentration increased over time
Jampeetong, A., H. Brix and S. Kantawanichkul. 2012. Response of *Salvinia cucullata* to high NH4(+) concentrations at laboratory scales. Ecotoxicol. Environ. Saf. 79: 69-74.		NonRes, Con	

Appendix L. Unused (Non-influential) Acute and Chronic Studies for Freshwater Ammonia Criteria Development			
Citation	**ECOTOX or Other Ref. No**	**Rejection Code(s)**	**Comment(s)**
Jampeetong, A., H. Brix and S. Kantawanichkul. 2012. Effects of inorganic nitrogen forms on growth, morphology, nitrogen uptake capacity and nutrient allocation of four tropical aquatic macrophytes (*Salvinia cucullata*, *Ipomoea aquatica*, *Cyperus involucratus* and *Vetiveria zizanioides*). Aquatic Botany 97(1): 10-16.		NonRes, No Dose	Plant
Jensen, R.A. 1978. A simplified bioassay using finfish for estimating potential spill damage. In: Proc. Control of Hazardous Material Spills. Rockville, MD. pp. 104-108.	5773	AF, Dur - 1d	
Jha, B.K. and B.S. Jha. 1995. Urea and ammonium sulfate induced changes in the stomach of the fish *Heteropneustes fossilis*. Environ. Ecol. 13(1): 179-181.	17562	NonRes, AF, UEndp	
Joy, K.P. 1977. Ammonium sulphate as a thyroid inhibitor in the freshwater teleost *Clarias batrachus* (L.). Curr. Sci. 46(19): 671-673.	7513	NonRes, AF, UEndp	
Kawabata, Z., T. Yoshida and H. Nakagawa. 1997. Effect of ammonia on the survival of *Zacco platypus* (Temminck and Schlegel) at each developmental stage. Environ. Pollut. 95(2): 213-218.	17963	NonRes, UEndp, Dur	
Khatami, S.H., D. Pascoe and M.A. Learner. 1998. The acute toxicity of phenol and unionized ammonia, separately and together, to the ephemeropteran *Baetis rhodani* (Pictet). Environ. Pollut. 99: 379-387.	19651	NonRes, Dur - 1d	
Kim, J.K., G.P. Kraemer, C.D. Neefus, I.K. Chung and C. Yarish. 2007. Effects of temperature and ammonium on growth, pigment production and nitrogen uptake by four species of porphyra (Bangiales, Rhodophyta) native to the New England Coast. J. App. Phycol. 19(5): 431-440.		UEndp	Plant
Kirk, R.S. and J.W. Lewis. 1993. An evaluation of pollutant induced changes in the gills of rainbow trout using scanning electron microscopy. Environ. Technol. 14(6): 577-585.	4931	UEndp, Dur	
Knepp, G.L. and G.F. Arkin. 1973. Ammonia toxicity levels and nitrate tolerance of channel catfish. Prog. Fish Cult. 35(4): 221-224.	8606	Dur - 7d, Form	
Konnerup, D. and H. Brix. 2010. Nitrogen nutrition of *Canna indica*: Effects of ammonium versus nitrate on growth, biomass allocation, photosynthesis, nitrate reductase activity and N uptake rates. Aquatic Botany 92(2): 142-148.		NonRes	Plant
Krainara, T. 1988. Effects of ammonia on walking catfish, *Clarias batrachus* (Linnaeus). Abstr. M.S. Thesis, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand 13:6.	17533	NonRes, AF	
Kulkarni, K.M. and S.V. Kamath. 1980. The metabolic response of *Paratelphusa jacquemontii* to some pollutants. Geobios 7(2): 70-73 (Author Communication Used).	5036	NonRes, AF, UEndp, Dur	
Kwok, K.W.H., K.M. Y Leung, G.S.G. Lui, V.K.H. Chu, P.K. S. Lam, D. Morritt, L. Maltby, T.C.M. Brock, P.J. Van den Brink, M.S.J. Warne and M. Crane. 2007. Comparison of tropical and temperate freshwater animal species‘ acute sensitivities to chemical: Implications for deriving safe extrapolation factors. Integr. Environ. Assess. Manag. 3(1): 49-67.		Sec	
Lang, T., G. Peters, R. Hoffmann and E. Meyer. 1987. Experimental investigations on the toxicity of ammonia: Effects on ventilation frequency, growth, epidermal mucous cells, and gill structure of rainbow trout. Dis. Aquat. Org. 3: 159-165.	4106	UEndp	

Appendix L. Unused (Non-influential) Acute and Chronic Studies for Freshwater Ammonia Criteria Development			
Citation	**ECOTOX or Other Ref. No**	**Rejection Code(s)**	**Comment(s)**
Larson J.H., P.C. Frost and G.A. Lamberti. 2008. Variable toxicity of ionic liquid-forming chemicals to *Lemna minor* and the influence of dissolved organic matter. Environ. Toxicol. Chem. 27(3): 676-681.		Tox	Plant
Lay, J.P., A. Peither, I. Juttner and K. Weiss. 1993. *In situ* pond mesocosms for ecotoxicological long-term studies. Chemosphere 26(6): 1137-1150.	7048	No Org	
Lazorchak, J.M. and M.E. Smith. 2007. Rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) 7-day survival and growth test method. Arch. Environ. Contam. Toxicol. 53(3): 397-405.	100026	Det, AF	7-day tests (S,U) with ammonia chloride; pH not reported
Lee, D.R. 1976. Development of an invertebrate bioassay to screen petroleum refinery effluents discharged into freshwater. Ph.D. Thesis, Virginia Polytechnic Inst. and State University, Blacksburg, VA.	3402	Det	This thesis appears to provide appropriate 48 h LC_{50} data for *D. pulex*, but details are lacking.
Leung, J., M. Kumar, P. Glatz and K. Kind. 2011. Impacts of unionized ammonia in digested piggery effluent on reproductive performance and longevity of *Daphnia carinata* and *Moina australiensis*. 310: 401-406.		NonRes, Efflu	
Lewis, J.W., A.N. Kay and N.S. Hanna. 1995. Responses of electric fish (family Mormyridae) to inorganic nutrients and tributyltin oxide. Chemosphere 31(7): 3753-3769.	16156	NonRes, UEndp, Dur	
Li, W.E.I., Z. Zhang and E. Jeppesen. 2008. The response of *Vallisneria spinulosa* (Hydrocharitaceae) to different loadings of ammonia and nitrate at moderate phosphorus concentration: A mesocosm approach. Freshw. Biol. 53(11): 2321-2330.		Tox	Plant
Linton, T.K., I.J. Morgan, P.J. Walsh and C.M. Wood. 1998. Chronic exposure of rainbow trout (*Oncorhynchus mykiss*) to simulated climate warming and sublethal ammonia: A year-long study of their appetite. Can. J. Fish. Aquat. Sci. 55(3): 576-586.	19144	No Dose	Only one exposure concentration
Litav, M. and Y. Lehrer. 1978. The effects of ammonium in water on *Potamogeton lucens*. Aquat. Bot. 5(2): 127-138.	7093	AF, UEndp, Dur	Plant
Lloyd, R. and D.W.M. Herbert. 1960. The influence of carbon dioxide on the toxicity of unionized ammonia to rainbow trout (*Salmo gairdnerii* Richardson). Ann. Appl. Biol. 48(2): 399-404.	10018	Dur - 8h	
Lloyd, R. and L.D. Orr. 1969. The diuretic response by rainbow trout to sublethal concentrations of ammonia. Water Res. 3(5): 335-344.	10019	Eff, UEndp, Dur - 1d	
Loong, A.M., J.Y.L. Tan, W.P. Wong, S.F. Chew and Y.K. Ip. 2007. Defense against environmental ammonia toxicity in the African lungfish, *Protopterus aethiopicus*: Bimodal breathing, skin ammonia permeability and urea synthesis. Aquat. Toxicol. 85(1): 76-86.		NonRes, No Dose	Only one exposure concentration
Loon, A.M., Y.R. Chng, S.F. Chew, W.P. Wong and Y.K. Ip. 2012. Molecular characterization and MRNA expression of carbamoyl phosphate synthetase III in the liver of the African lungish, *Protopterus annectens*, during aestivation of exposure to ammonia. J. Comp. Physiol. B. 182(3): 367-379.		NonRes	
Loppes, R. 1970. Growth inhibition by NH4+ ions in arginine-requiring mutants of *Chlamydomonas reinhardi*. Mol. Gen. Genet. 109(3): 233-240.	9619	AF, UEndp, Dur	Plant

Appendix L. Unused (Non-influential) Acute and Chronic Studies for Freshwater Ammonia Criteria Development

Citation	ECOTOX or Other Ref. No	Rejection Code(s)	Comment(s)
Magalhaes Bastos, J.A. 1954. Importance of ammonia as an ichthyotoxic substance. (Importancia da amonia como substacia ictiotoxica.). No.159, Serv. Piscicultura, Publ. Ser. 1-C, Dep. Nacl. Onbras Contra Secas, Ministerio Viacao E Onbras Publicas, Brazil. pp. 115-132.	10302	UEndp, Dur	
Malacea, I. 1966. Studies on the acclimation of fish to high concentrations of toxic substances. Arch. Hydrobiol. 65(1): 74-95 (GER) (ENG TRANSL).	10020	Dur	
Manissery, J.K. and M.N. Madhyastha. 1993. Hematological and histopathological effect of ammonia at sublethal levels on fingerlings of common carp *Cyprinus carpio*. Sci. Total Environ. (Suppl .): 913-920.	4314	Eff, UEndp	
McDonald, S.F., S.J. Hamilton, K.J. Buhl and J.F. Heisinger. 1997. Acute toxicity of fire-retardant and foam-suppressant chemicals to *Hyalella azteca* (Saussure). Environ. Toxicol. Chem. 16(7): 1370-1376.	18102	Tox	
McIntyre, M., M. Davis and A. Shawl. 2006. The effects of ammonia on the development, survival and metamorphic success of *Strombus gigas* veligers. 98th Annu. Meet. Natl. Shellfish. Assoc., Monterery, CA (ABS).		Det	Abstract only
Meador, M.R. and D.M. Carlisle. 2007. Quantifying tolerance indicator values for common stream fish species of the United States. Ecol. Indic. 7(2): 329-338.		Tox	
Melching, C.S., V. Novotny, J.B. Schilling, J. Chen and M. B. Beck. (Eds). 2006. Probabilistic evaluation of ammonia toxicity in Milwaukee's Outer Harbor. Alliance House. IWA Publishing, London, UK.		Tox	
Merkens, J.C. and K.M. Downing. 1957. The effect of tension of dissolved oxygen on the toxicity of unionized ammonia to several species of fish. Ann. Appl. Biol. 45(3): 521-527.	10021	UEndp, Dur	
Merkens, J.C. 1958. Studies on the toxicity of chlorine and chloramines to the rainbow trout. Water Waste Treat. J. 7: 150-151.	7404	UEndp, Dur	
Mitchell, S.J., Jr. 1983. Ammonia-caused gill damage in channel catfish (*Ictalurus punctatus*): Confounding effects of residual chlorine. Can. J. Fish. Aquat. Sci. 40(2): 242-247.	10543	UEndp, Dur	
Morris, J.M., E. Snyder-Conn, J.S. Foott, R.A. Holt, M.J. Suedkamp, H.M. Lease, S.J. Clearwater and J.S. Meyer. 2006. Survival of Lost River suckers (*Deltistes luxatus*) challenged with *Flavobacterium columnare* during exposure to sublethal ammonia concentrations at pH 9.5. Arch. Environ. Contam. Toxicol. 50(2): 256-263.	97379	WatQual	
Mosier, A.R. 1978. Inhibition of photosynthesis and nitrogen fixation in algae by volatile nitrogen bases. J. Environ. Qual. 7(2): 237-240.	15860	Dur	Plant
Mukherjee, S. and S. Bhattacharya. 1974. Effects of some industrial pollutants on fish brain cholinesterase activity. Environ. Physiol. Biochem. 4: 226-231.	668	Eff. Dur - 2d	
Muturi E.J., B.G. Jacob, J. Shililu and R. Novak. 2007. Laboratory studies on the effect of inorganic fertilizers on survival and development of immature *Culex quinquefasciatus* (Diptera: Culicidae). J Vector Borne Dis. 44(4): 259-65.		Dilut	Deionized water

Appendix L. Unused (Non-influential) Acute and Chronic Studies for Freshwater Ammonia Criteria Development			
Citation	**ECOTOX or Other Ref. No**	**Rejection Code(s)**	**Comment(s)**
Nawata, C.M. and C.M. Wood. 2009. MRNA expression analysis of the physiological responses to ammonia infusion in rainbow trout. J. Comp. Physiol. B. 179(7): 799-810.		RouExp	
Nimptsch, J. and S. Pflugmacher. 2007. Ammonia triggers the promotion of oxidative stress in the aquatic macrophyte *Myriophyllum mattogrossense*. Chemosphere 66(4): 708-714.	100651	NonRes	Plant
Obiekezie, A.I. and P.O. Ajah. 1994. Chemotherapy of macrogyrodactylosis in the culture of African clariid catfishes *Clarias gariepinus* and *Heterobranchus longifiliis*. J. Aquacult. Trop. 9(3): 187-192.	16594	NonRes	
Ohmori, M., K. Ohmori and H. Strotmann. 1977. Inhibition of nitrate uptake by ammonia in a blue-green alga, *Anabaena cylindrica*. Arch. Microbiol. 114(3): 225-229.	7605	UEndp, Dur	Plant
Okelsrud, A. and R.G. Pearson. 2007. Acute and postexposure effects of ammonia toxicity on juvenile barramundi (*Lates calcarifer*). Arch. Environ. Contam. Toxicol. 53(4): 624-631.		NonRes	
Olson, K.R., and P.O. Fromm. 1971. Excretion of urea by two teleosts exposed to different concentrations of ambient ammonia. Comp. Biochem. Physiol. A 40:999-1007.	10243	Eff, AF, UEndp, Dur - 1d	
Oromi, N. D. Sanuy and M. Vilches. 2009. Effects of nitrate and ammonium on larvae of *Rana temporaria* from the Pyrenees. Bull. Environ. Contam. Toxicol. 82(5): 534-537.		NonRes	
Ortiz-Santaliestra, M. E., A. Marco, M.J. Fernandez and M. Lizana. 2006. Influence of developmental stage on sensitivity to ammonium nitrate of aquatic stages of amphibians. Environ. Toxicol. Chem. 25(1): 105-111.		NonRes	
Ortiz-Santaliestra, M. E., A. Marco, M.J. Fernandez and M. Lizana. 2007. Effects of ammonium nitrate exposure and water acidification on the dwarf newt: The protective effect of oviposition behaviour on embryonic survival. Aquat. Toxicol. 85(4): 251-257.		NonRes	
Pagliarani, A., P. Bandiera, V. Ventrella, F. Trombetti, M.P. Manuzzi, M. Pirini and A.R. Borgatti. 2008. Response of Na+-dependent ATPase activities to the contaminant ammonia nitrogen in *Tapes philippinarum*: Possible ATPase involvement in ammonium transport. Arch. Environ. Contam. Toxicol. 55(1): 49-56.		No Dose	Only 2 exposure concentrations
Palanichamy, S., S. Arunachalam and M.P. Balasubramanian. 1985. Food consumption of *Sarotherodon mossambicus* (Trewaves) exposed to sublethal concentration of diammonium phosphate. Hydrobiologia 128(3): 233-237.	11516	NonRes, AF, UEndp, Dur	
Palanisamy, R. and G. Kalaiselvi. 1992. Acute toxicity of agricultural fertilizers to fish *Labeo rohita*. Environ. Ecol. 10(4): 869-873.	8278	NonRes, AF, Dur	
Paley, R.K., I.D. Twitchen and F.B. Eddy. 1993. Ammonia, Na+, K+ and Cl- levels in rainbow trout yolk-sac fry in response to external ammonia. J. Exp. Biol.180:273-284.	7746	Eff, UEndp, Dur - 1d	
Pascoa, I., A. Fontainhas-Fernandes and J. Wilson. 2008. Ammonia tolerance in the zebrafish (*Danio rerio*): Effects of ionic strength and ontogeny: Abstracts of the Annual Main Meeting of the Society of Experimental Biology, 6th-10th July 2008, Marseille, France. Comp. Biochem. Physiol. A: Mol. Integr. Physiol. 150(3 Supp.): 106-107.		Det, INV	

Appendix L. Unused (Non-influential) Acute and Chronic Studies for Freshwater Ammonia Criteria Development			
Citation	**ECOTOX or Other Ref. No**	**Rejection Code(s)**	**Comment(s)**
Patrick, R., J. Cairns, Jr. and A. Scheier. 1968. The relative sensitivity of diatoms, snails, and fish to twenty common constituents of industrial wastes. Prog. Fish-Cult. 30(3): 137-140 (Author Communication Used) (Publ in Part As 2406).	949	AF	
Paul, V.I. and T.K. Banerjee. 1995. Acute toxicity of ammonium sulphate to the air-breathing organ of the live fish *Heteropneustes (Saccobranchus) fossilis* (Bloch). Curr. Sci. 68(8): 845-849.	19532	NonRes, UEndp, Dur	
Penaz, M. 1965. The influence of ammonia on the eggs and young of the common trout, *Salmo trutta var. fario*. Zool. Listy 14(1): 47-54.	10307	UEndp, Dur	
Phillips, B.M., B.S. Anderson, J.W. Hunt, S.L. Clark, J.P. Voorhees, R.S. Tjeerdema, J. Casteline and M. Stewart. 2009. Evaluation of phase II toxicity identification evaluation methods for freshwater whole sediment and interstitial water. Chemosphere. 74(5): 648-653.		Tox	
Puchaslski, M.A., M.E. Sather, J.T. Walker, C.M. Lehmann, D.A. Gay, J. Mathew and W.P. Robarge. 2011. Passive ammonia monitoring in the United States: Comparing three different sampling devices. J. Environ. Monit. 13(11): 3156-3167.		No Dose	Fate
Puglis H.J. and M.D. Boone. 2007. Effects of a fertilizer, an insecticide, and a pathogenic fungus on hatching and survival of bullfrog (*Rana catesbeiana*) tadpoles. Environ. Toxicol. Chem. 26(10): 2198-2201.		Tox	
Ram, R. and A.G. Sathyanesan. 1987. Effect of chronic exposure of commercial nitrogenous fertilizer, ammonium sulfate, on testicular development of a teleost *Channa punctatus* (Bloch). Indian J. Exp. Biol. 25(10): 667-670.	24	NonRes, AF, UEndp, Dur	
Ram, R.N. and A.G. Sathyanesan. 1986. Ammonium sulfate induced nuclear changes in the oocyte of the fish, *Channa punctatus* (Bl.). Bull. Environ. Contam. Toxicol. 36(6): 871-875.	11793	NonRes, AF, UEndp, Dur	
Ram, R.N. and A.G. Sathyanesan. 1986. Inclusion bodies: Formation and degeneration of the oocytes in the fish *Channa punctatus* (Bloch) in response to ammonium sulfate treatment. Ecotoxicol. Environ. Saf. 11(3): 272-276.	12428	NonRes, AF, UEndp, Dur	
Ram, R.N. and A.G. Sathyanesan. 1987. Histopathological changes in liver and thyroid of the teleost fish, *Channa punctatus* (Bloch), in response to ammonium sulfate fertilizer treatment. Ecotoxicol. Environ. Saf. 13(2): 185-190.	12684	NonRes, AF, UEndp, Dur	
Ram, R.N. and S.K. Singh. 1988. Long-term effect of ammonium sulfate fertilizer on histophysiology of adrenal in the teleost, *Channa punctatus* (Bloch). Bull. Environ. Contam. Toxicol. 41(6): 880-887.	2649	NonRes, AF, UEndp, Dur	
Ramachandran, V. 1960. Observations on the use of ammonia for the eradication of aquatic vegetation. J. Sci. Ind. Res.19C: 284-285; Chem. Abstr. 55 (1961).	626	AF, UEndp, Dur	Plant
Rani, E.F., M. Elumalal and M.P. Balasubramanian. 1998. Toxic and sublethal effects of ammonium chloride on a freshwater fish *Oreochromis mossambicus*. Water Air Soil Pollut. 104(1/2): 1-8.	19157	UChron	
Rao, V.N.R. and G. Ragothaman. 1978. Studies on *Amphora coffeaeformis* II. Inorganic and organic nitrogen and phosphorus sources for growth. Acta Bot. Indica 6(Supp l): 146-154.	5449	AF, UEndp, Dur	Plant

