United States
Environmental Protection
Agency

Office of Water
4304

EPA 822-R-98-008
August 1998

EPA

1998 Update of Ambient Water Quality Criteria for

Ammonia

SFUND RECORDS CTR
98893

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

August 1998

OFFICE OF

WATER

Office of Science and Technology Policy Recommendations

The criteria recommendations provided here under Clean Water Act (CWA) Section 304(a)(1) serve as guidance to States, Territories, and authorized Tribes in developing water quality standards under CWA Section 303(c), used as a basis for controlling discharges or releases of pollutants. The material provided in this document constitutes the Agency's current Section 304(a)(1) guidance, and will continue to serve as such until EPA publishes a revision.

Freshwater Ammonia Criteria Guidance

EPA prepared this guidance as a revision of its 1984/1985 and 1992 freshwater ammonia criteria. This document revises (a) the pH and temperature relationship of the Criteria Maximum Concentration, (CMC or acute criterion) based on re-evaluation of the data in the 1984 criteria document, (b) the Criteria Continuous Concentration (CCC or chronic criterion), including its pH and temperature relationship, based on new data in addition to what was available for the 1984 document, and (c) the averaging period applicable to the CCC. The document does not address, and is not intended to modify (d) the averaging period applicable to the CMC, or (e) the recommended frequencies for excursions of the CMC or CCC, which remain as set forth in the 1985 "Guidelines for Deriving...Criteria for the Protection of Aquatic Organisms...".

Cold-Season Risk Management Policy Recommendations

Because the costs of biological treatment of ammonia increase substantially as the water temperature drops, establishing the cold-season ammonia concentrations necessary for protecting aquatic life uses is of particular importance. Two factors affect the appropriateness of the update document's CCC during cold seasons. First, with respect to chronic toxicity of ammonia to fish, the most sensitive life stages are early life stages, which in many, but not all water bodies, do not occur in during the cold season. Second, for the most sensitive invertebrates, the toxicity of ammonia appears to decrease with decreasing temperature. For this reason, EPA has concluded that under some circumstances the cold-season CCC could be relaxed somewhat, although setting the appropriate criteria value involves uncertainties.

In light of the evidence available, EPA recommends the following risk management policies with regard to cold-season ammonia criteria:

- While the cold-season ammonia criterion may in some cases be different than the criterion applicable to other seasons, all periods of the year should be covered by some ammonia criterion.

- If a state can make a finding that identifies a time of year when no sensitive life stages of any fish species are ordinarily present in numbers affecting the sustainability of populations, the criterion applicable to that time of year may be set as much as 3-fold higher than the criterion applicable to the remainder of the year. Baseline and subsequent biological monitoring in accordance with currently available EPA guidance should be conducted to assure that the integrity of the aquatic community being protected is maintained when these higher cold-season concentrations are allowed.

- If a state can demonstrate, based on rigorous baseline and subsequent instream biological monitoring, that particular eco-regions can fully support beneficial fisheries uses, defined by appropriate biological measures, under the cold-season concentration regimes occurring at monitored sites in the eco-region, then the state may set the cold-season criterion more than 3-fold higher than the applicable criterion to accord with the results of such analysis. In judging the adequacy of the instream biological monitoring, EPA would rely on its May 1996 guidance "Biological Criteria, Technical Guidance for Streams and Small Rivers" (EPA 822-B-96-001) or later updates when they become available.

Endangered or Threatened Species Policy Recommendations

Because the criteria are generally designed to protect 95 percent of all fish and aquatic invertebrate taxa, there remains a small possibility that the criteria will not protect all listed endangered or threatened species. Consequently, EPA recommends the following:

> In adopting ammonia criteria for specific water bodies, States and Tribes may need to develop site-specific modifications of the criteria to protect listed endangered or threatened species, where sufficient data exist indicating that endangered or threatened species are more sensitive to a pollutant than the species upon which the criteria are based. Such modifications may be accomplished using either of the following two procedures: (1) If the CMC is greater than 0.5 times the Species Mean Acute Value for a listed threatened or endangered species, or a surrogate for such species, obtained from flow-through, measured-concentration tests, then the CMC should be reset equal to 0.5 times that Species Mean Acute Value. (The empirical factor 0.5 converts from a 50 percent lethality concentration to a minimal-lethality concentration.) If CCC is greater than the Species Mean Chronic Value of a listed threatened or endangered species or surrogate, then the CCC should be reset to that Species Mean Chronic Value. (2) The site-specific criteria may be calculated using the recalculation procedure for site-specific modifications described in Chapter 3 of the U.S. EPA Water Quality Standards Handbook, Second Edition--Revised (1994).

EPA encourages the submission of additional data relevant to the appropriateness of the guidance contained in this document. Questions or comments may be directed to Charles Stephan, U.S. EPA, 6201 Congdon Blvd., Duluth, MN 55804 (TEL: 218-529-5219; FAX: 218-529-5003) or Charles Delos, U.S. EPA, Mail Code 4304, 401 M Street SW, Washington, DC 20460 (E-mail: delos.charles@epamail.epa.gov).

Tudor T. Davies, Director
Office of Science and Technology
Office of Water

1998 Update of

Ambient Water Quality Criteria for Ammonia

August 1998

U.S. Environmental Protection Agency

Office of Water
Office of Science and Technology
Washington, D.C.

Office of Research and Development
Mid-Continent Ecology Division
Duluth, Minnesota

NOTICES

This update provides guidance to States and Tribes authorized to establish water quality standards under the Clean Water Act (CWA) concerning toxicity values that protect aquatic life from acute and chronic effects of ammonia. 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 this guidance when appropriate. While this update constitutes EPA's 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 this guidance in the future.

This update has been reviewed by the Mid-Continent Ecology Division, Duluth, MN (Office of Research and Development) and the Office of Science and Technology (Office of Water), U.S. Environmental Protection Agency, and approved for publication.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

ACKNOWLEDGMENT

This update was written by Charles Stephan, Russ Erickson, Charles Delos, Tom Willingham, Kent Ballentine, and Rob Pepin (with substantial input from Alex Barron of the Virginia Department of Environmental Quality, Dave Maschwitz of the Minnesota Pollution Control Agency, and Bob Mosher of the Illinois Environmental Protection Agency) under the auspices of the Aquatic Life Criteria Guideline Committee. Please submit comments or questions or both to: Charles Stephan, U.S. EPA, 6201 Congdon Blvd., Duluth, MN 55804 (TEL: 218-529-5219)(FAX: 218-529-5003).

CONTENTS

	Page
Notices	ii
Acknowledgment	ii
Appendices	iv
Tables	v
Figures	vi
Introduction	1
Overview of Ammonia Toxicology	2
Temperature-Dependence of Ammonia Toxicity	9
pH-Dependence of Ammonia Toxicity	21
Derivation of the New CMC	30
Review and Analysis of Chronic Data	38
Derivation of the New CCC	68
Cold-Weather Conditions	73
CCC Averaging Period	78
Water-Effect Ratios	81
A Field Study Relevant to the CCC	83
The National Criterion for Ammonia in Fresh Water	91
References	94

APPENDICES

Page

1 Review of Some Toxicity Tests 107

2 Methods for Regression Analysis of pH Data 111

3 Conversion of Results of Toxicity Tests 114

4 Acute Values 116

5 Histopathological Effects 126

6 Results of Regression Analyses of Chronic Data 131

7 Acute-Chronic Ratios 144

TABLES

		Page
1	Ranked Genus Mean Acute Values	31
2	EC20s from Acceptable Chronic Tests	65
3	Data for Fishes and Clams in the Monticello Study	86
4	Results Obtained using Simulated Samples	113
5	Genus Mean Acute-Chronic Ratios	146
6	Ordered Genus Mean Acute-Chronic Ratios	148

FIGURES

Page

1 The effect of temperature on ammonia toxicity in terms of un-ionized ammonia (DeGraeve et al. (1987) 12

2 The effect of temperature on acute ammonia toxicity in terms of total ammonia 15

3 The effect of temperature on pH-adjusted acute ammonia toxicity in terms of total ammonia 16

4 The effect of temperature on normalized acute ammonia toxicity in terms of total ammonia 18

5 The effect of temperature on chronic ammonia lethality to fathead minnows in terms of total ammonia (DeGraeve et al. 1987) 19

6 The effect of pH on acute ammonia toxicity in terms of total ammonia 25

7 The effect of pH on normalized acute ammonia toxicity in terms of total ammonia 27

8 The effect of pH on chronic ammonia toxicity in terms of total ammonia 28

9 Ranked Genus Mean Acute Values (GMAVs) with Criterion Maximum Concentrations (CMCs) 35

10 Acute LC50s used in criteria derivation in relationship to Final Acute Values (FAVs) and Criterion Maximum Concentrations (CMCs) 36

11 Ranked Genus Mean Chronic Values (GMCVs) with the Criterion Continuous Concentration (CCC) 69

12 Chronic EC20s used in criteria derivation in relationship to Criterion Continuous Concentrations (CCCs) 70

13 Monticello data compared with the new CCC statement . . . 89

INTRODUCTION

Since the U.S. EPA published "Ambient Water Quality Criteria for Ammonia - 1984" (U.S. EPA 1985a), it has issued additional information concerning aquatic life criteria for ammonia (Heber and Ballentine 1992; U.S. EPA 1989,1996). Also, results of additional toxicity tests on ammonia have been published since 1985, which could affect the freshwater criterion for ammonia. The purpose of this 1998 Update is to revise the 1984/1985 ammonia criteria document (U.S. EPA 1985a) and replace Heber and Ballentine (1992) and U.S. EPA (1996) by addressing selected important issues to the extent possible in a short-term effort without additional research.

This 1998 Update first presents an overview of ammonia toxicology in order to provide the background needed to explain the revisions of the freshwater ammonia criterion. Then the equations used in the 1984/1985 ammonia criteria document to address the temperature- and pH-dependence of ammonia toxicity in fresh water are revised to take into account newer data, better models, and improved statistical methods. Next, a new CMC is derived using these revised equations and the acute toxicity data in the 1984/1985 criteria document. Then, new and old chronic toxicity data are evaluated and used to derive a new CCC. Finally, cold-weather conditions, the CCC averaging period, water-effect ratios, and a field study relevant to the CCC are discussed. This 1998 Update does not address (1) the CMC averaging period, (2) the frequency of allowed exceedences, or (3) field studies other than the one mentioned above. This 1998 Update addresses only the freshwater criterion for ammonia and does not affect the saltwater criterion for ammonia (U.S. EPA 1989).

Concentrations of un-ionized ammonia and total ammonia are given herein in terms of nitrogen, i.e., as mg N/L, because most permit limits for ammonia are expressed in terms of nitrogen. CMCs and CCCs are given to three significant figures to minimize the effect of round-off error in the calculation of permit limits.

Three unpublished manuscripts that were cited in the 1984/1985 criteria document have been published as Broderius et al. (1985), Erickson (1985), and Thurston et al. (1986). West (1985) was published as Arthur et al. (1987).

OVERVIEW OF AMMONIA TOXICOLOGY

The 1984/1985 ammonia criteria document reviewed data regarding the dependence of the toxicity of ammonia to aquatic organisms on various physicochemical properties of the test water, especially temperature, pH, and ionic composition. A key factor in these relationships is the chemical speciation of ammonia. In aqueous solution, ammonia primarily exists in two forms, un-ionized ammonia (NH_3) and ammonium ion (NH_4^+), which are in equilibrium with each other according to the following expressions:

$$NH_4^+ \leftrightharpoons NH_3 + H^+ \qquad (1)$$

$$K = \frac{[NH_3][H^+]}{[NH_4^+]} \qquad (2)$$

The equilibrium constant K depends significantly on temperature; this relationship has been described by Emerson et al. (1975) with the following equation:

$$pK = 0.09018 + \frac{2729.92}{273.2 + T} \qquad (3)$$

where $pK = -\log_{10}K$ and T is temperature in degrees Celsius.

From equation 2, the definition of pK, and the definition $pH = -\log_{10}[H^+]$, the following expressions can be derived for the fraction of total ammonia in each of the two forms:

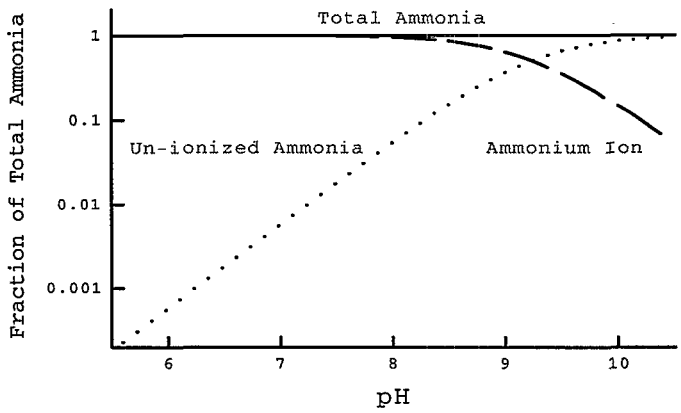
$$f_{NH_3} = \frac{1}{1 + 10^{pK-pH}}$$

$$f_{NH_4^+} = \frac{1}{1 + 10^{pH-pK}} \qquad (4)$$

$$f_{NH_3} + f_{NH_4^+} = 1$$

The individual fractions vary markedly with temperature and pH. The pH-dependence of the relative amounts of un-ionized ammonia and ammonium ion at 25°C, at which pK=9.24, is illustrated in the following graph:

Chemical Speciation of Ammonia



Ammonia speciation also depends on ionic strength, but in fresh water this effect is much smaller than the effects of pH and temperature (Soderberg and Meade 1991) and is sufficiently small compared to the typical uncertainty in LC50s that it will not be considered here as a variable affecting ammonia toxicity. (As discussed later, ionic composition might affect ammonia toxicity in ways other than its effect on ammonia speciation).

These speciation relationships are important to ammonia toxicity because un-ionized ammonia is much more toxic than ammonium ion. The importance of un-ionized ammonia was first recognized when it was observed that increased pH caused total ammonia to appear to be much more toxic (Chipman 1934; Wuhrmann and Woker 1948). It is not surprising that un-ionized ammonia is the more toxic form, because it is a neutral molecule and thus is able to diffuse across the epithelial membranes of aquatic organisms much more readily than the charged ammonium ion. 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 un-ionized ammonia from the gills. High external un-ionized ammonia concentrations reduce or reverse diffusive gradients and cause the buildup of ammonia in gill tissue and blood.

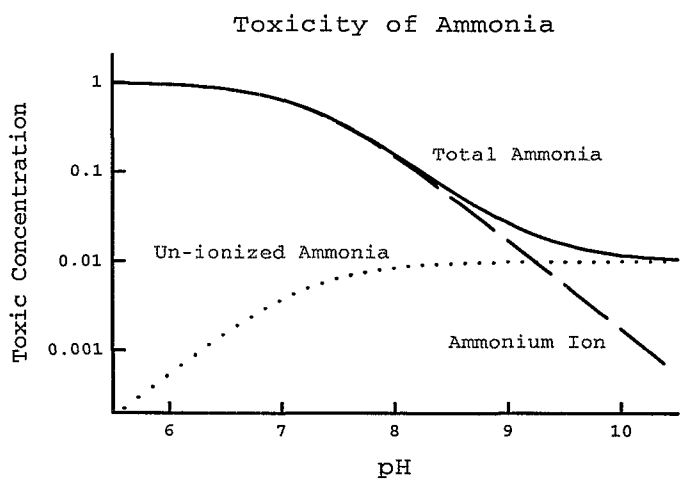
Because of the importance of un-ionized ammonia, it became a convention in the scientific literature to express ammonia toxicity in terms of un-ionized ammonia, and water quality criteria and standards followed this convention. However, there are reasons to believe that ammonium ion can contribute significantly to ammonia toxicity under some conditions. Observations that ammonia toxicity is relatively constant when expressed in terms of un-ionized ammonia come mainly from

toxicity tests conducted at pH>7.5. At lower pH, toxicity varies considerably when expressed in terms of un-ionized ammonia and under some conditions is relatively constant in terms of ammonium ion (Erickson 1985). Also, studies have established that mechanisms exist for the transport of ammonium ion across gill epithelia (Wood 1993), so this ion might contribute significantly to ammonia exchange at gills and affect the buildup of ammonia in tissues if its external concentration is sufficiently high. Thus, the very same arguments employed for the importance of un-ionized ammonia can also be applied in some degree to ammonium ion. This is not to say that ammonium ion is as toxic as un-ionized ammonia, but rather that, regardless of its lower toxicity, it can still be important because it is generally present in much greater concentrations than un-ionized ammonia.