Appendix L. Unused (Non-influential) Acute and Chronic Studies for Freshwater Ammonia Criteria Development			
Citation	**ECOTOX or Other Ref. No**	**Rejection Code(s)**	**Comment(s)**
Redner, B.D. and R.R. Stickney. 1979. Acclimation to ammonia by *Tilapia aurea*. Trans. Am. Fish. Soc. 108: 383-388.	2561	NonRes, Dur - 3d	
Redner, B.D., J.R. Tomasso and B.A. Simco. 1980. Short term alleviation of ammonia toxicity by environmental sodium chloride in channel catfish (*Ictalurus punctatus*). J. Tenn. Acad. Sci. 55: 54.	407	Dur - 2d	
Reichenbach-Klinke, H.H. 1967. Investigations on the influence of the ammonia content on the fish organism. Arch. Fischereiwiss. 17(2): 122-132 (GER) (ENG TRANSL).	10170	Uchron, UEndp	
Rice, S.D. and R.M. Stokes. 1975. Acute toxicity of ammonia to several developmental stages of rainbow trout, *Salmo gairdneri*. Fish. Bull. 73(1): 207-211 (Personal Communication Used).	667	Dur - 1d	
Rippon, G.D. and R.V. Hyne. 1992. Purple spotted gudgeon: Its use as a standard toxicity test animal in tropical northern Australia. Bull. Environ. Contam. Toxicol. 49(3): 471-476.	5770	NonRes, AF, Dur	
Robertson, E.L. and K. Liber. 2007. Bioassays with caged *Hyalella azteca* to determine *in situ* toxicity downstream of two Saskatchewan, Canada, uranium operations. Environ. Toxicol. Chem. 26(1): 2345-2355.		Field, Tox	
Robinette, H.R. 1976. Effects of selected sublethal levels of ammonia on the growth of channel catfish (*Ictalurus punctatus*). Prog. Fish-Cult. 38(1): 26-29.	524	Dur - 1d	
Ronan, P.J., M.P. Gaikowski, S.J. Hamilton, K.J. Buhl and C.H. Summers. 2007. Ammonia causes decreased brain monoamines in fathead minnows (*Pimephales promelas*). Brain Res. 1147:184-91.		Det, UChron	
Rubin, A.J. and G.A. Elmaraghy. 1977. Studies on the toxicity of ammonia, nitrate and their mixtures to guppy fry. Water Res. 11(10): 927-935.	7635	Sec	Other data used from an earlier report
Rushton, W. 1921. Biological notes. Salmon Trout Mag. 25: 101-117.	11164	Det, AF, UEndp, Dur	
Saha, N. and B.K. Ratha. 1994. Induction of ornithine-urea cycle in a freshwater teleost, *Heteropneustes fossilis*, exposed to high concentrations of ammonium chloride. Comp. Biochem. Physiol. B 108(3): 315-325.	16783	NonRes, UEndp	
Salin, D. and P. Williot. 1991. Acute toxicity of ammonia to Siberian sturgeon *Acipenser baeri*. In: P. Willot (Ed.), Proc.1st Symposium on Sturgeon, Bordeaux (Gironde, France), Oct.3-6, 1989: 153-167.	7491	Dur - 1d	
Samylin, A.F. 1969. Effect of ammonium carbonate on early stages of development of salmon. Uch. Zap. Leningr. Gos. Pedagog. Inst. Im. A. I. Gertsena. 422: 47-62 (RUS) (ENG TRANSL).	2606	UEndp, Dur	
Sarkar, S.K. and S.K. Konar. 1988. Dynamics of abiotic-biotic parameters of water and soil in relation to fish growth exposed to ammonium sulfate. Environ. Ecol. 6(3): 730-733.	804	UEndp, No Org	
Sarkar, S.K. 1988. Influence of ammonium sulphate on the feeding rate of fish under multivariate temperature. Comp. Physiol. Ecol. 13(1): 30-33.	3235	AF, UEndp, Dur	
Sarkar, S.K. 1991. Dynamics of aquatic ecosystem in relation to fish growth exposed to ammonium sulphate. J. Environ. Biol. 12(1): 37-43.	238	WatQual, UEndp, No Org	

Appendix L. Unused (Non-influential) Acute and Chronic Studies for Freshwater Ammonia Criteria Development

Citation	ECOTOX or Other Ref. No	Rejection Code(s)	Comment(s)
Sarkar, S.K. 1991. Toxicity evaluation of urea and ammonium sulphate to *Oreochromis mossambicus* (Peters). J. Ecobiol. 3(1): 79-80.	7535	Con	
Sathyanesan, A.G., K.P. Joy and R.S. Kulkarni. 1978. Endocrine changes in fishes in response to pollutants. Q. J. Surg. Sci. 14(1/2): 67-77.	10173	Eff, AF, UEndp	
Schram, E., J.A.C. Roques, W. Abbink, T. Spanings, P. de Vries, S. Bierman, H. van de Vis and G. Flik. 2010. The impact of elevated water ammonia concentration on physiology, growth and feed intake of African catfish (*Clarisa gariepinus*). Aquaculture 306(1-4): 108-115		NonRes	
Schubauer-Berigan, M.K., P.D. Monson, C.W. West and G.T. Ankley. 1995. Influence of pH on the toxicity of ammonia to *Chironomus tentans* and *Lumbriculus variegatus*. Environ. Toxicol. Chem. 14(4): 713-717.	15119	Dur - 10d	Data rejected for *C. tentans* as indicated
Schulze-Wiehenbrauck, H. 1976. Effects of sublethal ammonia concentrations on metabolism in juvenile rainbow trout (*Salmo gairdneri* Richardson). Ber. Dtsch. Wiss. Kommn. Meeresforsch. 24:234-250.	2616	UEndp, Dur	
Shedd, T.R., M.W. Widder, M.W. Toussaint, M.C. Sunkel and E. Hull. 1999. Evaluation of the annual killifish *Nothobranchius guentheri* as a tool for rapid acute toxicity screening. Environ. Toxicol. Chem. 18(10): 2258-2261.	20487	NonRes, Dur - 1d	
Sheehan, R.J. and W.M. Lewis. 1986. Influence of pH and ammonia salts on ammonia toxicity and water balance in young channel catfish. Trans. Am. Fish. Soc. 115(6): 891-899.	12194	Dur - 1d	
Singh, S.B., S.C. Banerjee and P.C. Chakrabarti. 1967. Preliminary observations on response of young ones of Chinese carps to various physico-chemical factors of water. Proc. Nat. Acad. Sci., India 37(3B): 320-324; Biol. Abstr. 51: 5159 (1970).	2629	INV, UEndp, Dur	
Slabbert, J.L. and J.P. Maree. 1986. Evaluation of interactive toxic effects of chemicals in water using a *Tetrahymena pyriformis* toxicity screening test. Water S. A. 12(2): 57-62.	12836	AF, UEndp, Dur	
Slabbert, J.L. and W.S.G. Morgan. 1982. A Bioassay technique using *Tetrahymena pyriformis* for the rapid assessment of toxicants in water. Water Res. 16(5): 517-523.	11048	AF, UEndp, Dur	
Smart, G. 1976. The effect of ammonia exposure on gill structure of the rainbow trout (*Salmo gairdneri*). J. Fish Biol. 8: 471-475 (Author Communication Used).	2631	Eff, UEndp, Dur	
Smith, C.E. and R.G. Piper. 1975. Lesions associated with chronic exposure to ammonia. In: The pathology of fishes. Ribelin W.E. and G. Migaki (Eds.). University of Wisconsin Press, Madison, WI. pp. 497-514.	2636	Eff, UEnpd, Dur	
Smith, C.E. 1984. Hyperplastic lesions of the primitive meninx of fathead minnows, *Pimephales promelas*, induced by ammonia: Species potential for carcinogen testing. In: Use of small fish species in carcinogenicity testing. Hoover, K.L. (Ed.). Monogr. Ser. Natl. Cancer Inst. No. 65, NIH Publ. No. 84-2653, U.S. Dep. Health Human Serv., Natl. Cancer Inst., Bethesda, MD. pp. 119-125.	10254	Eff, UEndp	

Appendix L. Unused (Non-influential) Acute and Chronic Studies for Freshwater Ammonia Criteria Development

Citation	ECOTOX or Other Ref. No	Rejection Code(s)	Comment(s)
Snell, T.W., B.D. Moffat, C. Janssen and G. Persoone. 1991. Acute toxicity tests using rotifers IV. Effects of cyst age, temperature, and salinity on the sensitivity of *Brachionus calyciflorus*. Ecotoxicol. Environ. Saf. 21(3): 308-317 (OECDG Data File).	9385	Sec, AF, Dur - 1d	Data provided in earlier report
Soderberg, R.W., J.B. Flynn and H.R. Schmittou. 1983. Effects of ammonia on growth and survival of rainbow trout in intensive static-water culture. Trans. Am. Fish. Soc. 112(3): 448-451.	15728	AF, UEndp, Dur	
Solomonson, L.P. 1970. Effects of ammonia and some of its derivatives on photosynthesis in the blue-green alga, *Plectonema boryanum*. Ph.D. Thesis, Univ. of Chicago, Chicago, IL.	5443	UEndp, Dur, Plant	Plant
Spadaro, D., T. Micevska and S.L. Simpson. 2008. Effect of nutrition on toxicity of contaminants to the epibenthic amphipod *Melita plumulosa*. Arch. Environ. Contam. Toxicol. 55(4): 593-602.		NonRes	
Speare, D. and S. Backman. 1988. Ammonia and nitrite waterborne toxicity of commercial rainbow trout. Can. Vet. J. 29: 666.	2958	AF, UEndp, Dur - 2d	
Spencer, P., R. Pollock and M. Dube. 2008. Effects of unionized ammonia on histological, endocrine, and whole organism endpoints in slimy sculpin (*Cottus Cognatus*). Aquat. Toxicol. 90(4): 300-309.		Det	Dilution water not described
Stanley, R.A. 1974. Toxicity of heavy metals and salts to Eurasian watermilfoil (*Myriophyllum spicatum* L.). Arch. Environ. Contam. Toxicol. 2(4): 331-341.	2262	AF	Plant
Sun, H., K. Lu, E.J.A. Minter, Y. Chen, Z. Yang and D.J.S. Montagnes. 2012. Combined effects of ammonia and microcystin on survival, growth, antioxidant responses, and lipid peroxidation of bighead carp *Hypophthalmyths nobilis* larvae. J. Hazard. Mater. 221-222: 213-219.		No Dose, INV	
Sun, H., W. Yang, Y. Chen and Z. Yang. 2011. Effect of purified microcystin on oxidative stress of silver carp *Hypophthalmyths nobilis* larvae under different ammonia concentrations. Biochem. System. Ecol. 39: 536-543.		No Dose, INV	
Suski, C.D., J. D. Kieffer, S.S. Killen and B.L. Tufts. 2007. Sub-lethal ammonia toxicity in largemouth bass. Comp. Biochem. Physiol., A: Mol. Integr. Physiol. 146(3): 381-389.		No Dose, Dur	Only 2 exposure concentrations
Tabata, K. 1962. Toxicity of ammonia to aquatic animals with reference to the effect of pH and carbonic acid. Bull. Tokai Reg. Fish. Res. Lab.(Tokai-ku Suisan Kenkyusho Kenkyu Hokoku) 34: 67-74 (ENG TRANSL).	14284	Dur - 1d	
Tarazona, J.V., M. Munoz, J.A. Ortiz, M. Nunez and J.A. Camargo. 1987. Fish mortality due to acute ammonia exposure. Aquacult. Fish. Manage. 18(2): 167-172.	12807	UEndp, Dur - 1d	
Taylor, J.E. 1973. Water quality and bioassay study from Crawford National Fish Hatchery. Trans. Nebr. Acad. Sci. 2: 176-181.	2531	UEndp, Dur - 2d	
Thomas, J.D., M. Powles and R. Lodge. 1976. The chemical ecology of *Biomphalaria glabrata*: The effects of ammonia on the growth rate of juvenile snails. Biol. Bull. 151(2): 386-397.	15962	NonRes, UEndp, Dur	

Appendix L. Unused (Non-influential) Acute and Chronic Studies for Freshwater Ammonia Criteria Development			
Citation	**ECOTOX or Other Ref. No**	**Rejection Code(s)**	**Comment(s)**
Thomas, P.C., C. Turner and D. Pascoe. 1991. An assessment of field and laboratory methods for evaluating the toxicity of ammonia to *Gammarus pulex* L. - Effects of water velocity. In: Bioindic. Environ. Manage. 6th Symposium. Jeffrey, D.W. and B. Madden (Eds.). Academic Press, London, UK. pp. 353-363.	6276	UEndp, Dur - 1d	
Thumann, M.E. 1950. The effect of ammonium salt solutions on rainbow and brook trout and some fish nutrient animals. Abh. Fischerei. Lieferung 2: 327-348.	2528	UEndp, Dur	
Tilak, K.S., K. Veeraiah and J.M.P. Raju. 2006. Toxicity and effects of ammonia, nitrite, nitrate and histopathological changes in the gill of freshwater fish *Cyprinus carpio*. J. Ecotoxicol. Environ. Monit. 16(6): 527-532.	105937	Det, AF, Dur	
Tng, Y.Y., S.F. Chew, N.L. Wee, F.K. Wong, W.P. Wong, C.Y. Tok and Y.K. Ip. 2009. Acute ammonia toxicity and the protective effects of methionine sulfoximine on the swamp eel, *Monopterus albus.* J. Exp. Zool. A Ecol. Genet. Physiol. 311(9): 676-688.		INV	
Tomasso, J.R., C.A. Goudie, B.A. Simco and K.B. Davis. 1980. Effects of environmental pH and calcium on ammonia toxicity in channel catfish. Trans. Am. Fish. Soc. 109(2): 229-234 (Personal Communication Used).	410	Dur - 1d	
Tonapi, G.T. and G. Varghese. 1984. Cardiophysiological responses of the crab, *Berytelphusa cunnicularis* (Westwood), to three common pollutants. Indian J. Exp. Biol. 22(10): 548-549.	12198	AF, UEndp, Dur	
Tonapi, G.T. and G. Varghese. 1987. Cardio-physiological responses of some selected cladocerans to three common pollutants. Arch. Hydrobiol. 110(1): 59-65.	2075	AF, UEndp, Dur	
Tsai, C.F. and J.A. McKee. 1980. Acute toxicity to goldfish of mixtures of chloramines, copper, and linear alkylate sulfonate. Trans. Am. Fish. Soc. 109(1): 132-141 (Personal Communication Used).	5619	Tox	
Twitchen, I.D. and F.B. Eddy. 1994. Effects of ammonia on sodium balance in juvenile rainbow trout *Oncorhynchus mykiss* Walbaum. Aquat. Toxicol. 30(1): 27-45.	14071	Eff, UEndp	
Twitchen, I.D. and F.B. Eddy. 1994. Sublethal effects of ammonia on freshwater fish. In: Sublethal and chronic effects of pollutants on freshwater fish. Chapter 12. Muller, R. and R. Lloyd (Eds.). Fishing News Books, London, UK. pp.135-147.	18512	Eff, UEndp, Dur - 2d	
Van Der Heide, T., A.J.P. Smolders, B.G.A. Rijkens, E.H. Van Nes, M.M. VanKatwijk and J.G.M. Roelofs. 2008. Toxicity of reduced nitrogen in eelgrass (*Zostera marina*) is highly dependent on shoot density and pH. Oecologia 158(3): 411-419.		VarExp	Substantial loss of ammonia; Plant
Van Vuren, J.H.J. 1986. The effects of toxicants on the haematology of *Labeo umbratus* (Teleostei: Cyprinidae). Comp. Biochem. Physiol. C 83(1): 155-159.	11744	NonRes, AF, UEndp, Dur	
Vedel, N.E., B. Korsgaard and F.B. Jensen. 1998. Isolated and combined exposure to ammonia and nitrite in rainbow trout (*Oncorhynchus mykiss*): Effects on electrolyte status, blood respiratory. Aquat. Toxicol. 41(4): 325-342.	19154	Eff, UEndp	