Also, when expressed in terms of un-ionized ammonia, ammonia toxicity is usually not constant with temperature, on average being about four-fold greater at 5°C than at 25°C for fish (Erickson 1985). Because the relative amount of ammonium ion is also higher at low temperatures, this raises the possibility that ammonium ion might be in part responsible for this temperature dependence. However, temperature might also alter ammonia toxicity by affecting membrane permeabilities, endogenous ammonia production, and other physiological processes.

Various authors have evaluated models that might explain the pH and temperature dependence of ammonia toxicity. Tabata (1962) and Armstrong et al. (1978) suggested that the observed pH dependence is due to joint toxicity of un-ionized ammonia and ammonium ion.

The adjacent graph shows an idealized picture of ammonia toxicity assuming that (a) ammonium ion and un-ionized ammonia jointly determine toxicity and (b) un-ionized ammonia is 100 times more toxic than ammonium ion. At sufficiently high pH, the more toxic un-ionized ammonia comprises a sufficiently large fraction of total ammonia to dominate



toxicity, and so toxicity is relatively constant when expressed in terms of un-ionized ammonia. As pH decreases, the relative amount of ammonium ion increases until it contributes significantly to toxicity, so that toxicity expressed in terms of un-ionized ammonia increases (i.e., it appears that less un-ionized ammonia is necessary to cause toxicity because ammonium ion is responsible for some of the toxicity). At sufficiently low pH, ammonium ion dominates toxicity, and so toxicity is relatively constant when expressed in terms of either ammonium ion or total ammonia.

In contrast to this theory, Lloyd and Herbert (1960) suggested that the apparent effect of pH on un-ionized ammonia toxicity is due to the data being plotted in terms of the pH of the bulk exposure water rather than the pH at the gill surface. The release of carbon dioxide at the gill lowers pH when pH is moderately alkaline, but has less effect when pH is already low; this results in an apparent effect of pH on toxicity when the pH of the bulk exposure water is used even if there is no such effect if the pH at the gill surface is used. Szumski et al. (1982) suggested that this theory explained not only much of the pH dependence of ammonia toxicity, but also the temperature dependence.

Erickson (1985) reviewed available information concerning the effects of pH and temperature on acute toxicity of ammonia when expressed in terms of un-ionized ammonia and tested its adherence to these theories. He concluded that effects associated with pH changes at the gill could not account for the effect of temperature and only a small part of the effect of pH. In contrast, the additive joint toxicity model explained a large part of the dependence of ammonia toxicity on pH and predicted important features of the data, specifically a slope of zero at high pH and a slope of one at low pH. The joint toxicity model could also be fit to the temperature data, but led to values of the model parameters that were questionable because they indicated that ammonium ion is as or more toxic than un-ionized ammonia. Clearly, joint toxicity could not possibly account for both pH and temperature effects, and Erickson (1985) concluded that joint toxicity is likely responsible for much of the pH effect, but not for the temperature effect. In the 1984/1985 criteria document, it was noted that the one available dataset concerning the dependence of chronic toxicity on pH (Broderius et al. 1985) also suggested joint toxicity of un-ionized ammonia and ammonium ion.

Therefore, a major consideration in deriving the aquatic life criterion for ammonia is whether the mathematical model used to describe pH dependence should be based on joint toxicity theory.

Since the 1984/1985 criteria document was issued, several additional studies (Sheehan and Lewis 1986; Schubauer-Berigan et al. 1995; Ankley et al. 1995; Johnson 1995) of the pH dependence of ammonia toxicity have provided more information regarding the relative importance of un-ionized ammonia and ammonium ion, including indications of more diversity among species than was apparent in the data reviewed by Erickson (1985).

The report of Sheehan and Lewis (1986) requires special consideration here because they suggest that the toxicity of ammonia at low pH is due to the effect of osmotic shock on unacclimated organisms and that this has major implications for the derivation of a criterion for ammonia. In their investigations concerning the pH-dependence of acute ammonia toxicity to channel catfish, Sheehan and Lewis (1986) found that LC50s expressed in terms of un-ionized ammonia increased with increasing pH, but less so than reported in most studies, although Tomasso et al. (1980) also reported little effect of pH≥7 on un-ionized ammonia toxicity to the channel catfish. Sheehan and Lewis noted that lethal concentrations at pH=6 were associated with very high total ammonia concentrations (2000 mg N/L) and exhibited steeper concentration-effect curves than at higher pH. They also reported that other salts were lethal at similar concentrations and suggested that the toxicity of ammonia at low pH was due to the effect of osmotic shock on unacclimated organisms rather than a specific action of the ammonium ion per se. However, the implication of this work for the ammonia criterion is doubtful for the following reasons:

1. Any concern that the effects of high concentrations of ammonia would be less for acclimated organisms is really not relevant. To be adequately protective, criteria cannot assume that acclimation takes place, because if such high ammonia concentrations are discharged, they would create a plume of high concentrations compared to ambient levels. Organisms entering that plume would not be acclimated to the high concentrations.
2. It is doubtful that the effects of high salt concentrations observed by Sheehan and Lewis were strictly due to osmotic effects. In their experiments, potassium chloride caused higher mortality than the physiologically balanced salt they also used. In fact, the toxicities of such salts vary quite widely, with potassium salts generally being more toxic (Mount et al. 1997), probably due to effects of potassium beyond any osmotic effects. Ammonium chloride also caused higher mortality than the physiologically balanced salt, although this might be in part due to effects of un-ionized ammonia.
3. As part of their evidence for supporting osmotic effects as a toxic mechanism at low pH, Sheehan and Lewis noted that the dose-response curves were steeper at low pH, suggestive not

only of a different mechanism, but one that is less variable among organisms within a test. However, Broderius et al. (1985) found the opposite effect of pH on dose-response curves.

4. The LC50s for channel catfish at low pH are generally much higher than those for other fishes that have been tested at low pH. When expressed in terms of total ammonia, the LC50 for channel catfish at pH=6 is four-fold higher than any other LC50 reported for a fish species. For many other fishes, LC50s at pH≈6.5 represent salt concentrations of only a few hundred mg/L and less than a factor of two greater than that of control water. A role of osmotic effects in such cases is doubtful. Of all of the fish species tested, the pH curves for channel catfish show the least indication for an effect of ammonium ion, so it is a very questionable species upon which to base broad conclusions.
5. In contrast to Sheehan and Lewis, Knoph (1992) reported no mortality of Atlantic salmon at pH=6 in KCl or in physiologically balanced salt solutions with concentrations equivalent to ammonium chloride solutions causing 45% mortality. Similarly, Mount et al. (1997) found acute LC50s for fathead minnows for various salts and combinations (except those including potassium) to be at least several-fold higher than the total ammonia LC50s reported at pH=6.5 by Thurston et al. (1981b). Although for an invertebrate, the likely role of ammonium ion other than in association with high salt concentrations is also evident in the daphnid data of Tabata (1962) and Mount et al. (1997).
6. Even if a different mechanism for toxicity exists at low pH, these tests still identify concentrations that are unacceptably toxic and this is still joint toxicity in the broad sense of the term. Although the joint toxicity might not be strictly additive, as would be expected if the two forms of ammonia operate by the same mechanism, it is joint toxicity nonetheless and should exhibit a similar pH dependence and be considered in criteria derivation.

Although there is considerable reason to consider the effects of pH on ammonia toxicity to be largely due to the joint toxicity of ammonium ion and un-ionized ammonia, pH can have other effects on membrane function and other physiological processes that could also alter ammonia toxicity, especially at very low and high pHs, and these are poorly established. The state of knowledge for the pH dependence is incomplete in terms of understanding specific mechanisms, variation among species, and interactions with various physicochemical processes. Lacking a definitive, thorough theoretical approach for describing pH effects, the most reasonable approach is to adopt the best empirical description that can be obtained from available data. However, the shape of

this empirical equation can be guided by consideration of the evidence for the role of speciation in ammonia toxicity.

The effects of temperature on ammonia toxicity are even less well understood, and there is no adequate theoretical basis or scientific understanding for specifying how temperature adjustments to the ammonia criterion should be made. Therefore, an empirical approach will also be used for temperature dependence, as developed in the next section.

As reviewed in the 1984/1985 ammonia criteria document, ammonia toxicity can also depend on various aspects of the ionic composition of the exposure water, but the effects were not clear and consistent enough to warrant inclusion of other variables in the criterion. Although Soderberg and Meade (1992), Yesaki and Iwama (1992), Ankley et al. (1995), Johnson (1995), Borgmann and Borgmann (1997), and Iwama et al. (1997) have provided new data concerning interactions between various ions and ammonia toxicity and excretion, there is still insufficient understanding and information to account for these effects in the criterion and they will have to be addressed using water-effect ratios or other site-specific approaches.

In summary, the available evidence indicates that the toxicity of ammonia can depend on ionic composition, pH, and temperature. The mechanisms of these effects are poorly understood, but the pH dependence strongly suggests that joint toxicity of un-ionized ammonia and ammonium ion is an important component. For the reasons presented above, the following approach will be used to account for these effects.

1. Because its effects on ammonia speciation in fresh water are small and its other effects on toxicity are poorly established, the ionic composition of the exposure water will not be considered in the derivation of the criterion.
2. Even though temperature can strongly affect the relative amounts of un-ionized ammonia and ammonium ion, its effect on the toxicity of ammonia is not strongly indicative of joint toxicity and will be described strictly by an empirical approach.
3. The effect of pH will be described by equations that include basic features of joint toxicity of un-ionized ammonia and ammonium ion, but with an empirical component that recognizes the incomplete knowledge of these effects.

TEMPERATURE-DEPENDENCE OF AMMONIA TOXICITY

The 1984/1985 ammonia criteria document identified temperature as an important factor affecting the toxicity of ammonia. When expressed in terms of un-ionized 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 un-ionized 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 un-ionized 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 un-ionized 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 un-ionized 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 un-ionized 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 un-ionized 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 un-ionized criterion at low temperature is warranted. Data used include those analyzed by Erickson (1985), which are shown in Figure 2 of the criteria 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 un-ionized 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), 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 un-ionized 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 surprising because three other studies (Reinbold and Pescitelli 1982a; Thurston et al. 1983; DeGraeve et al. 1987) reported a significant effect of temperature on the acute toxicity of un-ionized ammonia to the fathead minnow. This discrepancy might be due to other factors confounding temperature effects in the tests by Arthur et al. (1987) because these tests were not conducted

simultaneously; rather they were conducted during different seasons. For five invertebrate species tested over a temperature range of at least 10°C, there was no consistent relationship between temperature and un-ionized 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.

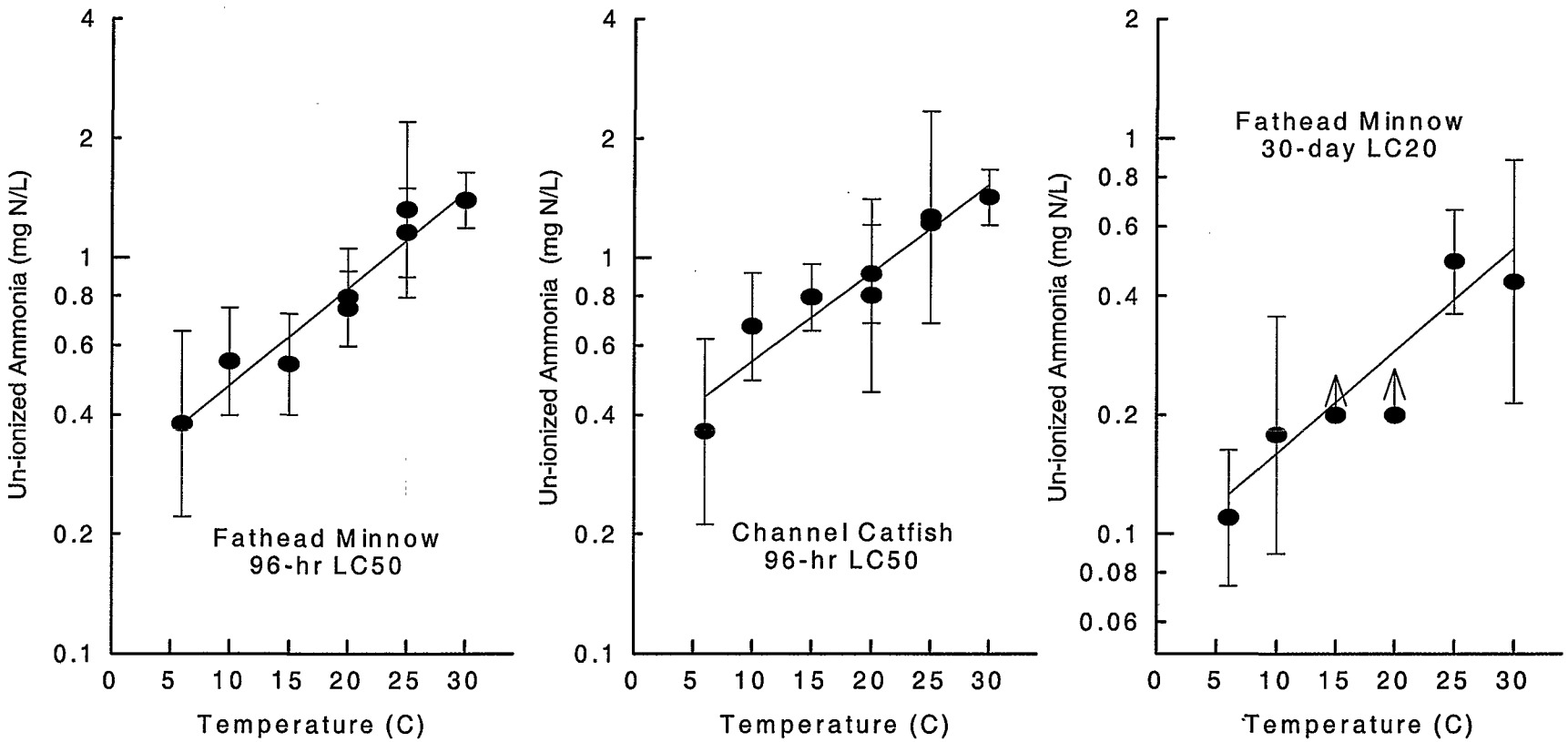
DeGraeve et al. (1987) studied the effect of temperature (from 6 to 30°C) on the toxicity of ammonia to juvenile fathead minnows and channel catfish using acute (4-day) and chronic (30-day) ammonia exposures. As shown for both fish species in Figure 1, log(96-hr un-ionized ammonia LC50) versus temperature was linear within the reported uncertainty in the LC50s; 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 un-ionized 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 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, LC50s expressed in terms of un-ionized 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, LC50s expressed in terms of un-ionized ammonia increased substantially with temperature.

Even with these additional data, the shape of the temperature relationship is incompletely resolved and more research is needed, especially regarding chronic toxicity and differences among species. Nevertheless, the acute data for fishes overwhelmingly indicate that ammonia toxicity, expressed in terms of un-ionized ammonia, decreases with increasing temperature.

Figure 1. The effect of temperature on ammonia toxicity in terms of un-ionized ammonia (DeGraeve et al. 1987). Symbols denote LC50s or LC20s and 95% confidence limits and lines denote linear regressions of logLC versus temperature.



Most importantly, the data of DeGraeve et al. (1987) show (Figure 1) that (a) a linear relationship of log un-ionized ammonia LC50 versus temperature applies within the reported uncertainty in the LC50s 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 un-ionized 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. An un-ionized ammonia criterion should continue to be lower at lower temperatures, consistent with the observed temperature dependence of ammonia toxicity to the most sensitive species, i.e., fishes. The need for this is well established for the CMC, based on the acute toxicity of ammonia to several species of fish. 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. For the CCC, the only available dataset concerns chronic mortality, and it supports a relationship similar to that for acute toxicity.

Therefore, for a criterion expressed in terms of un-ionized 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.

This raises 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 un-ionized ammonia and is not indicative of any established, significant trend. The expression and implementation of the ammonia criterion would be considerably simplified if temperature

was dropped as a modifying factor, which might be 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 un-ionized ammonia. Because of such benefits and because there are no compelling scientific or practical reasons for expressing the criterion in terms of un-ionized 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 un-ionized ammonia, pH, and temperature using the speciation relationship of Emerson et al. (1975). The data are presented in Figure 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 LC50 was adjusted to the mean pH of the data for the study using the pH relationship discussed in the next section of this 1998 Update. These adjusted data are shown in Figure 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 LC50 versus temperature was conducted (Draper and Smith 1981) and the resulting regression lines are plotted as solid lines in Figures 2 and 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 pHs were lower in the low-temperature tests than in the high-temperature tests. Slopes from regression analyses of datasets in Figure 3 range from -0.015 to 0.013, compared to a range from 0.015 to 0.054 when expressed in terms of un-ionized ammonia (Erickson 1985). This narrower range of slopes in terms of total ammonia nitrogen also argues for use of total ammonia, rather than un-ionized ammonia, because there is less uncertainty associated with the generic

Figure 2. The effect of temperature on acute ammonia toxicity in terms of total ammonia. Symbols denote LC50s, solid lines denote regressions for individual datasets, and dotted lines denote pooled regressions over all datasets.