Appendix L. Unused (Non-influential) Acute and Chronic Studies for Freshwater Ammonia Criteria Development			
Citation	**ECOTOX or Other Ref. No**	**Rejection Code(s)**	**Comment(s)**
Vijayavel, K., E.F. Rani, C. Anbuselvam and M.P. Balasubramanian. 2006. Interactive effect of monocrotophos and ammonium chloride on the freshwater fish Oreochromis mossambicus with reference to lactate/pyruvate ratio. Pestic. Biochem. Physiol. 86(3): 157-161.	108153	Det, AF	Detail (pH, temp, etc. not reported)
Wallen, I.E., W.C. Greer and R. Lasater. 1957. Toxicity to *Gambusia affinis* of certain pure chemicals in turbid waters. Sewage Ind. Wastes 29(6): 695-711.	508	Dur, Con, UEndp	
Wang, C., S.H. Zhang, P.F. Wang, J. Hou, W. Li, and W.J. Zhang. 2008. Metabolic adaptations to ammonia-induced oxidative stress in leaves of the submerged macrophyte *Vallisneria natans* (Lour.) Hara. Aquat. Toxicol. 87(2): 88-98.		NonRes	
Ward, S., T.O.M. Augspurger, F.J. Dwyer, C. Kane and C.G. Ingersoll. 2007. Risk assessment of water quality in three North Carolina, USA, streams supporting federally endangered freshwater mussels (Unionidae). Environ. Toxicol. Chem. 26(10): 2075-2085.		Tox	
Ward, D.J., V. Perez-Landa, D.A. Spadaro, S.L. Simpson and D.F. Jolley. 2011. An assessment of three harpacticoid copepod species for use in ecotoxicological testing. Arch. Environ. Contam. Toxicol. 61(3): 414-425.		NonRes	
Water Pollution Research Board. 1961. Effects of pollution on fish: Toxicity of gas liquors. In: Water pollution research 1960, Water Pollution Research Board, Dep. of Scientific and Industrial Research, H.M. Stationery Office, London, UK. pp. 76-81.	2514	UEndp, Dur	
Water Pollution Research Board. 1968. Effects of pollution on fish: Chronic toxicity of ammonia to rainbow trout. In: Water pollution research 1967, Water Pollution Research Board, Dep. of Scientific and Industrial Research, H.M. Stationery Office, London, UK. pp. 56-65.	10185	AF, Dur - 2d, UEndp	
Watt, P.J. and R.S. Oldham. 1995. The effect of ammonium nitrate on the feeding and development of larvae of the smooth newt, *Triturus vulgaris* (L.), and on the behaviour of its food. Freshw. Biol. 33(2): 319-324.	14883	UEndp	
Wee, N.L.J., Y.Y.M. Tng, H.T. Cheng, S.M.L. Lee, S.F. Chew and Y.K. Ip. 2007. Ammonia toxicity and tolerance in the brain of the African sharptooth catfish, *Clarias gariepinus*. Aquat. Toxicol. 82(3): 204-213.		NonRes, No Dose	Only 2 exposure concentrations
Weiss, L.A. and E. Zaniboni-Filho. 2009. Survival of diploid and triploid *Rhamdia quelen* juveniles in different ammonia concentrations. Aquaculture 298(1-2): 153-156.		NonRes	
Wells, M.M. 1915. The reactions an resistance of fishes in their natural environment to salts. J. Exp. Zool. 19(3): 243-283.		Det, No Dose	
Wickins, J.F. 1976. The tolerance of warm-water prawns to recirculated water. Aquaculture 9(1): 19-37.	2320	AF, UEndp, Dur	
Wilkie, M.P., M.E. Pamenter, S. Duquette, H. Dhiyebi, N. Sangha, G. Skelton, M.D. Smith and L.T. Buck. 2011. The relationship between NMDA receptor function and the high ammonia tolerance of anoxia-tolerant goldfish. J. Exp. Biol. 214(24): 4107-4120.		Eff, UEndp	
Williams, J.E. Jr. 1948. The toxicity of some inorganic salts to game fish. M.S. Thesis, Louisiana State University, Baton Rouge, LA, 71 p.		No Org	

Appendix L. Unused (Non-influential) Acute and Chronic Studies for Freshwater Ammonia Criteria Development			
Citation	**ECOTOX or Other Ref. No**	**Rejection Code(s)**	**Comment(s)**
Woltering, D.M., J.L. Hedtke and L.J. Weber. 1978. Predator-prey interactions of fishes under the influence of ammonia. Trans. Am. Fish. Soc. 107(3): 500-504.	7218	UEndp, Dur	
Xu, Q. and R.S. Oldham. 1997. Lethal and sublethal effects of nitrogen fertilizer ammonium nitrate on common toad (*Bufo bufo*) tadpoles. Arch. Environ. Contam. Toxicol. 32(3): 298-303.	17840	AF, UEndp, Dur	
Zhang, L. and C.M. Wood. 2009. Ammonia as a stimulant to ventilation in rainbow trout *Oncorhynchus mykiss*. Respir. Physiol. Neurobiol. 168(3): 261-271.		RouExp	
Zhang, L.J., G.G. Ying, F. Chen, J.L. Zhao, L. Wang and Y.X. Fang. 2012. Development and application of whole-sediment toxicity test using immobilized freshwater microalgae *Pseudokirchneriella subcapitata*. Environ. Toxicol. Chem. 31(2): 377-386.		SedExp	Plant

Corresponding Code List

ABIOTIC FACTOR (AF)	Studies where one or both of the two abiotic factors (pH and temperature) important for ammonia criteria derivation are not reported.
ACELLULAR (Ace)	Studies of acellular organisms (protozoa) and yeast.
BACTERIA (Bact)	Studies describing only the results on bacteria.
BIOMARKER (Biom)	Studies reporting results for a biomarker having no reported association with a biologically significant adverse effect (survival, growth, or reproduction of an individual or population) and an exposure dose (or concentration).
CONTROL (Con)	Studies where control mortality is insufficient or unsatisfactory, i.e., where survival is less than 90% in acute tests or 80% in chronic tests; or where no control is used.
DETAIL (Det)	Insufficient detail regarding test methodology or statistical analysis.
DURATION (Dur)	Laboratory and field studies where duration of exposure is inappropriate (e.g., too short) for the type of test (i.e., acute or chronic), or was not reported or could not be easily estimated.
EFFLUENT (Efflu)	Studies reporting only effects of effluent, sewage, or polluted runoff where individual pollutants are not measured.
EFFECT (Eff)	Studies where the biologically significant adverse effect was not survival, growth, or reproduction of an individual or population.
ENDPOINT (UEndp)	Studies reported in ECOTOX where an endpoint (LC50, EC50, NOEC, LOEC, MATC, EC20, etc.) was not provided, where none of the concentrations tested in a chronic test were deleterious (no LOEC); or where all concentrations tested in a chronic test caused a statistically significant adverse effect (no NOEC).
FIELD (Field)	Chronic, long-term studies conducted in a field setting (stream segment, pond, etc.) where source/dilution water is not characterized for other possible contaminants.
FORMULATION (Form)	Studies where the chemical is a primary ingredient in a commercial formulation, e.g., biocide, fertilizer, etc.
INVASIVE [Harmful] (INV)	Defined in this document as a species that is non-native to the ecosystem under consideration and whose introduction causes or is likely to cause economic or environmental harm or harm to human health (see ISAC 2006).
IN VITRO (In Vit)	*In vitro* studies, including only exposure of the chemical to cell cultures and excised tissues and not related to whole organism toxicity.
LETHAL TIME (LT)	Laboratory studies reporting only lethal time to mortality, except under special conditions (no other applicable information is available for species pivotal in making a finding).
NO DOSE or CONC (No Dose or Conc)	Studies with too few concentrations to establish a dose-response, or no usable dose or concentration reported in either primary or sister article(s), except under special conditions (no other applicable information is available for species pivotal in making a finding).
NOMINAL (Nom)	Chronic studies where test concentrations were not measured.
NON-RESIDENT (NonRes)	Species that are not resident to North America, or where there is no reported evidence of their reproducing naturally in North America.
NO ORGANISM (No Org)	Laboratory and field studies where no one organism is studied (e.g., periphyton community) or where no scientific/common name is given in either a primary or sister article(s).
PURITY (Pur)	Studies where the chemical purity of the toxicant was less than 80% pure (active ingredient).
ROUTE OF EXPOSURE (RouExp)	Dietary or un-natural exposure routes for aquatic chemicals, e.g., injection, spray, inhalation.
Secondary (Sec)	Non-original data first reported elsewhere.
Sediment Exposure (SedExp)	Sediment-based toxicity test and method.
TOXICANT (Tox)	Inappropriate form of toxicant used or none identified in a laboratory or field study. Note: Inappropriate form includes mixtures.
UNACCEPTABLE CHRONIC (UChron)	Chronic studies which were not based on flow-through exposures (exception for cladocerans and other small, planktonic organisms where test water is continuously renewed), where test concentrations were not measured, or when the chronic test did not include the appropriate test duration for the organism and life-stage tested.

UNUSUAL DILUTION WATER (Dilut)	Laboratory or field studies where the dilution water contained unusual amounts or ratios of inorganic ions or was without addition of appropriate salts (i.e., distilled or de-ionized water).
VARIABLE EXPOSURE (VarExp)	Excessive variability in contaminant concentrations during the exposure period.
WATER QUALITY (WatQual)	Studies where the measured test pH is below 6 or greater than 9, where dissolved oxygen was less than 40% saturation for any length of time, or where total or dissolved organic carbon is greater than 5 mg/L.

Appendix M. 1999 Re-examination of Temperature Dependence of Ammonia Toxicity.

This section presents the temperature analysis published in the 1998 Update, followed by the re-analysis performed for the 1999 Update and reproduced here as background information. Figure and table numbers are preceded by an 'M' in this appendix, in order to distinguish them from tables and figures in the main document.

1998 Analysis of Temperature-Dependence

The 1984/1985 ammonia criteria document identified temperature as an important factor affecting the toxicity of ammonia. When expressed in terms of *unionized* ammonia, the acute toxicity of ammonia was reported in the criteria document to be inversely related to temperature for several species of fish, whereas limited data on acute ammonia toxicity to invertebrates showed no significant temperature dependence. No direct data were available concerning the temperature dependence of chronic toxicity. It was noted, however, that the differences between chronic values for salmonid fish species tested at low temperatures and chronic values for warmwater fish species tested at higher temperatures paralleled differences in acute toxicity known to be caused by temperature.

In the 1984/1985 criteria document, an average temperature relationship observed for fish was used to adjust fish acute toxicity data to a common temperature (20°C) for derivation of the CMC for *unionized* ammonia; this same relationship was used to extrapolate this CMC to other temperatures. (Invertebrate toxicity data were not adjusted, but invertebrates were sufficiently resistant to ammonia that adjustment of invertebrate data was not important in the derivation of the CMC.) This temperature relationship for fish resulted in the unionized ammonia CMC being higher at warm temperatures than at cold temperatures. Additionally, because of concerns about the validity of extrapolating the temperature relationship to high temperatures, the unionized ammonia CMC was "capped" to be no higher than its value at a temperature, called TCAP, near the upper end of the temperature range of the acute toxicity data available for warmwater and coldwater fishes. Similarly, the CCC was capped at a temperature near the upper end of the temperature range of the available chronic toxicity data.

Although the unionized ammonia criterion is lower at low temperatures, this does not result in more restrictive permit limits for ammonia because the ratio of ammonium ion to unionized ammonia increases at low temperatures, resulting in the total ammonia criterion being essentially constant at temperatures below TCAP. In practice, however, the criterion at low temperatures can be more limiting for dischargers than the criterion at high temperatures because biological treatment of ammonia is more difficult at low temperatures. Above TCAP, the constant unionized ammonia criterion results in the total ammonia criterion becoming progressively lower with increasing temperature, which can also result in restrictive discharge limitations.

Because more data are available at moderate temperatures than at lower and higher temperatures, the ammonia criterion is most uncertain for circumstances when compliance can be most difficult, either because of the low total ammonia criterion at high temperatures or because of treatment difficulties at low temperatures. This section examines the data used in the 1984/1985 criteria document and newer data to determine (1) whether the use of TCAPs should be continued and (2) whether a lower unionized criterion at low temperature is warranted. Data used include those analyzed by Erickson (1985), which are shown in Figure 2 of the 1984/1985 document, and more recent data reported by Arthur et al. (1987), DeGraeve et al. (1987), Nimmo et al. (1989), and Knoph (1992).

Data not used include those reported by the following:

1. Bianchini et al. (1996) conducted acute tests at 12 and 25°C, but one test was in fresh water, whereas the other was in salt water.
2. Diamond et al. (1993) conducted acute and chronic toxicity tests on ammonia at 12 and 20°C using several vertebrate and invertebrate species. When expressed in terms of unionized ammonia, they reported that vertebrates (i.e., fishes and amphibians) were more sensitive to ammonia at 12°C than at 20°C, whereas invertebrates were either less

sensitive or no more sensitive at 12°C, compatible with the relationships used in the 1984/1985 criteria document. However, such factors as dilution water and test duration varied between tests at different temperatures and possibly confounded the results (see Appendix 1 of the 1999 update), raising doubts about the temperature comparisons for the vertebrates and invertebrates.

Arthur et al. (1987) measured the acute toxicity of ammonia to several fish and invertebrate species at ambient temperature during different seasons of the year. For three of the five fish species (rainbow trout, channel catfish, and white sucker), the relationship of toxicity to temperature was similar to that used in the 1984/1985 criteria document. When expressed in terms of *unionized* ammonia, no clear relationship existed between temperature and toxicity for the other fish species (fathead minnow and walleye). This result for the fathead minnow is different from those of three other studies (Reinbold and Pescitelli 1982b; Thurston et al. 1983; DeGraeve et al. 1987) reporting a significant effect of temperature on the acute toxicity of unionized ammonia to the fathead minnow. For five invertebrate species, each tested over a temperature range of at least 10°C, there was no consistent relationship between temperature and unionized ammonia toxicity. An initial report of these results (West 1985) was the basis for no temperature adjustment being used for invertebrate data in the 1984/1985 criteria document. Further analysis of the Arthur (1987) data is discussed later.

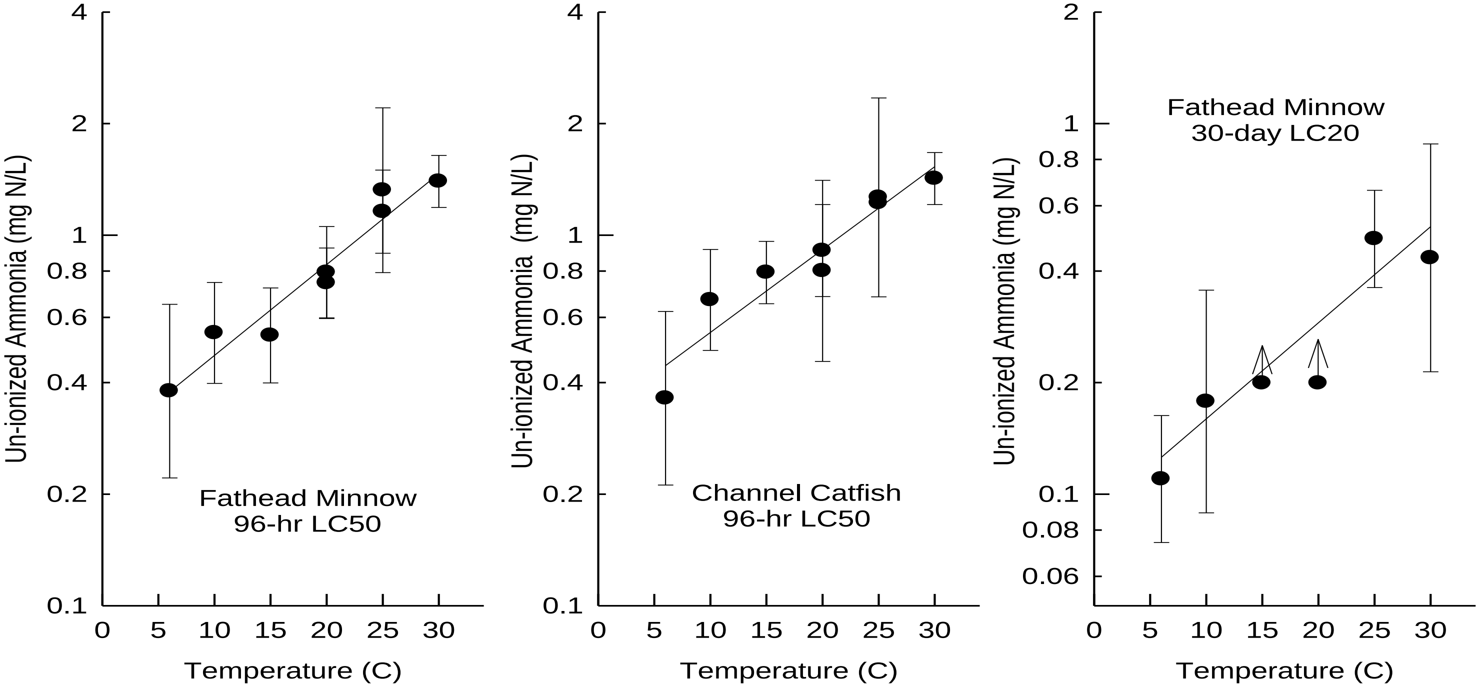
DeGraeve et al. (1987) studied the effect of temperature (from 6 to 30°C) on the toxicity of ammonia to juvenile fathead minnows and channel catfish using acute (4-day) and chronic (30-day) ammonia exposures. As shown for both fish species in Figure M.1, log(96-hr unionized ammonia LC_{50}) versus temperature was linear within the reported uncertainty in the LC_{50}s; the slopes were similar to those reported in the 1984/1985 criteria document. Problems with the channel catfish chronic tests precluded effective use of those data and the highest tested ammonia concentrations in the fathead minnow chronic tests at 15 and 20°C did not cause sufficient mortality to be useful. However, sufficient mortality did occur in the fathead minnow chronic tests at 6, 10, 25, and 30°C. Based on regression analysis of survival versus log concentration (discussed in more detail in the section concerning the CCC below), 30-day LC20s for unionized ammonia were 0.11, 0.18, 0.48, and 0.44 mg N/L at 6, 10, 25, and 30°C, respectively. This temperature dependence (Figure M.1) is similar to that for acute toxicity and that used in the 1984/1985 criteria document. The actual effect of temperature on these 30-day LC20s is probably somewhat greater, because test pH decreased with increasing temperature.

Nimmo et al. (1989) conducted acute toxicity tests on ammonia at 6 and 20°C in a well water using Johnny darters and in a river water using both Johnny darters and juvenile fathead minnows. In all three sets of tests, LC_{50}s expressed in terms of unionized ammonia were significantly higher at the warmer temperature, by factors ranging from 3.5 to 6.2.

Knoph (1992) conducted acute toxicity tests at temperatures ranging from 2 to 17°C using Atlantic salmon parr, one series of tests at pH≈6.0 and the other at pH≈6.4. In both series of tests, LC_{50}s expressed in terms of unionized ammonia increased substantially with temperature.

Even with these additional data, the shape of the temperature relationship is not completely resolved, especially for chronic toxicity. Nevertheless, the acute data for fishes overwhelmingly indicate that ammonia toxicity, expressed in terms of *unionized* ammonia, decreases with increasing temperature.

Figure M.1. The Effect of Temperature on Ammonia Toxicity in Terms of Unionized Ammonia (DeGraeve et al. 1987). Symbols denote LC_{50}s or LC_{20}s and 95% confidence limits and lines denote linear regressions of log LC versus temperature.



Most importantly, the data of DeGraeve et al. (1987) show (Figure M.1) that (a) a linear relationship of log unionized ammonia LC_{50} versus temperature applies within the reported uncertainty in the LC_{50}s over the range of 6 to 30°C and (b) temperature effects on long-term mortality are similar to those on acute mortality. For invertebrates, acute toxicity data suggest that ammonia toxicity, when expressed in terms of unionized ammonia, does not decrease, and possibly even increases, with increasing temperature. Quantifying and adjusting data for this relationship is not necessary because even at warm temperatures invertebrates are generally more resistant to acute ammonia toxicity than fishes and thus their precise sensitivities are of limited importance to the criterion. At low temperatures, they are even more resistant relative to fishes and thus their precise sensitivity is even less important to the criterion.

Based on this information, the two issues raised above were resolved as follows:

1. TCAPs will not be used in the ammonia criterion. This does not mean that the notion of high temperature exacerbating ammonia toxicity is wrong; rather, it reflects the fact that such an effect is not evident in the available data, which cover a wide temperature range.
2. A CMC, if it were expressed as unionized ammonia (rather than total ammonia, used in this document) would continue to be lower at lower temperatures, consistent with the observed temperature dependence of ammonia toxicity to the most sensitive species, i.e., fishes. Although it is possible that the temperature relationship differs among fish species and that using the same relationship for all fish species introduces some uncertainty, specifying a relationship for each fish species is not possible with current data and would also introduce considerable uncertainty.

Therefore, for a criterion expressed in terms of unionized ammonia, available data support the continued use of a generic temperature relationship similar to that in the 1984/1985 ammonia criteria document, but without TCAPs.

Figure M.2. The Effect of Temperature on Acute Ammonia Toxicity in Terms of Total Ammonia Nitrogen. Symbols denote LC_{50}s, solid lines denote regressions for individual datasets, and dotted lines denote pooled regressions over all datasets.

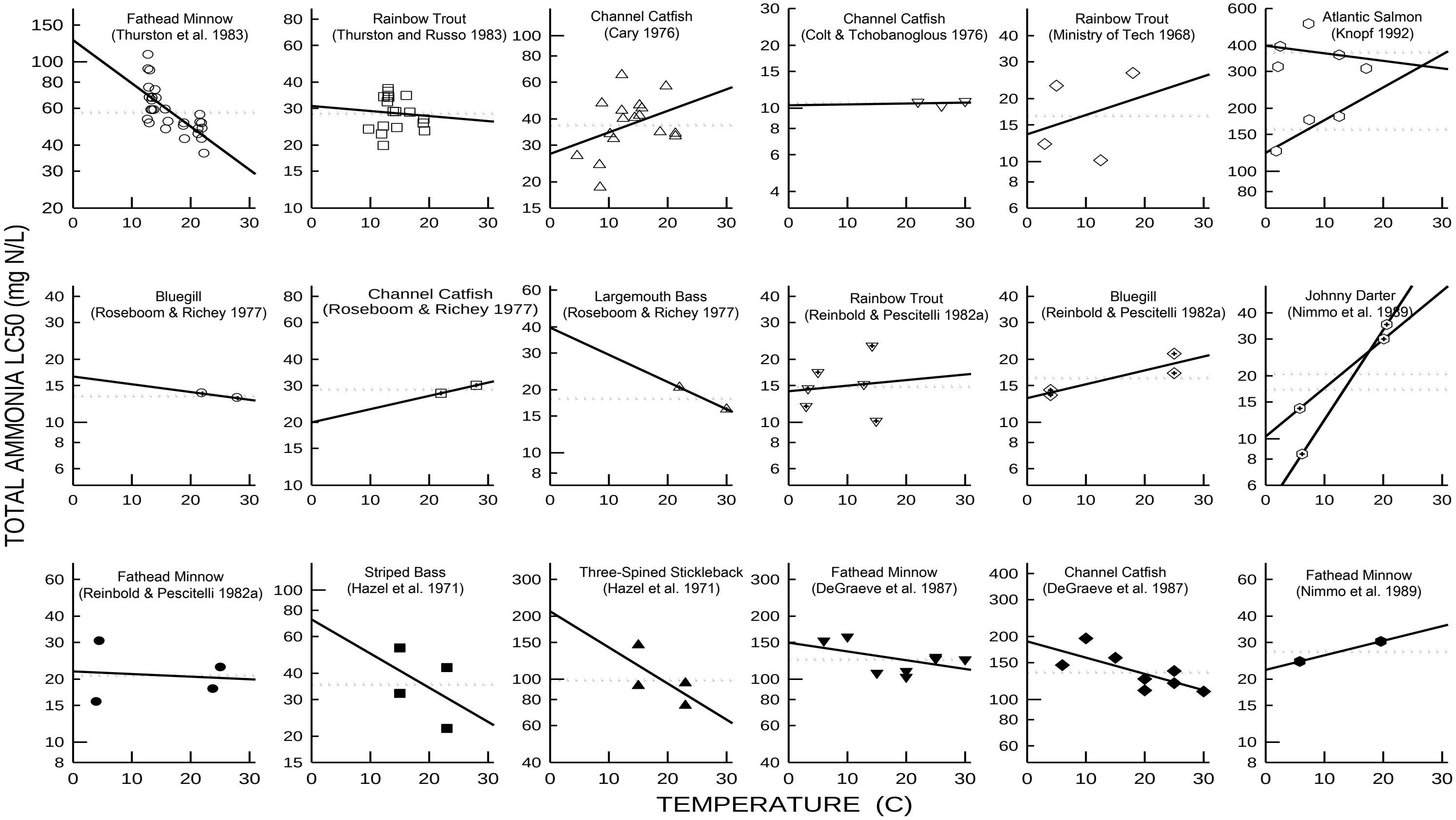
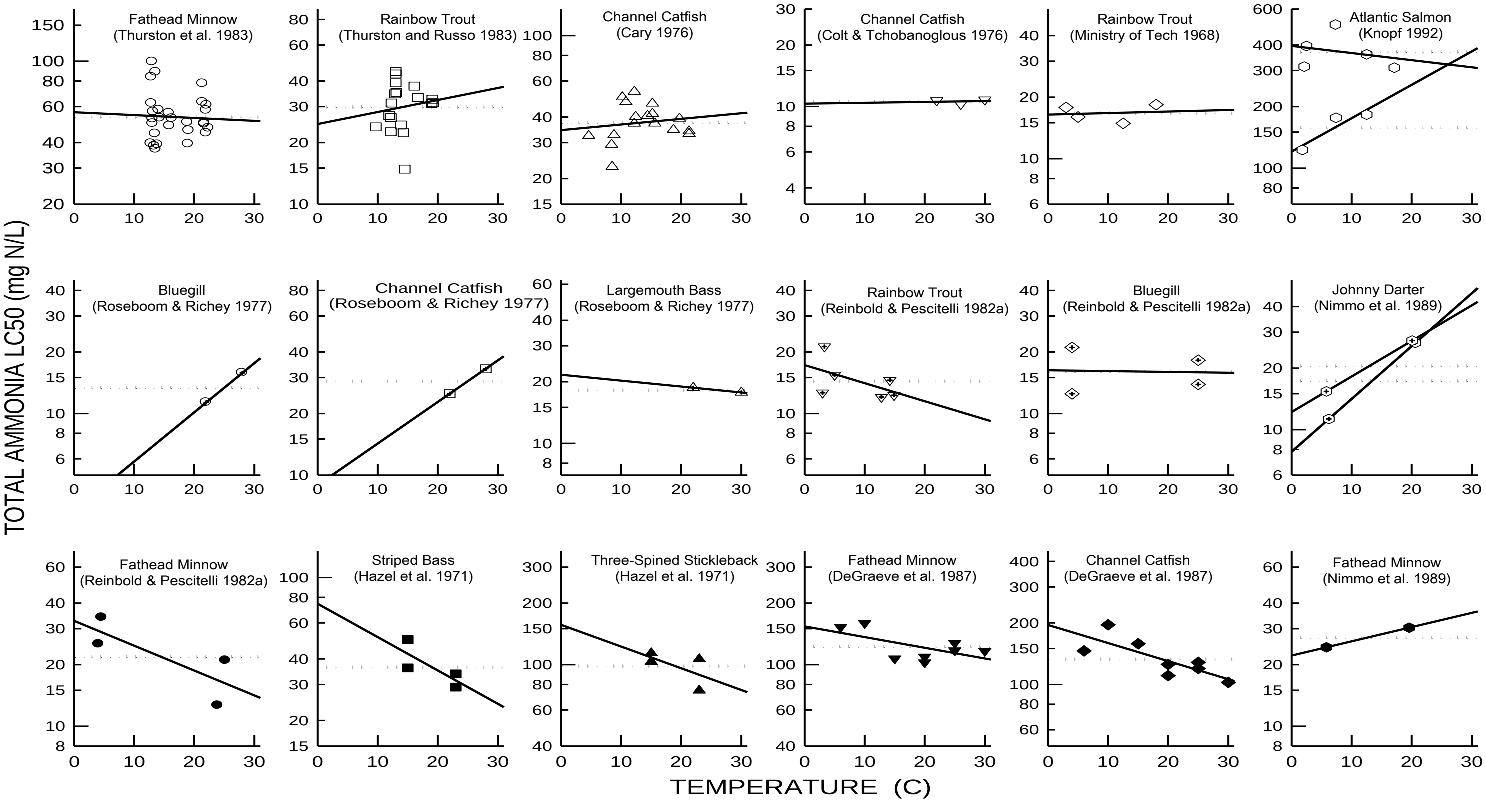


Figure M.3. The Effect of Temperature on pH-Adjusted Acute Ammonia Toxicity in Terms of Total Ammonia Nitrogen. LC_{50}s are adjusted to the mean pH of the dataset based on the pooled relationship of acute toxicity to pH. Symbols denote LC_{50}s, solid lines denote regressions for individual datasets, and dotted lines denote pooled regression over all datasets



This raised a new issue, however, because the criterion expressed in terms of total ammonia is nearly constant over all tested temperatures, and the small effect of temperature on the total ammonia criterion in the 1984/1985 criteria document is largely an artifact of conducting regression analyses in terms of unionized ammonia and is not indicative of any established, significant trend. It was thought that the expression and implementation of the ammonia criterion might have been simplified if temperature were dropped as a modifying factor, which might have been possible if ammonia toxicity is expressed in terms of total ammonia. Furthermore, permit limits and compliance are usually expressed in terms of total ammonia nitrogen, and so expressing the criterion in terms of total ammonia nitrogen would simplify its implementation by eliminating conversions to and from unionized ammonia. Because of such benefits and because there are no compelling scientific or practical reasons for expressing the criterion in terms of unionized ammonia, the freshwater toxicity data concerning temperature dependence were reanalyzed in terms of total ammonia nitrogen.

The data analyzed are from the studies included in the 1984/1985 ammonia criteria document and the studies of DeGraeve et al. (1987), Nimmo et al. (1989), and Knoph (1992). All analyses were conducted in terms of total ammonia nitrogen, either as reported by the authors or as converted by us from reported values for unionized ammonia, pH, and temperature using the speciation relationship of Emerson et al. (1975). The data are presented in Figure M.2 and show considerable diversity, with some datasets showing decreasing toxicity with increasing temperature, some showing increasing toxicity, and some showing virtually no change. There are even differences among studies using the same test species. However, in no case is the effect of temperature particularly large, being no more than a factor of 1.5 over the range of any dataset, except for the Johnny darter data of Nimmo et al. (1989). In some studies, test pH was correlated with test temperature. To reduce the confounding effect of pH, the total ammonia LC_{50} was adjusted to the mean pH of the data for the study using the pH relationship discussed in the next section of this appendix. These adjusted data are shown in Figure M.3 and also show neither large effects nor any clear consistency among or within species or studies.

For each dataset containing at least three data points, a linear regression of log LC_{50} versus temperature was conducted (Draper and Smith 1981) and the resulting regression lines are plotted as solid lines in Figures M.2 and M.3. These regressions are significant at the 0.05 level for only one dataset (the unadjusted fathead minnow data of Thurston et al. 1983); for this dataset, however, the regression is not significant when the data are adjusted for the fact that pH values were lower in the low-temperature tests than in the high-temperature tests. Slopes from regression analyses of datasets in Figure M.3 range from -0.015 to 0.013, compared to a range from 0.015 to 0.054 when expressed in terms of unionized ammonia (Erickson 1985). This narrower range of slopes in terms of total ammonia nitrogen also argues for use of total ammonia, rather than unionized ammonia, because there is less uncertainty associated with the generic relationship. For datasets with just two points, Figures M.2 and M.3 also show the slopes for comparative purposes. Based on the typical uncertainty of LC_{50}s, these slopes also would not be expected to be significant, except perhaps for the Johnny darter data of Nimmo et al. (1989).

A multiple least-squares linear regression (Draper and Smith 1981) using all datasets (with a common slope for all datasets and separate intercept for each dataset) was conducted, both with and without pH adjustment. The results of these pooled analyses are plotted as dotted lines in Figures M.2 and M.3 to show that the residual errors for the common regression line compared to the individual regression lines are not large relative to the typical uncertainty of LC_{50}s. To better show the overall fit of the common regression line, the data are also plotted together in Figure M.4 by dividing each point by the regression estimate of the LC_{50} at 20°C for its dataset. This normalization is done strictly for data display purposes because it allows all of the datasets to be overlaid without changing their temperature dependence, so that the overall scatter around the common regression line can be better examined. The data show no obvious trend, with the best-fit slope explaining only 1% of the sum of squares around the means for the pH-adjusted data and 0% for the unadjusted data. The one available chronic dataset (DeGraeve et al. 1987) also shows no significant temperature effect when expressed in terms of total ammonia nitrogen (Figure M.5) and adjusted for pH differences among the tests. (These tests and the calculation of the LC20s are discussed in detail later.)

Based on the small magnitude and the variability of the effect of temperature on total ammonia acute and chronic toxicity values for fish, the 1998 Update did not include temperature as a modifying factor for a total ammonia criterion. For invertebrates, it should be noted that the 1998 Update's assumption that temperature had no effect on the toxicity of *total* ammonia differs from the 1984/1985 criteria document's assumption that temperature has no effect on the toxicity of *unionized* ammonia. This inconsistency is resolved during the 1999 re-examination of data, to be discussed shortly, by incorporating a relationship between temperature and total ammonia toxicity to invertebrates. That relationship, however, does not affect the (1999 update) CMC because invertebrates are not among the acutely sensitive taxa.

Figure M.4. The Effect of Temperature on Normalized Acute Ammonia Toxicity in Terms of Total Ammonia Nitrogen. Data were normalized by dividing measured LC_{50}s by regression estimates of LC_{50}s at 20°C for individual datasets for Figure M.2 (top plot) and Figure M.3 (bottom plot).

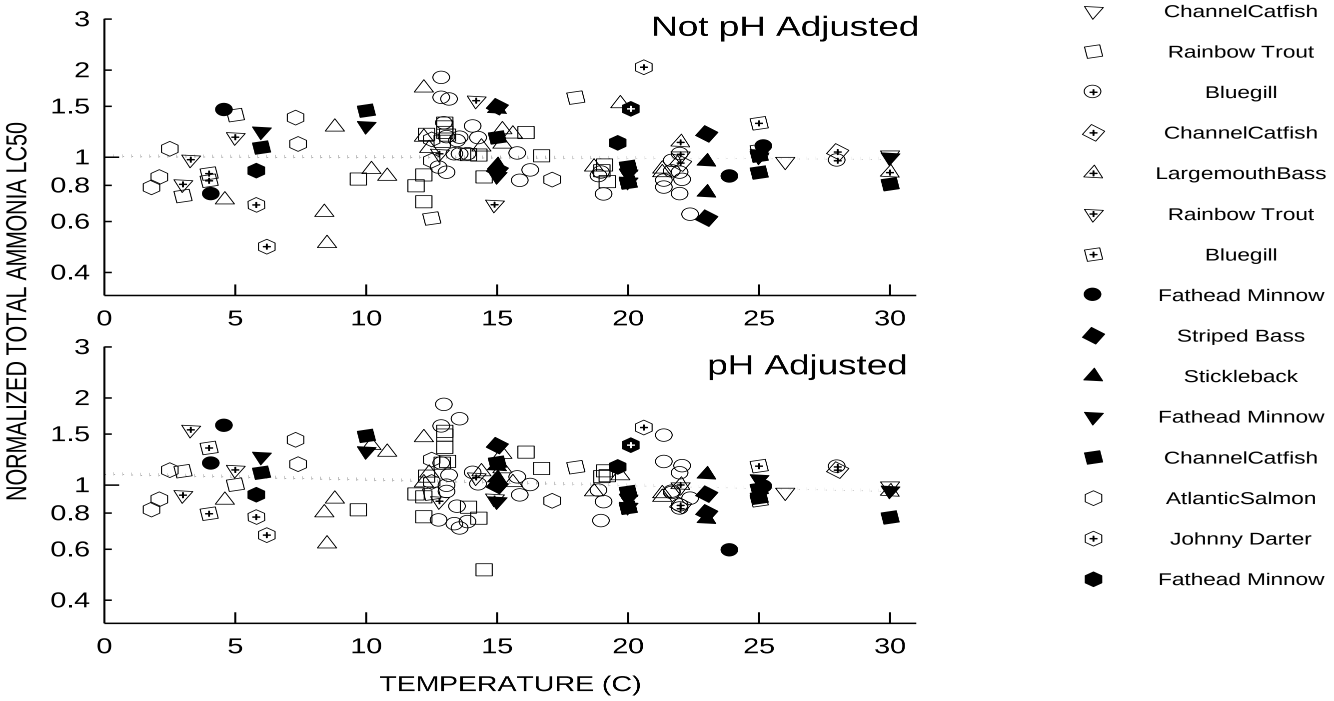
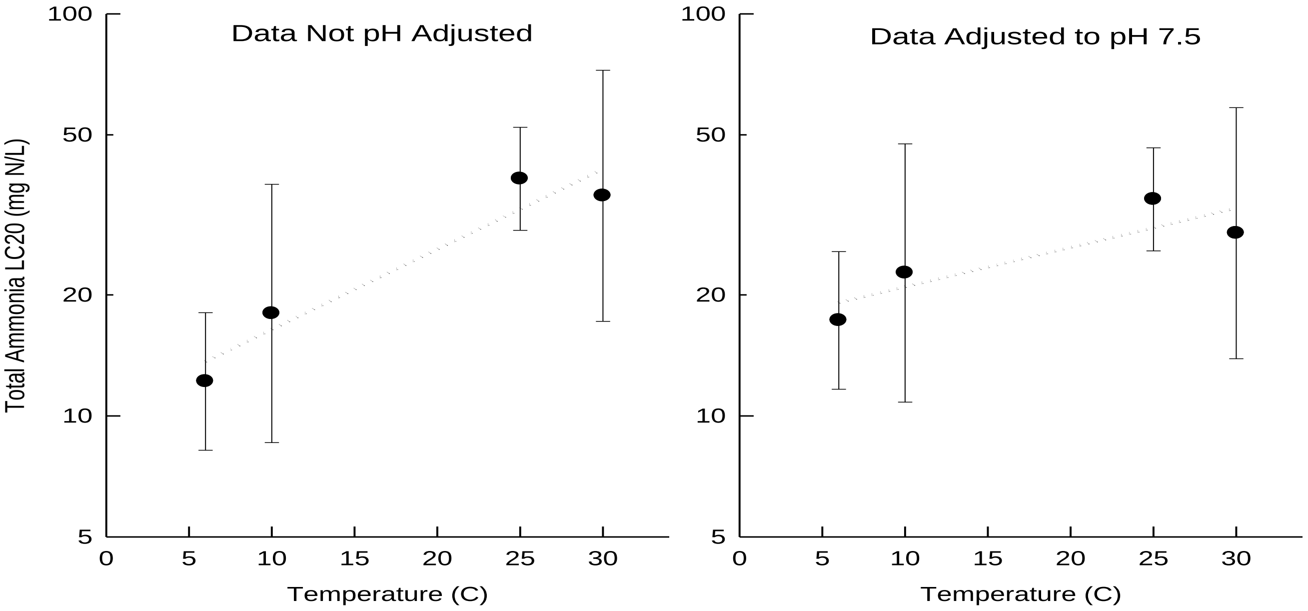


Figure M.5. The Effect of Temperature on Chronic Ammonia Lethality to Fathead Minnows in Terms of Total Ammonia Nitrogen (DeGraeve et al. 1987).