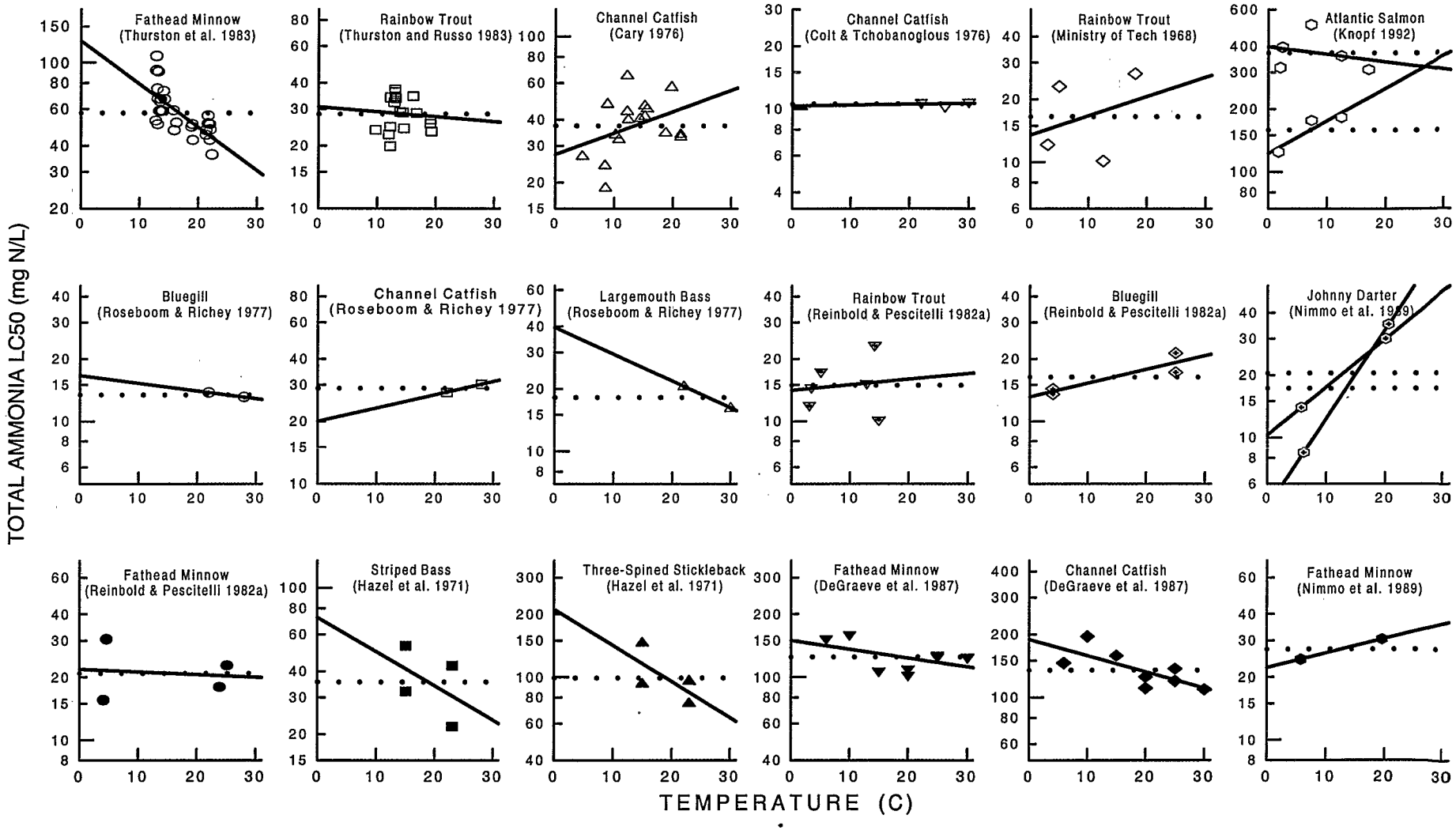
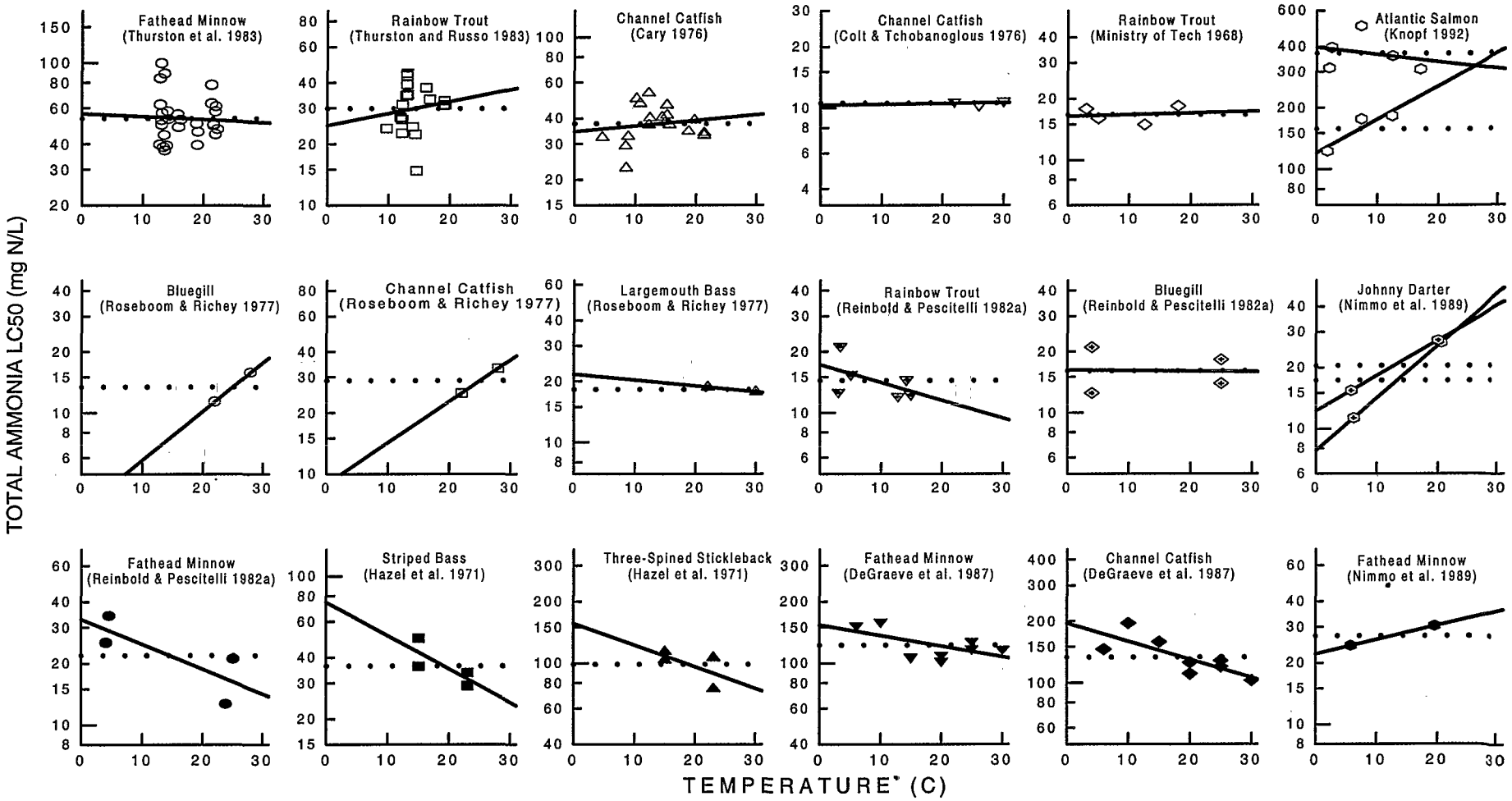


Figure 3. The effect of temperature on pH-adjusted acute ammonia toxicity in terms of total ammonia. LC50s are adjusted to the mean pH of the dataset based on the pooled relationship of acute toxicity to pH. Symbols denote LC50s, solid lines denote regressions for individual datasets, and dotted lines denote pooled regression over all datasets.



relationship. For datasets with just two points, Figures 2 and 3 also show the slopes for comparative purposes. Based on the typical uncertainty of LC50s, 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 2 and 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 LC50s. To better show the overall fit of the common regression line, the data are also plotted together in Figure 4 by dividing each point by the regression estimate of the LC50 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 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, including temperature as a modifying factor for a total ammonia criterion is not justified, and the criterion derived below is based on the acute and chronic toxicity of total ammonia without adjustment for test temperature. It is not argued that total ammonia toxicity is absolutely constant with temperature or that whatever temperature dependence exists is the same for all life stages of all species, but rather it is argued that the available data do not show temperature effects that are sufficiently large or consistent enough to allow a worthwhile, reliable temperature adjustment, either generically for all species or for individual species. For invertebrates, it should be noted that this update's assumption that temperature has 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 *un-ionized* ammonia. However, the available data do not contradict either assumption. Fortunately, most invertebrate species are resistant to the acute toxicity of

Figure 4. The effect of temperature on normalized acute ammonia toxicity in terms of total ammonia. Data were normalized by dividing measured LC50s by regression estimates of LC50s at 20°C for individual datasets for Figure 2 (top plot) and Figure 3 (bottom plot).