Symbols denote LC20s and 95% confidence limits and lines denote linear regressions of log LC versus temperature. Figure on left is for estimated LC_{50}s at test pH and figure on right is for LC_{50}s adjusted to pH=7.5 based on pooled relationship of chronic toxicity to pH.



The amount of uncertainty in this approach to the CMC can be demonstrated to be small by considering how the criterion would differ if total ammonia toxicity was adjusted based on the slopes in various datasets. Because the bulk of the toxicity data used in the derivation of the criterion is within a few degrees of 20°C, the temperature relationship used has very little effect on the criterion near this temperature, but rather has the greatest effect on the criterion at much higher or lower temperatures. If the average slope for the pH-adjusted acute data from Figure M.4 is used, the total ammonia CMC at 5°C would be only about 6% higher than at 20°C. The smallest and largest slopes from the acute regressions for individual species in Figure M.3 would produce a range from 40% lower to 68% higher at 5°C than at 20°C, but this greatly overstates the uncertainty because effects on a CMC derived from many datasets should not be near these extremes.

1999 Re-examination of Temperature Dependence – Acute Toxicity

The previous section, reproduced with relatively few changes from the 1998 Update, included an analysis of available data on the temperature dependence of acute ammonia toxicity to fish. These data (in Figures M.2, M.3, and M.4) consisted of 20 different data sets drawn from 11 different studies and included nine different species, four of these species being in more than one study. Data from Arthur et al. (1987) were not used in the 1998 analysis because those authors reported concerns about factors confounding temperature in their data set. Linear regression analysis of log LC_{50} (total ammonia basis) versus temperature was conducted on each data set, both with and without correcting for pH as a confounding factor. No consistent trend with temperature was observed and only one data set showed a slope different than zero at the 0.05 level of statistical significance. Therefore, a pooled linear regression analysis was conducted across all data to derive an average slope, which was very close to zero and also not statistically significant. On the basis of this analysis, the 1998 Update did not include any temperature dependence for criteria to protect fish from acute ammonia toxicity.

In response to public comment (U.S. EPA, 1999), the 1998 analysis was re-examined. This re-examination indicated that it is appropriate to handle the temperature dependencies of fish and invertebrates separately. For invertebrates, the inclusion of the Arthur et al. (1987) data in the regression analysis yields a change in the temperature dependency that is ultimately reflected in the difference between the 1999 CCC and the 1998 CCC.

In the 1998 Update, EPA did not use the Arthur et al. (1987) data because of those authors concerns that other variable factors in their tests, conducted during different seasons, might have had a potential to confound their results. In re-examining their data in response to comments, however, EPA found that most of the *fish* data from Arthur et al. showed behavior similar to that from numerous other investigators: that is, little relationship with temperature when expressed as total ammonia. Consequently, it was concluded that the other variable factors were unlikely to be confounding the results.

For fish, although the temperature dependency is unchanged from 1998, additional documentation is provided here, primarily because the apparent difference between fish acute and chronic temperature dependencies is now used in the projection of the invertebrate chronic temperature dependency.

First presented here will be more details on the regression analyses of the individual data sets conducted for the Update, plus similar analyses of the data of Arthur et al. A linear regression was conducted on each data set using the equation:

$$\log(LC50_T) = \log(LC50_{20}) + S \cdot (T- 20)$$

where $LC50_T$ is the total ammonia LC50 at temperature T, S is the slope of log LC50 versus temperature, and $LC50_{20}$ is the estimated total ammonia LC50 at 20°C. For completeness, this effort included data sets with just two points, although the regression analysis then provides a perfect fit and has no residual error, so that confidence limits, significance levels, etc. cannot be evaluated using normal methods. In such cases, the mean squared error (MSE) of data around the regression was assumed to be equal to the weighted mean residual MSE for the larger data sets, so that approximate significance levels could be determined.

Fish acute data:

Table M.1 presents the results of the regression analysis for each data set, with data adjusted to pH=8 based on the average pH relationship used in the 1998 and 1999 Updates. Plots of these relationships (except for Arthur et al. 1987) are in Figure M.3 in the previous section.

Of the 24 entries in Table M.1, nearly half (11) have very small slopes of between -0.006 and +0.006, a range which corresponds to a factor of 1.3 change or less in LC_{50} for a 20°C temperature change and is less than normal data variability. Of these 11, five have positive slopes and six have negative slopes. Of the 13 entries with steeper slopes, five have positive slopes and eight have negative slopes. Among the data sets used in the Update, only two of the regressions are statistically significant at the 0.05 level, one with a negative slope and one with a positive slope, although two other sets (for fathead minnows from DeGraeve et al. 1987 and Reinbold and Pescitelli 1982b) are close to being significant. (The level of significance for the Johnny darter data set differs from what was reported in the previous section because it consists of two different sets which were analyzed separately in the 1998 analysis, but combined here because they were not significantly different.) Of the five data sets from Arthur et al. (1987), only one is significant at the 0.05 level. For species with more than one entry, slopes vary considerably. This general lack of statistical significance and consistency precludes any reliable assessments based on these individual analyses.

The 1998 Update therefore conducted a pooled regression analysis to determine whether the combined acute toxicity data sets indicated any significant average trends with temperature. Table M.2 summarizes the mean trends determined in various pooled analyses. The first entry is the pooled analysis conducted for the 1998 Update, which included all the data in Table M.1 above except the fish data of Arthur et al. (1987). The slope from this pooled analysis was very small (-0.0023), and was not statistically significant despite the large number of data. The second entry adds the fish data of Arthur et al.; it does result in a statistically significant trend. The mean slope (-0.0058) is still small, but does amount to a 23% decrease in LC_{50} per 20°C increase in temperature. However, this slope is heavily influenced by two points with high residual ($>3\sigma$) deviations. One of these points is a test at 3.4°C by Arthur et al. (1987) with fathead minnows, which showed much greater effects of low temperature than other studies with the same species. The other point is for a test at 22.6°C by Arthur et al. (1987) with walleye, which showed very high sensitivity and was part of a set of three tests which used fish from different sources, potentially confounding the temperature effects. Without these two data, the regression has an even lower slope and is not significant at the 0.05 level (third entry in Table M.2). Overall, these analyses of the fish acute data suggest a weak overall trend of higher LC_{50}s at low temperatures, with a $\log LC_{50}$ versus temperature slope in the -0.002 to -0.006 range, but of questionable statistical significance.

Table M.1. Results of Regression Analysis of $\log LC_{50}$ (mg/L total ammonia nitrogen) Versus Temperature (°C) for Individual Data Sets on the Temperature Dependence of Acute Ammonia Toxicity.

Table M.1

Reference/ Species	Slope (95% CL)	$\log LC50_{20}$ (95% CL)	Residual SD (r^2)	F_{REGR} (α)
Thurston et al. (1983) Fathead Minnow	-0.0014 (-0.013,+0.013)	1.641 (1.582,1.700)	0.112 (<1%)	0.06 (0.81)
Thurston and Russo (1983) Rainbow Trout	+0.0059 (-0.017,+0.029)	1.350 (1.204,1.495)	0.121 (2%)	0.30 (0.59)
Cary (1976) Channel Catfish	+0.0028 (-0.008,+0.013)	1.676 (1.593,1.758)	0.093 (2%)	0.32 (0.58)
Colt and Tchobanoglous (1976) Channel Catfish	+0.0004 (-0.037,+0.038)	1.604 (1.350,1.858)	0.016 (2%)	0.02 (0.91)
Ministry of Technology (1967) Rainbow Trout	+0.0008 (-0.018,+0.019)	1.231 (1.010,1.452)	0.051 (1%)	0.03 (0.88)
Roseboom and Richey (1977) Bluegill Sunfish	+0.024 (-0.025,+0.073)	1.089 (0.803,1.375)	-	0.95 (0.33)
Roseboom and Richey (1977) Channel Catfish	+0.020 (-0.029,+0.069)	1.482 (1.196,1.768)	-	0.68 (0.41)
Roseboom and Richey (1977) Largemouth Bass	-0.0029 (-0.040,+0.034)	1.237 (0.972,1.502)	-	0.02 (0.88)
Reinbold and Pescitelli (1982b) Rainbow Trout	-0.0088 (-0.028,+0.010)	1.396 (1.159,1.632)	0.088 (29%)	1.63 (0.27)
Reinbold and Pescitelli (1982b) Bluegill Sunfish	-0.0004 (-0.027,+0.026)	1.370 (1.059,1.681)	0.128 (0%)	0.00 (0.96)
Reinbold and Pescitelli (1982b) Fathead Minnow	-0.0153 (-0.031,+0.009)	1.429 (1.243,1.615)	0.076 (89%)	16.6 (0.06)
Hazel et al. (1971) Striped Bass	-0.0163 (-0.057,+0.025)	1.274 (1.105,1.443)	0.076 (60%)	2.93 (0.23)
Hazel et al. (1971) Three-Spined Stickleback	-0.0106 (-0.053,+0.032)	1.390 (1.214,1.567)	0.081 (36%)	1.14 (0.40)
DeGraeve et al. (1987) Fathead Minnow	-0.0052 (-0.012,+0.002)	1.617 (1.563,1.670)	0.061 (36%)	3.33 (0.12)
DeGraeve et al. (1987) Channel Catfish	-0.0088 (-0.016,-0.002)	1.648 (1.595,1.701)	0.061 (62%)	9.76 (0.02)
Knopf (1992) Atlantic Salmon	-0.0035 (-0.027,+0.020)	1.715 (1.406,2.025)	0.097 (7%)	0.22 (0.067)
Knopf (1992) Atlantic Salmon	+0.0163 (-0.075,+0.108)	1.636 (0.405,2.866)	0.054 (84%)	5.18 (0.26)
Nimmo et al. (1989) Johnny Darter	+0.021 (+0.000,+0.043)	1.463 (1.248,1.678)	0.072 (90%)	18.1 (0.05)
Nimmo et al. (1989) Fathead Minnow	+0.0070 (-0.014,+0.028)	1.568 (1.353,1.782)	-	0.42 (0.52)
Arthur et al. (1987) Fathead Minnow	-0.032 (-0.059,-0.004)	1.762 (1.493,2.030)	0.105 (92%)	24.8 (0.04)

Table M.1

Reference/ Species	Slope (95% CL)	logLC50$_{20}$ (95% CL)	Residual SD (r^2)	F$_{REGR}$ (α)
Arthur et al. (1987) Rainbow Trout	-0.0100 (-0.053,+0.033)	1.348 (0.937,1.758)	0.158 (16%)	0.56 (0.51)
Arthur et al. (1987) Channel Catfish	-0.0058 (-0.038,+0.027)	1.558 (1.230,1.886)	0.030 (84%)	5.15 (0.26)
Arthur et al. (1987) White Sucker	+0.0007 (-0.23,+0.25)	1.902 (1.657,2.147)	0.048 (1%)	0.01 (0.92)
Arthur et al. (1987) Walleye	-0.038 (-0.327,+0.250)	1.216 (-1.911,4.343)	0.306 (74%)	2.84 (0.34)

Table M.2. Results of Regression Analysis of log LC$_{50}$ (mg/L total ammonia nitrogen) Versus Temperature (°C) for Pooled Data Sets on the Temperature Dependence of Acute Ammonia Toxicity to Fish.

Data Sets Pooled	Slope (95% CL)	Residual SD (r^2)	F$_{REGR}$ (α)
All Data excluding Arthur et al.	-0.0023 (-0.0057,+0.0011)	0.105 (2%)	1.79 (0.18)
All Data including Arthur et al.	-0.0058 (-0.0094,-0.0022)	0.122 (8%)	10.3 (<0.01)
All Data including Arthur et al. except "Outliers"	-0.0030 (-0.0063,+0.0002))	0.105 (3%)	3.52 (0.06)
Fathead Minnow excluding Arthur et al	-0.0063 (-0.0122,-0.0005)	0.106 (11%)	4.76 (0.04)
Fathead Minnow including Arthur et al.	-0.0105 (-0.0169,-0.0049)	0.120 (25%)	13.4 (<0.01)
Fathead Minnow including Arthur et al. excl "Outlier"	-0.0073 (-0.0129,-0.0017)	0.106 (15%)	6.85 (0.01)
Rainbow Trout excluding Arthur et al.	-0.0013 (0.0122,+0.0096)	0.109 (<1%)	0.06 (0.80)
Rainbow Trout including Arthur et al.	-0.0034 (-0.0133,+0.0064)	0.115 (2%)	0.51 (0.48)
Channel Catfish excluding Arthur et al.	-0.0030 (-0.0091,+0.0031)	0.088 (4%)	1.05 (0.32)
Channel Catfish including Arthur et al.	-0.0034 (-0.088,+0.021)	0.085 (6%)	1.64 0.21)
Bluegill Sunfish	+0.0006 (-0.0172,+0.0184)	0.120 (<1%)	0.01 (0.92)

It is also useful to consider separately the overall trends for different fish species. Table M.1 includes multiple studies with fathead minnows, rainbow trout, channel catfish, and bluegill sunfish. Table M.2 includes the results of pooled analyses for each of these species, both with and without data from Arthur et al. (1987). For rainbow trout, bluegill, and channel catfish, the regressions were not statistically significant. The bluegill data indicated virtually no temperature effect, whereas weak trends similar to the pooled analyses over all data sets were suggested in the channel catfish data (slope = -0.0030 without and -0.0034 with Arthur et al. data) and rainbow trout data (slope = -0.0014 without and -0.0034 with Arthur et al. data). For fathead minnow, the pooled analyses were statistically significant and stronger, with slopes ranging from -0.0063 to -0.0105 depending on the treatment of data from Arthur et al. Such slopes for fathead minnow would result in moderate effects over a broad temperature range: a 20°C decrease in temperature would result in a 33% to 62% increase in LC_{50}. However, this species is not sensitive enough that this would affect the acute criterion values. For the species used in the acute criterion calculations, no temperature correction for acute toxicity is appropriate due to the lack of any significant trend over all data sets.

Invertebrate acute data:

Unlike fish, available acute toxicity data for invertebrates indicates that their acute sensitivity to ammonia decreases substantially with decreasing temperature. The 1998 Update noted this temperature dependence, but did not present any analysis of it because tested invertebrates were sufficiently tolerant to acute ammonia exposures that this dependence would not affect the acute ammonia criterion. The 1998 Update also noted that this temperature dependence should be a consideration in setting low temperature chronic criterion, but did not provide any specific analysis regarding this. This section will provide an analysis of available information on the temperature-dependence of invertebrate *acute* ammonia toxicity, to be used later for estimating the temperature-dependence of *chronic* ammonia toxicity.

Arthur et al. (1987) provide the only available data on the temperature dependence of acute ammonia toxicity to invertebrates. As noted earlier, these toxicity tests did not specifically test temperature effects, but rather were seasonal tests in which various chemical characteristics of the tests water varied as well as temperature. Test organisms were whatever were available in outdoor experimental streams at the time of the test, so the size, life stage, and condition of the organisms also varied. The authors of this study expressed some doubt as to how much of the effects they observed were actually due to temperature. However, for invertebrates, they did observe strong correlations of total ammonia toxicity with temperature. Confounding factors might contribute somewhat to this correlation, but temperature is still likely the primary underlying cause. If other factors were largely responsible for the apparent effects of temperature, it would be expected that strong correlations with temperature would also be evident in their fish data. However, as discussed above, the fish data usually showed much weaker effects of temperature, similar to other studies with fewer confounding factors.

These invertebrate acute data were analyzed using the same regression model and techniques as discussed above for fish. The study of Arthur et al. (1987) included data sets for nine invertebrate species, but two of these sets were not included in the analysis because they consisted of two tests at temperatures only 3°C apart. For the other species, the number of tests ranged from 2 to 6, with temperature ranges of from 9°C to 21°C. Table M.1 summarizes the regression results for the data sets of each species and for pooled analyses conducted on (a) all seven species, and (b) three species that had more than two tests and a temperature range of at least 15°C. All data were corrected to pH 8 based on the average acute pH relationship (described later). All species show substantially greater tolerance to ammonia at lower temperatures, and in most cases the significance level of the regression is better than 0.05. (As for the analysis of the fish data, when there were just two tests for a species, the significance level for the individual analysis is based on the MSE from the pooled analysis.) The slope of log LC_{50} versus temperature does not vary widely, ranging from -0.028 to -0.046 and being -0.036 for both pooled analyses. Figure M.6 provides plots of this data and the regression lines comparable to those for fish previously shown in Figures M.3 and M.4.

Again, because invertebrates are not among the species acutely sensitive to ammonia (in the 1999 update), the invertebrate acute temperature slope does not affect the formulation of the acute criterion. It will be used subsequently, however, in formulating the invertebrate *chronic* temperature slope, which ultimately will affect the formulation of the chronic criterion.

1999 Re-examination of Temperature Dependence – Chronic Toxicity

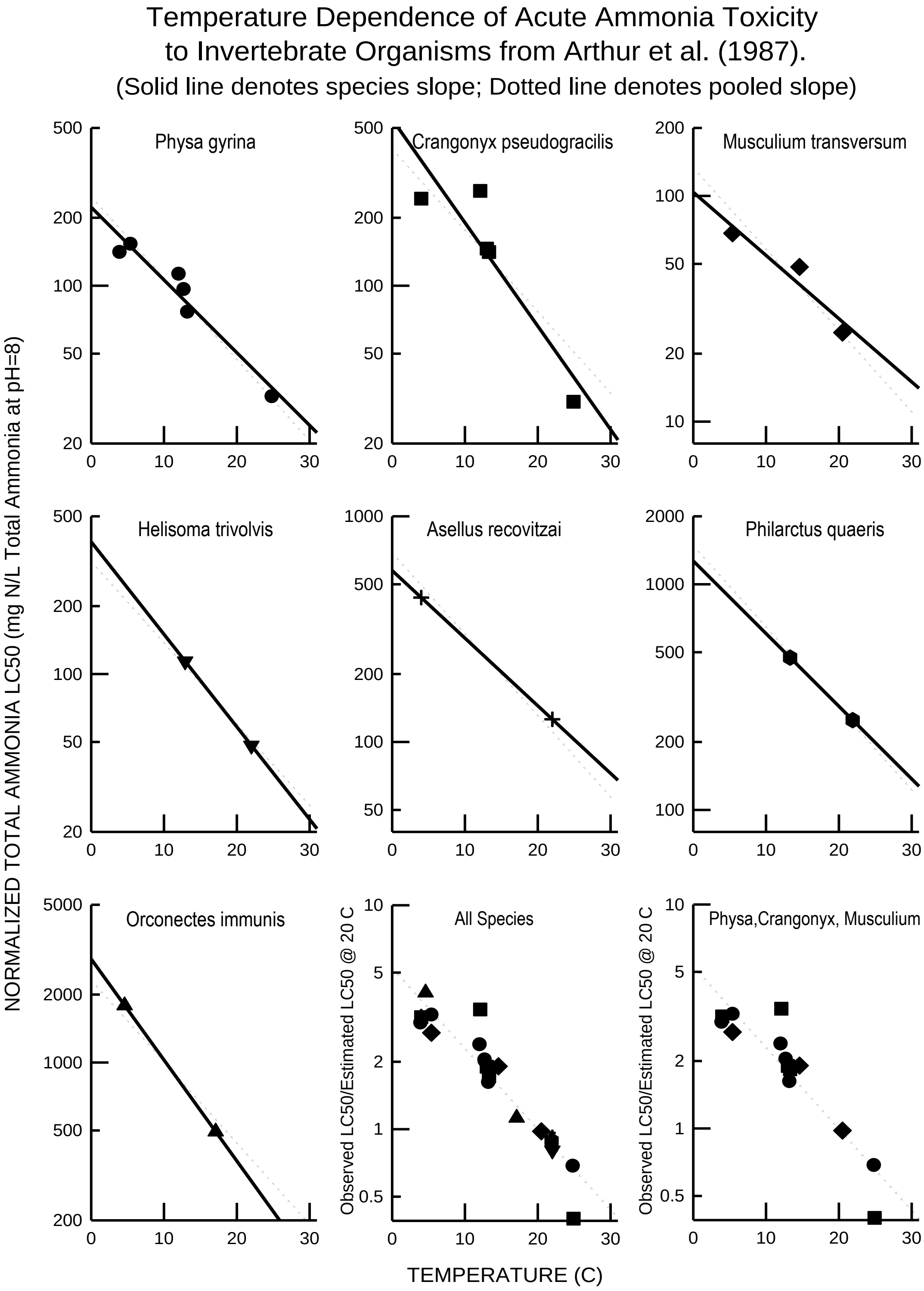
Fish chronic data

As in the 1998 Update, the only available data on the temperature dependence of chronic ammonia toxicity are from the study by DeGraeve et al. (1987) on survival of juvenile fathead minnows during 30-day exposures to ammonia at temperatures ranging from 6°C to 30°C. In contrast to acute toxicity, which for fathead minnows showed sensitivity to be slightly *reduced* at low temperatures, this data on chronic toxicity suggested *greater* sensitivity at low temperatures. However, this trend was small, at least once the confounding effect of pH was corrected for, and not statistically significant. Based on this analysis, the 1998 Update treated effect concentrations for chronic ammonia toxicity to fish as it did for acute toxicity: as being invariant with temperature. However, the 1998 Update also noted that, if seasonal variations in temperature cause a shift in what endpoints the criterion should be based on, the chronic criterion could have a seasonal temperature dependence even if effect concentrations for specific chronic endpoints do not vary with temperature (This is discussed in the 1999 AQWC document under the section named Seasonality of Chronic Toxicity Endpoints).

This section will provide more details regarding the analysis of the chronic toxicity data from DeGraeve et al. (1987), and a comparison of its temperature dependence to that of acute toxicity in the same study. Figure M.5 showed the temperature dependence of acute and chronic effect concentrations from this study.

An important issue in this analysis is the confounding effect of pH on the apparent effect of temperature, because pH increased with decreasing temperature in these chronic exposures. To examine what the effect of temperature is, the effect concentrations should be adjusted to a common pH using an equation that accounts for the effect of pH. A critical question then is what pH equation to use, because no study exists for the effect of pH on this particular chronic endpoint (juvenile 30-day survival), or on the interaction of pH and temperature effects. The 1998 Update used the pH relationship derived for the chronic criterion. Of the pH relationships available, that one is probably most appropriate, but entails some uncertainty. To evaluate how conclusions about temperature effects will vary if the true pH relationship is different, this analysis will also use the pH relationship for acute toxicity to fathead minnows from Thurston et al. (1983). This relationship likely represents an extreme possibility; i.e., it assumes that chronic toxicity pH relationships are the same acute ones, contrary to what is indicated by available chronic studies.

Figure M.6. Temperature Dependence of Acute Ammonia Toxicity to Invertebrate Organisms from Arthur et al. (1987).

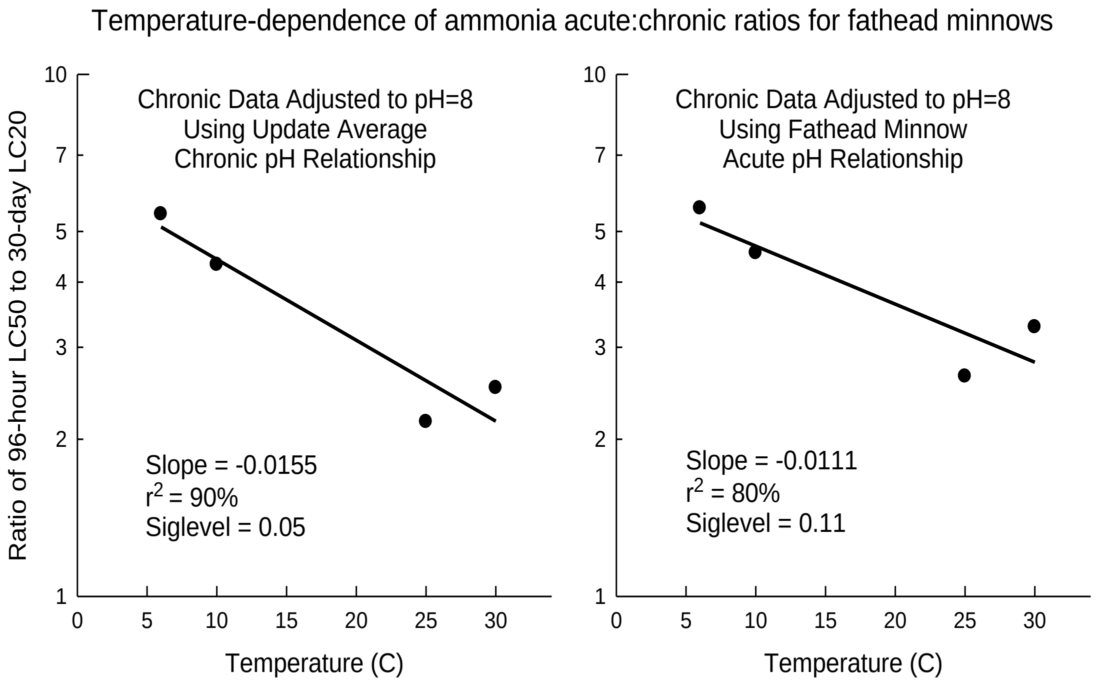


Using the pooled chronic pH relationship (presented later in this document), slope=0.010, significance=0.13, and r^2=0.76. Using the fathead minnow acute pH relationship, slope=0.0053, significance=0.32, and r^2=0.45. In neither case is the regression statistically significant at the 5 percent level, due to the amount and variability of the data. Nevertheless, it should be noted that, in both cases, the chronic data show an upward trend with temperature, in contrast to that observed for acute toxicity. Even under the extreme assumption that these data have a pH relationship similar to acute toxicity, the slope is 0.005, and is twice this under the assumption that these data follow the chronic pH relationship. Thus, even if fathead minnows show increased *acute* tolerance to ammonia at low temperatures, a similar assumption for *chronic* toxicity is contraindicated.

The difference between acute and chronic temperature relationships can be better assessed by looking at acute-chronic ratios. Figure M.7 shows the temperature dependence of the ratio of the acute LC_{50} to the chronic LC_{20}. The chronic LC_{20}s used for the ACRs were normalized via the above two alternative pH relationships, while the acute LC_{50}s were normalized only using the acute pH relationship. The results show that for either pH normalization alternative, the ACRs are substantially higher at lower temperatures than at higher temperatures. If the chronic data are pH-normalized using the chronic pH relationship, the regression is significant at the 0.05 level, with a slope of -0.0155. If normalized using the acute pH relationship, the slope is less (-0.0110), but even with this extreme assumption, there is only a 13 percent probability that the regression slope arose by chance.

It is not surprising that acute-chronic ratios are higher at low temperature. Temperature can affect toxicity in a variety of ways, one of which is simply to slow down responses. This is evident in some reports on the effect of temperature on ammonia toxicity. For example, for the rainbow trout data from Ministry of Technology (1967), there was little effect of temperature on total ammonia LC_{50}s at 96 hours, but at shorter durations LC_{50}s increased with decreasing temperature. The overall impact on the temperature dependence of LC_{50}s and ACRs will depend on the duration of the acute toxicity test and on the speed of action of acute ammonia toxicity in the species of concern. However, temperature is likely to affect ammonia toxicity in multiple ways, some of which would alter acute and chronic toxicity similarly. Nonetheless, to some degree the ratio of effect concentrations at different durations is expected to increase at lower temperatures. This expectation, as well as the empirical evidence, argues against the direct application of acute temperature relationships to chronic toxicity.

Figure M.7. Temperature-Dependence of Ammonia ACRs for Fathead Minnows. (The choice of reference condition, pH=8 here versus pH=7.5 in Figure M.5, has no effect on slope or significance.)



Invertebrate chronic projections

No data are available on the effect of temperature on chronic ammonia toxicity to invertebrates. Because invertebrates are much more acutely tolerant at low temperatures than at high temperatures, it is likely that their chronic toxicity would also show some temperature dependence. However, as discussed above, there is reason to expect acute and chronic toxicity to vary somewhat differently with temperature, with acute-chronic ratios increasing at low temperature, especially for organisms for which acute ammonia toxicity is not especially fast, which is the case for invertebrates (Thurston et al. 1984b). The observed trend in the fathead minnow ACRs provides support for this expectation.

The critical question then becomes, how much of the acute temperature slope for invertebrates should be assumed to apply to chronic toxicity? If this slope is predominantly due to temperature-induced delays of acute toxicity, chronic toxicity might have very little slope. If this slope is not at all due to such delays, then all the slope should be applied to chronic toxicity.

One option for an objective mathematical prediction of the invertebrate chronic slope is to assume that the difference between acute and chronic slopes will be the same for fish and invertebrates, potentially implying that the effect of temperature on the kinetics of toxicity is roughly the same for fish and invertebrates. In this case the invertebrate chronic slope would be the difference between -0.036, the average invertebrate acute slope, and -0.016, the observed slope for fish acute-chronic ratios. This would yield an invertebrate chronic slope of -0.020. This correction still applies most of the acute slope to chronic toxicity, but recognizes that the chronic slope should probably be less steep.

It is recognized that few data are available to define the Figure M.7 fish ACR slope, and that the assumption the invertebrate ACR slope would equal the fish ACR slope is quite uncertain despite having some theoretical underpinning in the kinetics of toxicity. Consequently a second option is to equate the invertebrate chronic slope to the invertebrate acute slope (-0.036) minus one-half the fish ACR slope (-0.016/2). This splits the difference between no correction and full correction for the fish ACR slope, resulting in an invertebrate chronic slope of -0.028.

A third, related option is suggested from the appearance of data in the last two plots in Figure M.6, plots of "All Species" and "Physa, Crangonyx, Musculium". These plots suggest a steeper invertebrate acute slope at higher temperatures than at very low temperature. At greater than 10°C, these data also comfortably fit a slope of -0.044. If such a slope were used to fit those data, however, a concentration plateau would need to be imposed between 5 and 10°C to avoid over-estimating the acute effect concentrations measured near 5°C. If the invertebrate chronic slope is obtained by subtracting the full value of the fish ACR slope (-0.016), this would yield the same invertebrate chronic slope, -0.028, as the option in the previous paragraph. In this case, however, concentrations would be capped between 5 and 10°C in order to reflect the implied attenuation of slope at low temperature relative to higher temperatures.

EPA selected this third option, a compromise between the first two options, for defining the invertebrate chronic temperature slope in formulating the CCC, discussed later. This provides a good fit to the available information.

Appendix N. Site-Specific Criteria for Ammonia.

Recalculation Procedure for Site-specific Criteria Derivation

The water quality standards (WQS) regulation at 40 CFR § 131.11(b)(1)(ii) provides states with the opportunity to adopt water quality criteria that are "…modified to reflect site-specific conditions." As with any criteria, site-specific criteria must be based on a sound scientific rationale in order to protect the designated use and are subject to review and approval or disapproval by EPA.

The recalculation procedure for site-specific criteria derivation is intended to allow site-specific criteria that differ from national criteria recommendations (i.e., concentrations that are higher or lower than national recommendations) where there are demonstrated differences in sensitivity between the aquatic species that occur at the site and those that were used to derive the national criteria recommendations. The national dataset may contain aquatic species that are sensitive to a particular pollutant, but these or comparably sensitive species might not occur at the site (e.g., freshwater mussels are included in the national ammonia dataset but may not be present at a particular site). On the other hand, a species that is critical at the site might be sensitive to the pollutant and require site-specific criteria that are lower than the national recommended criteria.

In the case of ammonia, where a state demonstrates that mussels are not present on a site-specific basis, the recalculation procedure may be used to remove the mussel species from the national criteria dataset to better represent the species present at the site. For example, many of the commonly occurring freshwater bivalves (e.g., pea clam) are more closely related to the non-unionid fingernail clam *Musculium* (which is the fourth most sensitive genus in the national dataset for the chronic criterion) than to the unionid mussels *Lampsilis* and *Villosa* (which are the two most sensitive genera in the national dataset for the chronic criterion). At sites where all bivalves present are more closely related to *Musculium* than to *Lampsilis* and *Villosa* (i.e., where unionid mussels are not present at the site), the recalculation procedure may be used to remove *Lampsilis* and *Villosa* from the dataset because they would not be representative of the species present at the site. With removal of *Lampsilis* and *Villosa* from the national dataset, the recalculation procedure could result in criteria (and associated water quality-based effluent limits (WQBELs) based on such criteria) with higher concentrations than EPA's recommendations but that are still protective of the designated use. The retention of *Musculium* in the dataset would represent the other non-unionid bivalves present at the site, so the non-unionid bivalves would still be protected if *Lampsilis* and *Villosa* were removed from the chronic dataset. However, at sites where both unionid and non-unionid bivalves are present, all three bivalves in the national chronic dataset (i.e., *Lampsilis*, *Villosa*, and *Musculium*) would be retained because they would represent the species present at the site. The recalculation procedure describes how to compare the taxonomy of species present at the site with the taxonomy of species in the national dataset.

The number of tested genera (N) in the criteria calculations must be updated where genera such as *Lampsilis* and *Villosa* are removed from the dataset. For example, if only the two unionid mussels are removed from the dataset for the national chronic ammonia criterion, N would be reduced from 16 genera in the national dataset to 14 genera in the site-specific dataset, and this would affect the site-specific criteria values.

Freshwater snails represent another sensitive freshwater species group for which acute and chronic toxicity data exist and are used in criteria derivation. Because freshwater snails tend to be more ubiquitous in the environment, however, the existing data for these animals are not likely to be deleted from the datasets in a criteria recalculation.

As with any criteria, states choosing to utilize the recalculation procedure should ensure that their site-specific criteria "…provide for the attainment and maintenance of the water quality standards of downstream waters." 40 CFR § 131.10(b). In addition, states should consider how they will demonstrate that mussels are not present at the site before selecting this approach. For additional information on the recalculation procedure, see EPA's *Water Quality Standards Handbook* at http://www.epa.gov/wqshandbook.

Acute Criterion Magnitude Recalculation for Ammonia

Unionid Mussels Present and Oncorhynchus species Absent

Where *Oncorhynchus* species are absent, EPA does not lower its acute criteria for ammonia below the 5th percentile in order to protect the commercially and recreationally important adult rainbow trout, but instead, retains all tested species in the Order Salmoniformes as tested surrogate species representing untested freshwater fish resident in the U.S. from another Order. The lowest GMAV for a freshwater fish (vertebrate species) is 51.93 mg TAN/L for *Prosopium* (Table 3). Therefore, in this case, the CMC equals the lower of: a) 0.7249 times the temperature adjusted lowest invertebrate GMAV (e.g., 17 mg TAN/L at pH 7.0 and 20º C), or (b) 0.7249 times the lowest freshwater fish GMAV (e.g., 38 mg TAN/L at pH 7.0), according to the following temperature relationship:

$$CMC = 0.7249 \times MIN\left(51.93, 23.12 \times 10^{0.036\times(20-T)}\right)$$

Thus, the CMC increases with decreasing temperature as a result of increased invertebrate insensitivity until it reaches a plateau of 37.65 mg TAN/L at 10.2°C and below (51.93 mg TAN/L x 0.7249), where the most sensitive taxa switches to the temperature invariant fish genus *Prosopium* (Tables 5b and N.1; see also *Oncorhynchus* absent line in Figure 5a). Note: while the mountain whitefish (*Prosopium williamsoni*) is a species in the same family as *Oncorhynchus* sp. (i.e., Family: Salmonidae), it is also an appropriately sensitive surrogate species amongst all freshwater fish in the Class Actinopterygii.

The CMC where *Oncorhynchus* sp. are absent extrapolated across both temperature and pH is as follows:

$$CMC = 0.7249 \times \left(\frac{0.0114}{1+10^{7.204-pH}} + \frac{1.6181}{1+10^{pH-7.204}}\right) \times MIN\left(51.93, 23.12 \times 10^{0.036\times(20-T)}\right)$$

When a threatened or endangered species occurs at a site and sufficient data indicate that it is sensitive at 1-hour average concentrations below the CMC, it is appropriate to consider deriving a site-specific criterion. It should be noted that the dataset used to derive these new ammonia criteria included some threatened or endangered species, none of which were the most sensitive of the species tested. Extrapolated values across a range of temperatures and pH values are presented in Table N.1.

Unionid Mussels Absent and Oncorhynchus spp. Present

If a state can demonstrate that unionid mussels are not present at a site, a site-specific criteria can be calculated for waters with mussels absent. It is important to recognize that for site-specific criteria derived where unionid mussels are absent, the commercially and recreationally important adult rainbow trout (*Oncorhynchus mykiss*) is the most acutely sensitive species. Thus, when *Oncorhynchus* spp. are present, the acute criterion cannot exceed 24 mg TAN/L (the SMAV for adult rainbow trout 48.21 mg TAN/L divided by two – see also *Acute Criterion Calculation* section in this document).

At pH 7, the temperature relationship is expressed as follows:

$$CMC = MIN\left(24.10, \left(45.05 \times 10^{0.036\times(20-T)}\right)\right)$$

Where 24.10 mg TAN/L is one half the SMAV of 48.21 mg TAN/L for adult rainbow trout, and 45.05 is 0.7249 (the CMC divided by the lowest GMAV in the complete acute dataset) multiplied by 62.15 mg TAN/L, the GMAV of the temperature dependent pebblesnail (*Fluminicola* sp.), the most sensitive non-mussel invertebrate (Table N.2).