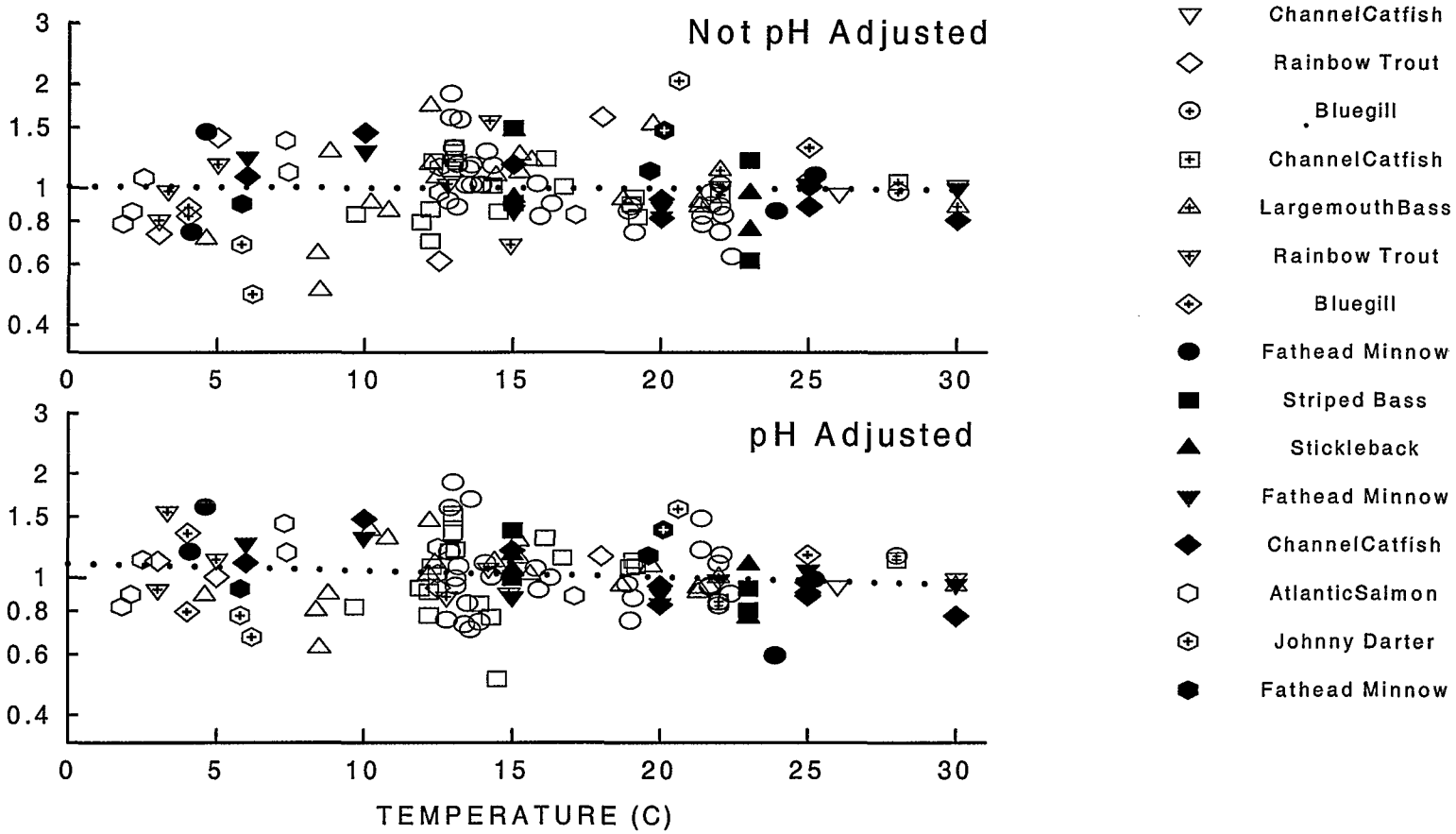
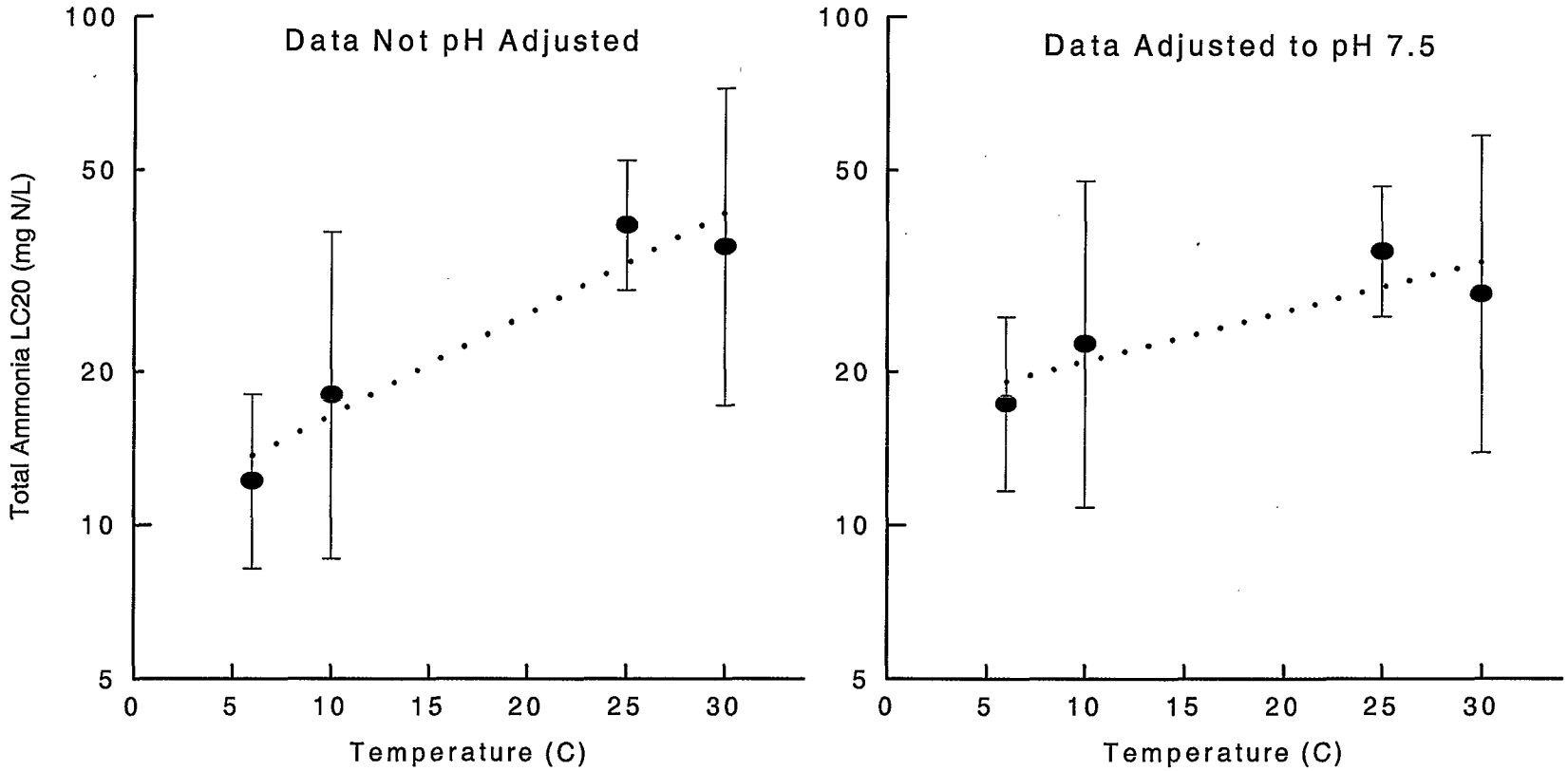


Figure 5. The effect of temperature on chronic ammonia lethality to fathead minnows in terms of total ammonia (DeGraeve et al. 1987). Symbols denote LC20s and 95% confidence limits and lines denote linear regressions of logLC versus temperature. Figure on left is for estimated LC50s at test pH and figure on right is for LC50s adjusted to pH=7.5 based on pooled relationship of chronic toxicity to pH.



ammonia, although some are sensitive to the chronic toxicity of ammonia.