At temperatures 0 - 27.5°C, the CMC with mussels absent and *Oncorhynchus* spp. present is 24.10 mg TAN/L, because adult rainbow trout remain the most sensitive species group in this temperature range. At temperatures greater than 27.5°C, however, the GMAV for *Fluminicola* species (62.15 mg TAN/L) becomes the most sensitive GMAV because invertebrates are increasingly more acutely-sensitive to ammonia as temperature increases, and thus, the CMC equals that of the mussels absent, *Oncorhynchus* sp. absent temperature relationship (Figure N.1). Consistent with the approach followed with the unionid mussels present, *Oncorhynchus* species absent CMC calculation in the *Acute Criterion Calculations* section of this document, the site-specific criteria should 1) retains all tested species in the Order Salmoniformes as tested surrogate species representing untested freshwater fish resident in the U.S. from another Order; and 2) maintains the SSD relationship from the complete acute dataset (i.e., CMC is equal to the lowest GMAV times 0.7249).

The CMC, where mussels are absent and *Oncorhynchus* spp. are present, extrapolated across both temperature and pH is as follows (extrapolated values provided Table N.3):

$$CMC = MIN\left(\left(\frac{0.275}{1+10^{7.204-pH}}+\frac{39}{1+10^{pH-7.204}}\right), \left(0.7249 \times \left(\frac{0.0114}{1+10^{7.204-pH}}+\frac{1.6181}{1+10^{pH-7.204}}\right) \times 62.15 \times 10^{0.036\times(20-T)}\right)\right)$$

Unionid Mussels Absent and Oncorhynchus spp. Absent

If both unionid mussels and *Oncorhynchus spp.* are absent, the CMC calculated using the Guidelines algorithm is 30.25 mg TAN/L at pH 7 and 20°C and is based on the four most sensitive GMAVs in the following rank order: mountain whitefish, Lost River sucker, pebblesnail, and golden shiner (see Table N.2). The ratio of the mussels absent and *Oncorhynchus* spp. absent CMC to the most sensitive GMAV (i.e., mountain whitefish; *Prosopium* sp.) is 0.5825, or 30.25 mg TAN/L divided by 51.93 mg TAN/L. However, this would result in a more protective criterion than when *Oncorhynchus* spp. are absent but mussels are present (see *Acute Criterion Calculations*). Because the unionid mussels absent and *Oncorhynchus* spp. absent CMC cannot be more protective than the unionid mussels present and *Oncorhynchus* spp. absent CMC, the CMC to lowest GMAV ratio of 0.7249 from the complete acute dataset is multiplied by 51.93 mg TAN/L for *Prosopium* sp., the lowest GMAV in the unionid mussels absent dataset, resulting in a calculated CMC of 37.65 mg TAN/L at pH 7 and 20°C. This is equivalent to the maximum plateau CMC when mussels are present and *Oncorhynchus* spp. are absent at temperatures of 10.2°C and below (compare in Figures 5a and N.1).

At pH 7, the temperature relationship is expressed as follows:

$$CMC = 0.7249 \times MIN\left(51.93, \left(62.15 \times 10^{0.036\times(20-T)}\right)\right)$$

At temperatures between 0-22.1°C the CMC with unionid mussels and *Oncorhynchus* spp. absent is 37.65 mg TAN/L. At temperatures greater than 22.1°C, the temperature dependent pebblesnail (*Fluminicola* sp.) becomes the most sensitive GMAV, and the CMC decreases with increasing temperature (Figure N.1).

The CMC, where both unionid mussels and *Oncorhynchus* spp. are absent, extrapolated across both temperature and pH is as follows (extrapolated values provided Table N.4):

$$CMC = 0.7249 \times \left(\frac{0.0114}{1 + 10^{7.204-pH}} + \frac{1.6181}{1 + 10^{pH-7.204}}\right) \times MIN\left(51.93, \left(62.15 \times 10^{0.036\times(20-T)}\right)\right)$$

A summary of the acute criterion recalculations for all four mussel and Salmonid present and absent combinations at pH 7 and 20°C is included in Table N.5.

Chronic Criterion Magnitude Recalculation for Ammonia

Unionid Mussels Absent, Early Life Stage (ELS) Protection Necessary

When unionid mussels are present, the CCC is the same regardless of whether early life stages (ELS) of fish genera require protection. This is because unionid mussels represent the two most sensitive genera in the chronic dataset, and at pH 7, the CCC at the invertebrate temperature plateau of 7°C is 4.363 mg TAN/L, which is lower than the GMCV for *Lepomis*, the most sensitive fish genera, multiplied by the CCC to lowest GMCV ratio (or 6.920 mg TAN/L x 0.8876 = 6.142 mg TAN/L – see *Chronic Criterion Calculations* for additional details).

When unionid mussels are absent and fish ELS require protection, however, the CCC is 6.508 mg TAN/L at pH 7 and 20°C (Tables N.6, N.7). The lowest GMCV is 6.920 mg TAN/L for the temperature invariant vertebrate genus *Lepomis*, and the most sensitive invertebrate GMCV is 7.547 mg TAN/L for *Musculium* (Table N.6). The ratio of the CCC to the most sensitive GMCV (*Lepomis* sp.) when unionid mussels are absent is 0.9405, or 6.508 mg TAN/L divided by 6.920 mg TAN/L. At pH 7 and 20°C, the CCC when mussels are absent and ELS protection is required is expressed as follows:

$$CCC = 0.9405 \times MIN\left(6.920, \left(7.547 \times 10^{0.028\times(20-T)}\right)\right)$$

This function remains constant at a CCC equal to 6.508 mg TAN/L at 0-21.3°C because the most sensitive GMCV is for the temperature invariant genera *Lepomis* (Figure N.2; Table N.6). At temperatures greater than 21.3°C, the GMCV for the invertebrate *Musculium* (i.e., 7.547 mg TAN/L) becomes the most sensitive, and the CCC decreases with increasing temperature (Figure N.2).

When unionid mussels are absent and ELS protection is required, the thirty-day average concentration of ammonia nitrogen (in mg TAN/L) does not exceed, more than once every three years on the average, the CCC calculated using the following equation:

$$CCC = 0.9405 \times \left(\frac{0.0278}{1 + 10^{7.688-pH}} + \frac{1.1994}{1 + 10^{pH-7.688}}\right) \times MIN\left(6.920, \left(7.547 \times 10^{0.028\times(20-T)}\right)\right)$$

Recalculated chronic criterion concentrations for the mussels absent, fish ELS protection necessary scenario across a range of temperatures and pH values are provided in Table N.8.

Unionid Mussels Absent, Early Life Stage (ELS) Protection Not Necessary

One approach for setting a chronic criterion for mussels absent and *fish ELS absent* is to modify the criterion for mussels absent and *fish ELS present*. The four most sensitive genera for the criterion to be modified are *Lepomis* (ELS), *Musculium*, *Fluminicola*, and *Pimephales* (ELS), which had yielded a criterion of 6.508 mg TAN/L at pH 7.0 and 20º C, or 0.9405 x the lowest GMCV (*Lepomis*). Since the *Lepomis* GMCV, 6.920 mg TAN/L, is based on ELS sensitivity, consider that with ELS absent this value would increase to its juvenile and adult GMCV of 21.3 mg TAN/L (from U.S. EPA 1999, page 75 GMCVs, translated from pH 8 to pH 7). In this case, *Musculium*, with GMCV 7.547 mg TAN/L, would now be the most sensitive genus in the dataset, such that at pH 7 and 20ºC the criterion could be calculated as 0.9405 x 7.547 = 7.098 mg TAN/L. Because *Musculium* remains the most sensitive genus throughout the full range of temperatures, the criterion follows the invertebrate temperature relationship, increasing with decreasing temperature until it reaches its maximum at the built-in 7ºC plateau, which is 16.41 mg TAN/L at pH 7, fully protective of the lowest juvenile-adult fish GMCV, that for *Lepomis*, 21.3 mg TAN/L shown above.

Mussels absent ELS protection not required at pH 7

$$CCC = 0.9405 \times \left(7.547 \times 10^{0.028\times\left(20-MAX(T,7)\right)}\right)$$

Overall

$$CCC = 0.9405 \times \left(\frac{0.0278}{1 + 10^{7.688-pH}} + \frac{1.1994}{1 + 10^{pH-7.688}}\right) \times \left(7.547 \times 10^{0.028\times\left(20-MAX(T,7)\right)}\right)$$

Recalculated chronic criterion concentrations for the mussels absent, fish ELS protection not required scenario across a range of temperatures and pH values are provided in Table N.9. A summary of the chronic criterion recalculations for all four mussel and fish ELS present and absent combinations at pH 7 and 20°C is included in Table N.7.

Table N.1. Temperature and pH-Dependent Values of the CMC (Acute Criterion Magnitude) – Unionid Mussels Present, *Oncorhynchus* Absent.

	Temperature (°C)																				
pH	0-10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
6.5	51	48	44	41	37	34	32	29	27	25	23	21	19	18	16	15	14	13	12	11	9.9
6.6	49	46	42	39	36	33	30	28	26	24	22	20	18	17	16	14	13	12	11	10	9.5
6.7	46	44	40	37	34	31	29	27	24	22	21	19	18	16	15	14	13	12	11	9.8	9.0
6.8	44	41	38	35	32	30	27	25	23	21	20	18	17	15	14	13	12	11	10	9.2	8.5
6.9	41	38	35	32	30	28	25	23	21	20	18	17	15	14	13	12	11	10	9.4	8.6	7.9
7.0	38	35	33	30	28	25	23	21	20	18	**17**	15	14	13	12	11	10	9.4	8.6	7.9	7.3
7.1	34	32	30	27	25	23	21	20	18	17	15	14	13	12	11	10	9.3	8.5	7.9	7.2	6.7
7.2	31	29	27	25	23	21	19	18	16	15	14	13	12	11	9.8	9.1	8.3	7.7	7.1	6.5	6.0
7.3	27	26	24	22	20	18	17	16	14	13	12	11	10	9.5	8.7	8.0	7.4	6.8	6.3	5.8	5.3
7.4	24	22	21	19	18	16	15	14	13	12	11	9.8	9.0	8.3	7.7	7.0	6.5	6.0	5.5	5.1	4.7
7.5	21	19	18	17	15	14	13	12	11	10	9.2	8.5	7.8	7.2	6.6	6.1	5.6	5.2	4.8	4.4	4.0
7.6	18	17	15	14	13	12	11	10	9.3	8.6	7.9	7.3	6.7	6.2	5.7	5.2	4.8	4.4	4.1	3.8	3.5
7.7	15	14	13	12	11	10	9.3	8.6	7.9	7.3	6.7	6.2	5.7	5.2	4.8	4.4	4.1	3.8	3.5	3.2	2.9
7.8	13	12	11	10	9.3	8.5	7.9	7.2	6.7	6.1	5.6	5.2	4.8	4.4	4.0	3.7	3.4	3.2	2.9	2.7	2.5
7.9	11	9.9	9.1	8.4	7.7	7.1	6.6	3.0	5.6	5.1	4.7	4.3	4.0	3.7	3.4	3.1	2.9	2.6	2.4	2.2	2.1
8.0	8.8	8.2	7.6	7.0	6.4	5.9	5.4	5.0	4.6	4.2	3.9	3.6	3.3	3.0	2.8	2.6	2.4	2.2	2.0	1.9	1.7
8.1	7.2	6.8	6.3	5.8	5.3	4.9	4.5	4.1	3.8	3.5	3.2	3.0	2.7	2.5	2.3	2.1	2.0	1.8	1.7	1.5	1.4
8.2	6.0	5.6	5.2	4.8	4.4	4.0	3.7	3.4	3.1	2.9	2.7	2.4	2.3	2.1	1.9	1.8	1.6	1.5	1.4	1.3	1.2
8.3	4.9	4.6	4.3	3.9	3.6	3.3	3.1	2.8	2.6	2.4	2.2	2.0	1.9	1.7	1.6	1.4	1.3	1.2	1.1	1.0	0.96
8.4	4.1	3.8	3.5	3.2	3.0	2.7	2.5	2.3	2.1	2.0	1.8	1.7	1.5	1.4	1.3	1.2	1.1	1.0	0.93	0.86	0.79
8.5	3.3	3.1	2.9	2.7	2.4	2.3	2.1	1.9	1.8	1.6	1.5	1.4	1.3	1.2	1.1	0.98	0.90	0.83	0.77	0.71	0.65
8.6	2.8	2.6	2.4	2.2	2.0	1.9	1.7	1.6	1.5	1.3	1.2	1.1	1.0	0.96	0.88	0.81	0.75	0.69	0.63	0.58	0.54
8.7	2.3	2.2	2.0	1.8	1.7	1.6	1.4	1.3	1.2	1.1	1.0	0.94	0.87	0.80	0.74	0.68	0.62	0.57	0.53	0.49	0.45
8.8	1.9	1.8	1.7	1.5	1.4	1.3	1.2	1.1	1.0	0.93	0.86	0.79	0.73	0.67	0.62	0.57	0.52	0.48	0.44	0.41	0.37
8.9	1.6	1.5	1.4	1.3	1.2	1.1	1.0	0.93	0.85	0.79	0.72	0.67	0.61	0.56	0.52	0.48	0.44	0.40	0.37	0.34	0.32
9.0	1.4	1.3	1.2	1.1	1.0	0.93	0.86	0.79	0.73	0.67	0.62	0.57	0.52	0.48	0.44	0.41	0.37	0.34	0.32	0.29	0.27

Table N.2. Acute Data Without Mussels: Comparison of the Four Taxa Used to Calculate the FAV and CMC in the 1999 AWQC and this Updated 2013 AWQC Excluding Data for Freshwater Unionid Mussels.

1999 Draft Update Acute Criterion (CMC) Magnitude (Salmonids [*Oncorhynchus* spp.] present)			**2013 Final Acute Criterion (CMC) Magnitude excluding Mussels (Salmonids [*Oncorhynchus* spp.] absent)**	
Species	**GMAV pH 8.0, T=25°C (mg TAN/L)**	**GMAV pH 7.0, T=20°C (mg TAN/L)**	**Species**	**GMAV pH 7.0, T=20°C (mg TAN/L)**
Oncorhynchus sp. (salmonids), includes: *O. aquabonita, O. clarkii, O. gorbuscha, O. kisutch*, O. mykiss**, and *O. tshawytscha**	21.95	99.15	Golden shiner, *Notemigonus crysoleucas*	63.02
Orangethroat darter, *Etheostoma spectabile*	17.96	74.25	Pebblesnail, *Fluminicola* sp.	62.15
Golden shiner, *Notemigonus crysoleucas*	14.67	63.02	Lost River sucker, *Deltistes luxatus**	56.62
Mountain whitefish, *Prosopium williamsoni*	12.11	51.93	Mountain whitefish, *Prosopium williamsoni*	51.93
FAV	11.23	48.21	FAV	76
CMC	5.6	**24**	**CMC**	**38****

*Federally-listed as endangered or threatened species

******CMC Excluding mussels, with Oncorhynchus present is 24 mg TAN/L to protect the recreationally and commercially important species Rainbow Trout. When Oncorhynchus is absent, the CMC is based on the mountain whitefish and is calculated by the ratio of the CMC to the lowest GMAV in the complete acute dataset (0.7249) times the lowest GMAV in the dataset excluding mussels (51.93 mg TAN/L for mountain whitefish) which results in a CMC of 37.65 mg TAN/L at pH 7 and 20°C.

Table N.3. Temperature and pH-Dependent Values of the CMC (Acute Criterion Magnitude) – Unionid Mussels Absent and *Oncorhynchus* Present.

	Temperature (°C)																
pH	0-14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
6.5	33	33	33	33	33	33	33	33	33	33	33	33	33	33	31	29	27
6.6	31	31	31	31	31	31	31	31	31	31	31	31	31	31	30	28	26
6.7	30	30	30	30	30	30	30	30	30	30	30	30	30	30	29	26	24
6.8	28	28	28	28	28	28	28	28	28	28	28	28	28	28	27	25	23
6.9	26	26	26	26	26	26	26	26	26	26	26	26	26	26	25	23	21
7.0	24	24	24	24	24	24	**24**	24	24	24	24	24	24	24	23	21	20
7.1	22	22	22	22	22	22	22	22	22	22	22	22	22	22	21	19	18
7.2	20	20	20	20	20	20	20	20	20	20	20	20	20	20	19	17	16
7.3	18	18	18	18	18	18	18	18	18	18	18	18	18	18	17	16	14
7.4	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	14	13
7.5	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	12	11
7.6	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	10	9.3
7.7	9.6	9.6	9.6	9.6	9.6	9.6	9.6	9.6	9.6	9.6	9.6	9.6	9.6	9.6	9.3	8.6	7.9
7.8	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	7.8	7.2	6.6
7.9	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.5	6.0	5.5
8.0	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.4	5.0	4.6
8.1	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.5	4.1	3.8
8.2	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.7	3.4	3.1
8.3	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.0	2.8	2.6
8.4	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.5	2.3	2.1
8.5	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	1.9	1.8
8.6	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.7	1.6	1.4
8.7	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.4	1.3	1.2
8.8	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.1	1.0
8.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.92	0.85
9.0	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.85	0.78	0.72

Table N.4. Temperature and pH-Dependent Values of the CMC (Acute Criterion Magnitude) – Unionid Mussels Absent and *Oncorhynchus* Absent.

	Temperature (°C)																
pH	0-14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
6.5	51	51	51	51	51	51	51	51	51	48	44	40	37	34	31	29	27
6.6	49	49	49	49	49	49	49	49	49	46	42	39	36	33	30	28	26
6.7	46	46	46	46	46	46	46	46	46	43	40	37	34	31	29	26	24
6.8	44	44	44	44	44	44	44	44	44	41	38	35	32	29	27	25	23
6.9	41	41	41	41	41	41	41	41	41	38	35	32	30	27	25	23	21
7.0	38	38	38	38	38	38	**38**	38	38	35	32	30	27	25	23	21	20
7.1	34	34	34	34	34	34	34	34	34	32	29	27	25	23	21	19	18
7.2	31	31	31	31	31	31	31	31	31	29	26	24	22	21	19	17	16
7.3	27	27	27	27	27	27	27	27	27	26	23	22	20	18	17	16	14
7.4	24	24	24	24	24	24	24	24	24	22	21	19	17	16	15	14	13
7.5	21	21	21	21	21	21	21	21	21	19	18	16	15	14	13	12	11
7.6	18	18	18	18	18	18	18	18	18	17	15	14	13	12	11	10	9.3
7.7	15	15	15	15	15	15	15	15	15	14	13	12	11	10	9.3	8.6	7.9
7.8	13	13	13	13	13	13	13	13	13	12	11	10	9.2	8.5	7.8	7.2	6.6
7.9	11	11	11	11	11	11	11	11	11	9.9	9.1	8.4	7.7	7.1	6.5	6.0	5.5
8.0	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.2	7.5	6.9	6.4	5.9	5.4	5.0	4.6
8.1	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	6.8	6.2	5.7	5.3	4.9	4.5	4.1	3.8
8.2	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	5.6	5.1	4.7	4.4	4.0	3.7	3.4	3.1
8.3	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.6	4.2	3.9	3.6	3.3	3.0	2.8	2.6
8.4	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	3.8	3.4	3.2	3.0	2.7	2.5	2.3	2.1
8.5	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.1	2.9	2.6	2.4	2.2	2.1	1.9	1.8
8.6	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.6	2.4	2.2	2.0	1.9	1.7	1.6	1.4
8.7	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.2	2.0	1.8	1.7	1.5	1.4	1.3	1.2
8.8	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.8	1.7	1.5	1.4	1.3	1.2	1.1	1.0
8.9	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.5	1.4	1.3	1.2	1.1	1.0	0.92	0.85
9.0	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.3	1.2	1.1	1.0	0.93	0.85	0.78	0.72

Table N.5. 2013 Acute Criterion Recalculations for Site-specific Criteria.