The amount of uncertainty in this approach 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 4 is used, the total ammonia CMC at 5°C would be only about 6% higher than at 20°C. In contrast, the chronic data in Figure 5 suggest that the total ammonia CCC should be about 20% lower at 5°C than at 20°C. The smallest and largest slopes from the acute regressions for individual species in Figure 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.

pH-DEPENDENCE OF AMMONIA TOXICITY

The 1984/1985 ammonia criteria document identified pH as an important factor affecting the toxicity of ammonia and used an empirical model to describe the pH-dependence of ammonia toxicity when expressed in terms of un-ionized ammonia. The major features of this empirical model were a slope for logLC50 versus pH which was approximately 1 at low pH and decreased as pH increased until pH≈8, above which the slope was 0. Such a model closely mimics a joint toxicity model, which also has a slope of 1 at low pH and a slope of 0 at high pH when ammonia toxicity is expressed in terms of un-ionized ammonia. The empirical model was parameterized based on a pooled analysis of four datasets concerning the effect of pH on the acute toxicity of ammonia. This effect of pH was generally supported by several additional datasets reviewed by Erickson (1985), although some variation among species was evident, especially for channel catfish. A dataset concerning chronic ammonia toxicity (Broderius et al. 1985) indicated a somewhat greater effect of pH than for acute toxicity and was used as the principal basis for the pH-dependence of the CCC.

As explained in the overview of this update, the effect of pH on the toxicity of ammonia will be described here largely in terms of the joint (combined) toxicity of un-ionized ammonia and ammonium ion. However, there is some dispute about whether ammonia toxicity merely involves such joint toxicity. Also, a variety of factors might affect the combined toxicity of the two forms. Therefore, use of a simple, mechanistic joint toxicity model is inadvisable, and the following "S-shaped" model will be used to describe the pH dependence of total ammonia toxicity:

$$LC50_t = \frac{LIM_H}{1 + 10^{pH_T - pH}} + \frac{LIM_L}{1 + 10^{pH - pH_T}} \qquad \textbf{(5)}$$

where the subscript t denotes total ammonia, LIM_H and LIM_L are asymptotic (limiting) LC50s at high and low pH respectively, and pH_T is the transition pH at which the LC50 is the arithmetic average of LIM_H and LIM_L. This model is justified by various data (see the overview) and is consistent with joint toxicity of un-ionized ammonia and ammonium ion. However, the model treats pH_T as a fitted parameter, whereas if joint toxicity were assumed it would be dictated by the pK of ammonia (see equation 4) and the relative toxicity of the two forms.

Use of LIM_H and LIM_L as model parameters results in a simple equation, but is inconvenient for data analysis for two reasons. First, when analyzing toxicological variables across multiple datasets, an important issue is whether the shapes of the curves are similar among the datasets. For making such comparisons and for estimating the best average shape, it is necessary that each parameter of the equation either is related only to the shape or is not related to the shape at all. For example, in linear regression, the equation is generally expressed in terms of a slope and an intercept (i.e., the value of y at a specified value of x, such as x=0). The slope completely defines the shape of the relationship, whereas the intercept anchors the relationship at a particular point and has no effect on the shape. For the nonlinear regression used here, there needs to be one, and only one, "intercept" parameter that specifies the LC50 at a particular pH, independent of the shape, whereas the other parameters must describe aspects of the shape and not affect the intercept. In the above equation, LIM_H and LIM_L are both "intercepts" (at high and low pH, respectively), and they also in part dictate the shape of the curve because the shape partly depends on the difference between the two intercepts. Thus, it is not possible to completely separate the shape from the intercepts. To eliminate this problem, the equation was reformulated so that LIM_L is the only intercept parameter. This was accomplished by using the parameter $R = LIM_H/LIM_L$, which, along with pH_T, defines the shape of the curve:

$$LC50_t = \left(LIM_L\right)\left(\frac{R}{1 + 10^{pH_T - pH}} + \frac{1}{1 + 10^{pH - pH_T}}\right) \qquad \textbf{(6)}$$

The second shortcoming of the use of LIM_H and/or LIM_L is that they are LC50s at extreme pHs which are not observed and are largely hypothetical; it is preferable to have an "intercept" parameter that lies in the range of the observed data. Therefore, the equation was reformulated to use the $LC50_t$ at pH=8 ($LC50_{t,8}$) as the intercept parameter instead of LIM_L. Switching from LIM_L to $LC50_{t,8}$ requires use of a term that is the ratio between $LC50_{t,8}$ and LIM_L:

$$LC50_t = \left(\frac{LC50_{t,8}}{\frac{R}{1+10^{pH_T-8}} + \frac{1}{1+10^{8-pH_T}}}\right)\left(\frac{R}{1 + 10^{pH_T - pH}} + \frac{1}{1 + 10^{pH - pH_T}}\right) \qquad \textbf{(7)}$$

All three of the above model equations are equivalent, differing only in the way in which the parameters are formulated.

Unfortunately, analyses based on any of these three model equations can be subject to serious problems with some datasets, especially for estimation of LIM_H or R. This is because $LC50_t$ is generally much greater than LIM_H even at the highest pH in most datasets (pH=8 to 9), so that the approach to this asymptotic value is very uncertain. However, the pH is usually sufficiently high that un-ionized ammonia, although only a small fraction of total ammonia, dominates toxicity and provides information about LIM_H and R that is not apparent when only total ammonia is examined. To address this problem, the formulation of the model was changed by splitting the equation into two parts:

$$LC50_u = \left(\frac{LC50_{t,8}}{\frac{R}{1+10^{pH_T-8}} + \frac{1}{1+10^{8-pH_T}}} \right) \left(\frac{R}{1 + 10^{pH_T-pH}} \right) \quad \textbf{(8)}$$

$$LC50_i = \left(\frac{LC50_{t,8}}{\frac{R}{1+10^{pH_T-8}} + \frac{1}{1+10^{8-pH_T}}} \right) \left(\frac{1}{1 + 10^{pH-pH_T}} \right) \quad \textbf{(9)}$$

where $LC50_u$ and $LC50_i$ are the LC50s expressed in terms of un-ionized ammonia and ammonium ion, respectively, and $LC50_u + LC50_i = LC50_t$. This approach more strongly emphasizes the notion of joint toxicity, but still is somewhat empirical because pH_T is a fitted parameter. Regression methods for multiple response variables (see Appendix 2) were used to fit this model to the available datasets.

Acute datasets evaluated included those cited in the 1984/1985 ammonia criteria document and Erickson (1985), as well as more recent studies by Sheehan and Lewis (1986), Schubauer-Berigan et al. (1995), Ankley et al. (1995), and Johnson (1995).