Acute Criterion Duration (1 hr average) at pH 7 and 20°C (mg TAN/L)	**Acute Criterion Magnitude (CMC) Oncorhynchus spp. (Rainbow Trout) Present**	**Acute Criterion Magnitude (CMC) Oncorhynchus spp. (Rainbow Trout) Absent**
Mussels Present	17	17
Mussels Absent	24	38
Frequency: Criteria values not to be exceeded more than once in three years.		

Table N.6. Chronic Dataset Without Mussels: Comparison of the Four Taxa used to Calculate the CCC in the 1999 AWQC and this Updated 2013 AWQC Excluding Data for Freshwater Unionid Mussels.

1999 Draft Update Chronic Criterion (CCC) Magnitude			**2013 Final Chronic Criterion (CCC) Magnitude excluding mussels**	
Species	**GMCV pH 8.0, T=25°C (mg TAN/L)**	**GMCV pH 7.0, T=20°C (mg TAN/L)**	**Species**	**GMCV pH 7.0, T=20°C (mg TAN/L)**
Fathead minnow, *Pimephales promelas*	3.09	7.503	Fathead minnow, *Pimephales promelas*	9.187
Lepomis sp. (Centrarchidae), includes: Bluegill sunfish, *L. macrochirus*, and Green sunfish, *L. cyanellus*	2.85	6.92	Pebblesnail, *Fluminicola* sp.	7.828
Long fingernailclam, *Musculium transversum*	<2.26	7.547	Long fingernailclam, *Musculium transversum*	7.547
Amphipod, *Hyalella azteca*	<1.45	4.865	*Lepomis* sp. (Centrarchidae), includes: Bluegill, *L. macrochirus* and Green sunfish, *L. cyanellus*	6.920
CCC	1.2	**4.5***	**CCC**	**6.5**

*Based on data renormalized to pH 7.0 and T 20°C

Table N.7. Chronic Criterion Recalculations for Site-Specific Criteria.

Chronic Criterion Duration (30-day average) at pH 7 and 20°C (mg TAN/L)	**Chronic Criterion Magnitude (CCC) Fish ELS Present**	**Chronic Criterion Magnitude (CCC) Fish ELS Absent**
Mussels Present	1.9	1.9
Mussels Absent	6.5	7.1
Not to exceed 2.5 times the CCC as a 4-day average within the 30-day averaging period.		
Frequency: Criteria values not to be exceeded more than once in three years.		

Table N.8. Temperature and pH-Dependent Values of the CCC (Chronic Criterion Magnitude) – Mussels Absent and Early Life Stage (ELS) Protection Necessary.

Temperature (°C)

pH	0-14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
6.5	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.0	6.6	6.2	5.8	5.4	5.1	4.8	4.5	4.2
6.6	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	6.9	6.5	6.1	5.7	5.4	5.0	4.7	4.4	4.1
6.7	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	6.8	6.4	6.0	5.6	5.3	4.9	4.6	4.3	4.1
6.8	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.6	6.2	5.8	5.5	5.1	4.8	4.5	4.2	4.0
6.9	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.5	6.1	5.7	5.3	5.0	4.7	4.4	4.1	3.9
7.0	6.5	6.5	6.5	6.5	6.5	6.5	6.5	**6.5**	6.2	5.8	5.5	5.1	4.8	4.5	4.2	4.0	3.7
7.1	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.0	5.6	5.3	4.9	4.6	4.3	4.1	3.8	3.6
7.2	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.7	5.3	5.0	4.7	4.4	4.1	3.9	3.6	3.4
7.3	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.4	5.0	4.7	4.4	4.1	3.9	3.6	3.4	3.2
7.4	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.0	4.7	4.4	4.1	3.9	3.6	3.4	3.2	3.0
7.5	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.6	4.3	4.1	3.8	3.6	3.3	3.1	2.9	2.8
7.6	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.2	3.9	3.7	3.5	3.2	3.0	2.9	2.7	2.5
7.7	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.8	3.5	3.3	3.1	2.9	2.7	2.6	2.4	2.3
7.8	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.4	3.2	3.0	2.8	2.6	2.4	2.3	2.1	2.0
7.9	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.0	2.8	2.6	2.4	2.3	2.1	2.0	1.9	1.8
8.0	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.6	2.4	2.3	2.1	2.0	1.9	1.7	1.6	1.5
8.1	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.2	2.1	1.9	1.8	1.7	1.6	1.5	1.4	1.3
8.2	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.1
8.3	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.6	1.5	1.4	1.3	1.2	1.2	1.1	1.0	0.96
8.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.3	1.2	1.1	1.1	0.99	0.93	0.87	0.81
8.5	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.1	1.0	0.95	0.89	0.83	0.78	0.73	0.69
8.6	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.97	0.91	0.85	0.80	0.75	0.70	0.66	0.62	0.58
8.7	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.82	0.77	0.72	0.68	0.64	0.60	0.56	0.52	0.49
8.8	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.70	0.65	0.61	0.58	0.54	0.51	0.47	0.44	0.42
8.9	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.60	0.56	0.52	0.49	0.46	0.43	0.41	0.38	0.36
9.0	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.51	0.48	0.45	0.42	0.40	0.37	0.35	0.33	0.31

Table N.9. Temperature and pH-Dependent Values of the CCC (Chronic Criterion Magnitude) – Mussels Absent and Early Life Stage (ELS) Protection not Necessary.

	Temperature (°C)																							
pH	0-7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
6.5	19	17	16	15	14	13	13	12	11	10	9.7	9.1	8.5	8.0	7.5	7.0	6.6	6.2	5.8	5.4	5.1	4.8	4.5	4.2
6.6	18	17	16	15	14	13	12	12	11	10	9.6	9.0	8.4	7.9	7.4	6.9	6.5	6.1	5.7	5.4	5.0	4.7	4.4	4.1
6.7	18	17	16	15	14	13	12	11	11	10	9.4	8.8	8.3	7.7	7.3	6.8	6.4	6.0	5.6	5.3	4.9	4.6	4.3	4.1
6.8	17	16	15	14	14	13	12	11	10	9.8	9.2	8.6	8.1	7.6	7.1	6.7	6.2	5.8	5.5	5.1	4.8	4.5	4.2	4.0
6.9	17	16	15	14	13	12	12	11	10	9.5	8.9	8.4	7.8	7.4	6.9	6.5	6.1	5.7	5.3	5.0	4.7	4.4	4.1	3.9
7.0	16	15	14	14	13	12	11	10	9.8	9.2	8.6	8.1	7.6	**7.1**	6.7	6.2	5.9	5.5	5.1	4.8	4.5	4.2	4.0	3.7
7.1	16	15	14	13	12	11	11	10	9.4	8.8	8.3	7.7	7.3	6.8	6.4	6.0	5.6	5.3	4.9	4.6	4.3	4.1	3.8	3.6
7.2	15	14	13	12	12	11	10	9.5	9.0	8.4	7.9	7.4	6.9	6.5	6.1	5.7	5.3	5.0	4.7	4.4	4.1	3.9	3.6	3.4
7.3	14	13	12	12	11	10	9.6	9.0	8.4	7.9	7.4	6.9	6.5	6.1	5.7	5.4	5.0	4.7	4.4	4.1	3.9	3.6	3.4	3.2
7.4	13	12	12	11	10	9.5	9.0	8.4	7.9	7.4	6.9	6.5	6.1	5.7	5.3	5.0	4.7	4.4	4.1	3.9	3.6	3.4	3.2	3.0
7.5	12	11	11	10	9.4	8.8	8.2	7.7	7.2	6.8	6.4	6.0	5.6	5.2	4.9	4.6	4.3	4.1	3.8	3.6	3.3	3.1	2.9	2.8
7.6	11	10	10	9.1	8.5	8.0	7.5	7.0	6.6	6.2	5.8	5.4	5.1	4.8	4.5	4.2	3.9	3.7	3.5	3.2	3.0	2.9	2.7	2.5
7.7	9.9	9.3	8.7	8.1	7.7	7.2	6.8	6.3	5.9	5.6	5.2	4.9	4.6	4.3	4.0	3.8	3.5	3.3	3.1	2.9	2.7	2.6	2.4	2.3
7.8	8.8	8.3	7.8	7.3	6.8	6.4	6.0	5.6	5.3	5.0	4.6	4.4	4.1	3.8	3.6	3.4	3.2	3.0	2.8	2.6	2.4	2.3	2.1	2.0
7.9	7.8	7.3	6.8	6.4	6.0	5.6	5.3	5.0	4.6	4.4	4.1	3.8	3.6	3.4	3.2	3.0	2.8	2.6	2.4	2.3	2.1	2.0	1.9	1.8
8.0	6.8	6.3	6.0	5.6	5.2	4.9	4.6	4.3	4.0	3.8	3.6	3.3	3.1	2.9	2.7	2.6	2.4	2.3	2.1	2.0	1.9	1.7	1.6	1.5
8.1	5.8	5.5	5.1	4.8	4.5	4.2	4.0	3.7	3.5	3.3	3.1	2.9	2.7	2.5	2.4	2.2	2.1	2.0	1.8	1.7	1.6	1.5	1.4	1.3
8.2	5.0	4.7	4.4	4.1	3.9	3.6	3.4	3.2	3.0	2.8	2.6	2.5	2.3	2.2	2.0	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.1
8.3	4.2	4.0	3.7	3.5	3.3	3.1	2.9	2.7	2.5	2.4	2.2	2.1	2.0	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.2	1.1	1.0	0.96
8.4	3.6	3.4	3.2	3.0	2.8	2.6	2.4	2.3	2.1	2.0	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.1	1.1	0.99	0.92	0.87	0.81
8.5	3.0	2.8	2.7	2.5	2.3	2.2	2.1	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.2	1.1	1.0	0.95	0.89	0.83	0.78	0.73	0.69
8.6	2.6	2.4	2.2	2.1	2.0	1.9	1.7	1.6	1.5	1.4	1.3	1.3	1.2	1.1	1.0	0.97	0.91	0.85	0.80	0.75	0.70	0.66	0.62	0.58
8.7	2.2	2.0	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.1	1.1	1.0	0.93	0.88	0.82	0.77	0.72	0.68	0.63	0.60	0.56	0.52	0.49
8.8	1.8	1.7	1.6	1.5	1.4	1.3	1.3	1.2	1.1	1.0	0.96	0.90	0.85	0.79	0.74	0.70	0.65	0.61	0.58	0.54	0.51	0.47	0.44	0.42
8.9	1.6	1.5	1.4	1.3	1.2	1.1	1.1	1.0	0.94	0.88	0.82	0.77	0.72	0.68	0.64	0.60	0.56	0.52	0.49	0.46	0.43	0.40	0.38	0.36
9.0	1.4	1.3	1.2	1.1	1.0	0.98	0.92	0.86	0.81	0.76	0.71	0.66	0.62	0.58	0.55	0.51	0.48	0.45	0.42	0.40	0.37	0.35	0.33	0.31

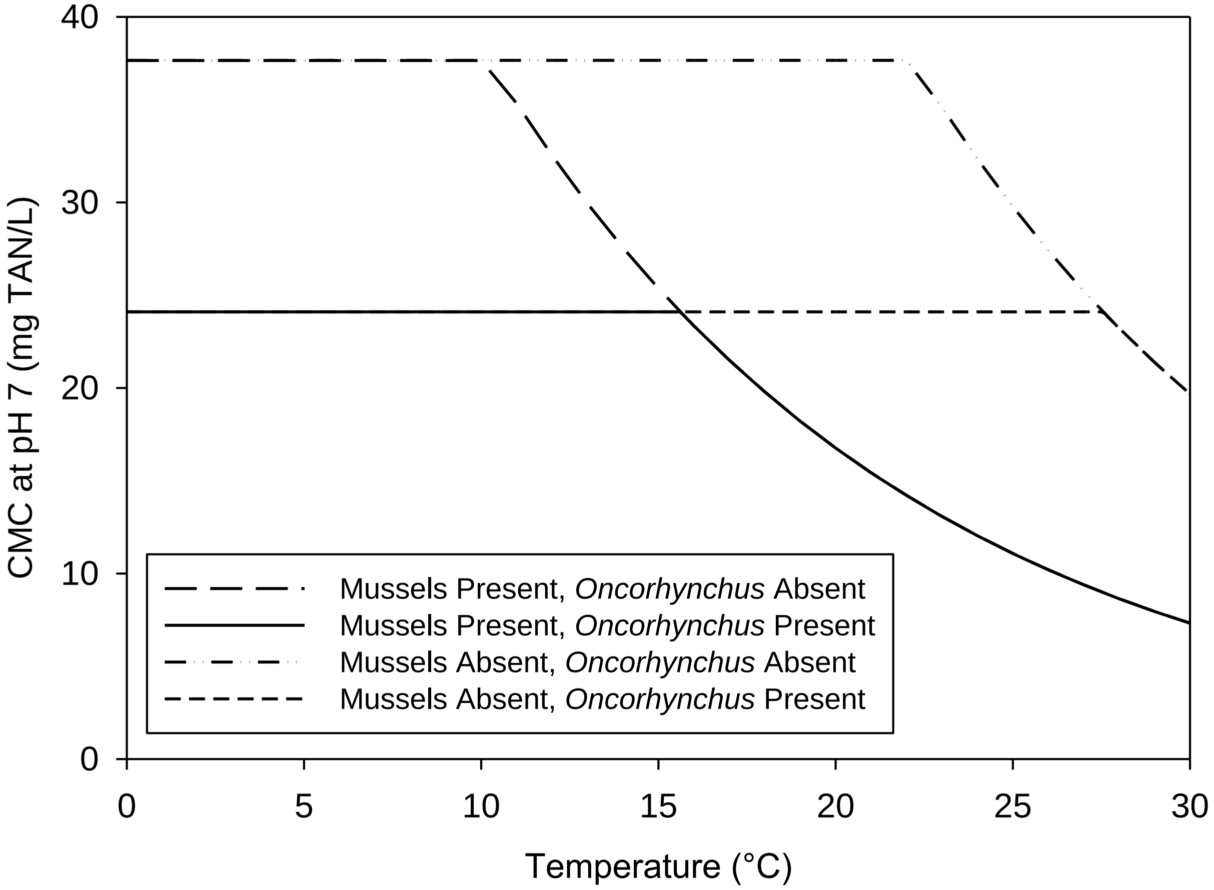


Figure N.1. Comparison of the 2013 CMC Extrapolated Across a Temperature Gradient at pH 7 Accounting for the Presence or Absence of Unionid Mussels and the Presence or Absence of *Oncorhynchus*.

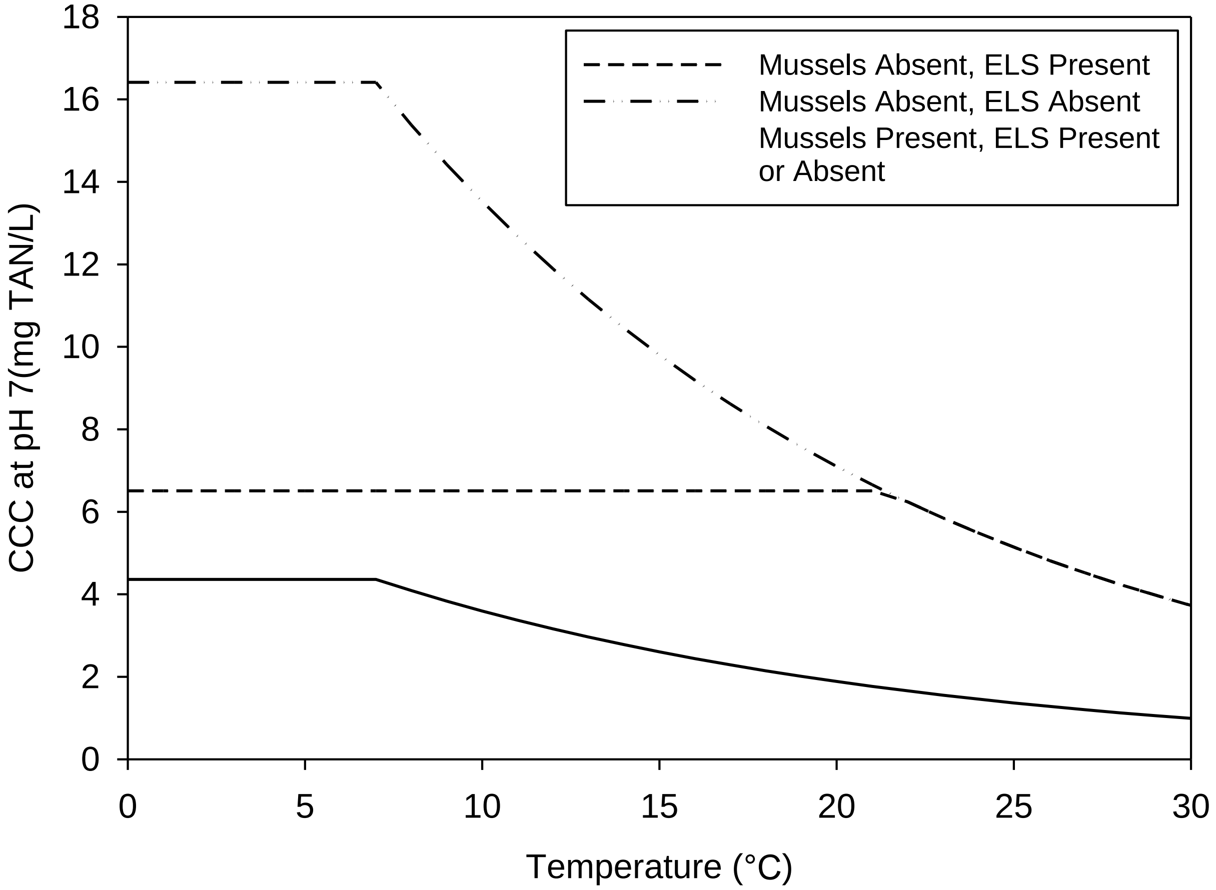


Figure N.2. Comparison of the 2013 CCC Extrapolated Across a Temperature Gradient at pH 7 Accounting for the Presence or Absence of Mussels and/or the Need for Early Life Stage (ELS) Protection of Fish Species.