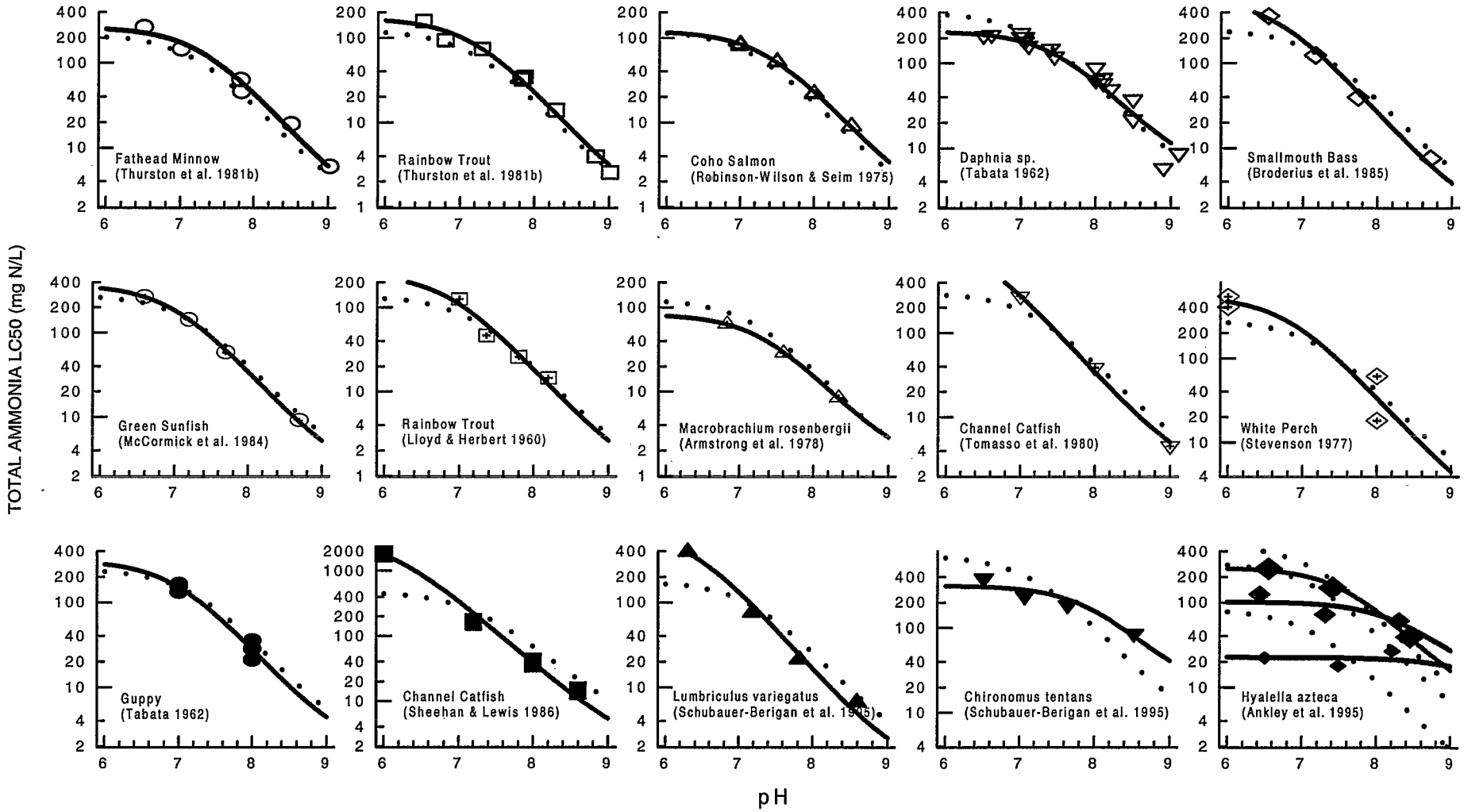
1. Sheehan and Lewis (1986) investigated the pH-dependence of acute ammonia toxicity to channel catfish. LC50s expressed in terms of un-ionized ammonia increased with increasing pH, but less so than reported in most studies, although Tomasso et al. (1980) also reported little effect of pH≥7 on un-ionized ammonia toxicity to the channel catfish.
2. Schubauer-Berigan et al. (1995) evaluated the effect of pH on the toxicity of ammonia to the oligochaete *Lumbriculus variegatus* and to larvae of the dipteran *Chironomus tentans*. Both species exhibited increases in 10-day un-ionized ammonia LC50s with increasing pH, but the increase for *C. tentans* was somewhat larger than those for other species for which data

are available, whereas those of *L. variegatus* were smaller. Such interspecies differences would be of concern in the derivation of the criterion if they substantially altered relationships for sensitive species; these particular species, however, are sufficiently resistant to ammonia that the pH relationship used for them has no impact on the criterion.

3. Ankley et al. (1995) tested the effect of pH on the toxicity of ammonia to the amphipod *Hyalella azteca* in waters of three different ionic compositions. In all three waters, 96-hr LC50s expressed in terms of un-ionized ammonia increased with pH, but the amount of increase was greater in waters with low ion concentrations. These waters differed with respect to a variety of ions, so it is uncertain which constituent is responsible for the difference in the effect of pH, although recent work by Borgmann and Borgmann (1997) suggests that the concentration of sodium is a major factor. These results not only indicate some effect of the ionic composition of the test water on ammonia toxicity, but also suggest that this composition might differentially affect the relative toxicity of un-ionized ammonia and ammonium ion. In the low ion concentration test water, *H. azteca* was one of the most sensitive species tested at low pH and consequences for the criterion will be considered later.
4. Johnson (1995) investigated the effect of pH on the chronic toxicity of ammonia to *Ceriodaphnia dubia* in test waters of three different ionic compositions. In all three waters, LC50s expressed in terms of un-ionized ammonia increased with increasing pH, but, unlike Ankley et al. (1995), the pH dependence was greater in waters with higher, rather than lower, hardness.

Acute total ammonia LC50s versus pH are presented in Figure 6 for all studies analyzed; for the study of Ankley et al. (1995) with *H. azteca*, the small, medium, and large symbols denote low, medium, and high ion concentrations in test waters. All analyses were conducted in terms of total ammonia nitrogen, either as reported by the authors or as converted by us from the reported un-ionized ammonia LC50, pH, and temperature using the speciation relationship of Emerson et al. (1975). All of the datasets show a strong trend of total ammonia LC50s decreasing with increasing pH, except that of *H. azteca* at low ion concentrations. There are, however, differences among the datasets in the magnitude and shape of the trend. Some datasets show an approach to an asymptote at low pH whereas others do not. In addition, *C. tentans* and *H. azteca* show lower slopes than other species. Nevertheless, it would be speculative to assign different relationships to different taxa, especially because the same or closely related species show some variation. Consequently, the

Figure 6. The effect of pH on acute ammonia toxicity in terms of total ammonia. Symbols denote LC50s, solid lines denote regressions for individual datasets, and dotted lines denote pooled regression over all datasets.



same as for temperature, all of the datasets were used to determine an average, generic shape for the pH dependence.

Regression analyses were conducted individually on each dataset, and on the pooled datasets assuming that only $LC50_{t,8}$ varied among datasets. The pooled analysis estimated pH_T to be 7.204 (95% confidence limits = 7.111 and 7.297) and R to be 0.00704 (95% confidence limits = 0.00548 and 0.00904). The individual regression results are plotted as solid lines and the pooled analysis as dotted lines in Figure 6. The data points and the common regression line from the pooled analysis are also plotted together in Figure 7 by dividing each point by the $LC50_{t,8}$ for its dataset (this normalized plot allows a different, combined perspective of the overall scatter of data from the shape of the generic relationship not possible in Figure 6). Except for the datasets for *L. variegatus* and *H. azteca* at low ion concentrations, the deviation of data from this generic relationship at pH>7 is rather small and consistent with the typical uncertainty of LC50s. At pH<7, however, some of the deviations are substantial; some species, most notably channel catfish and *L. variegatus*, have higher than expected total ammonia LC50s, whereas others, such as *Daphnia* sp. and *H. azteca* have lower than expected LC50s. Fortunately, these species are generally sufficiently resistant that more accurately describing their pH dependence is unimportant for deriving a CMC. Despite the variation among species at low pH, this generic relationship is appropriate for criteria derivation, because it provides significantly higher values at low pH, but not higher than those for fish species that are relatively sensitive at low pH, a suitably conservative assumption for sensitive species for which data do not exist at low pH.

For chronic toxicity, the data of Broderius et al. (1985) and Johnson (1995) were analyzed in terms of total ammonia nitrogen using the same pH model (Figure 8). The data used were EC25s reported by Johnson (1995) and EC20s calculated from the data of Broderius et al. (1985) by regression analyses discussed later. (Because Johnson's raw data were not available, EC20s could not be calculated, but the shape of the curve should be the same for EC20s and EC25s.) Because the uncertainty of the EC25s from Johnson (1995) was greater than that of Broderius et al. (1985) and to prevent the greater number of datapoints for the invertebrate from overwhelming the data for the fish, datapoints from Johnson (1995) were given a weighting factor of 0.5 in this analysis. These chronic data had a higher transition pH (7.688; 95% confidence limits = 7.554 and 7.821) and a higher R (0.0232; 95% confidence limits = 0.0160 and 0.0334) than the acute data. The higher pH_T is in accordance with differences previously noted

Figure 7. The effect of pH on normalized acute ammonia toxicity in terms of total ammonia. Data were normalized by dividing measured LC50s by regression estimates of LC50s at pH=8 for individual datasets from Figure 6.

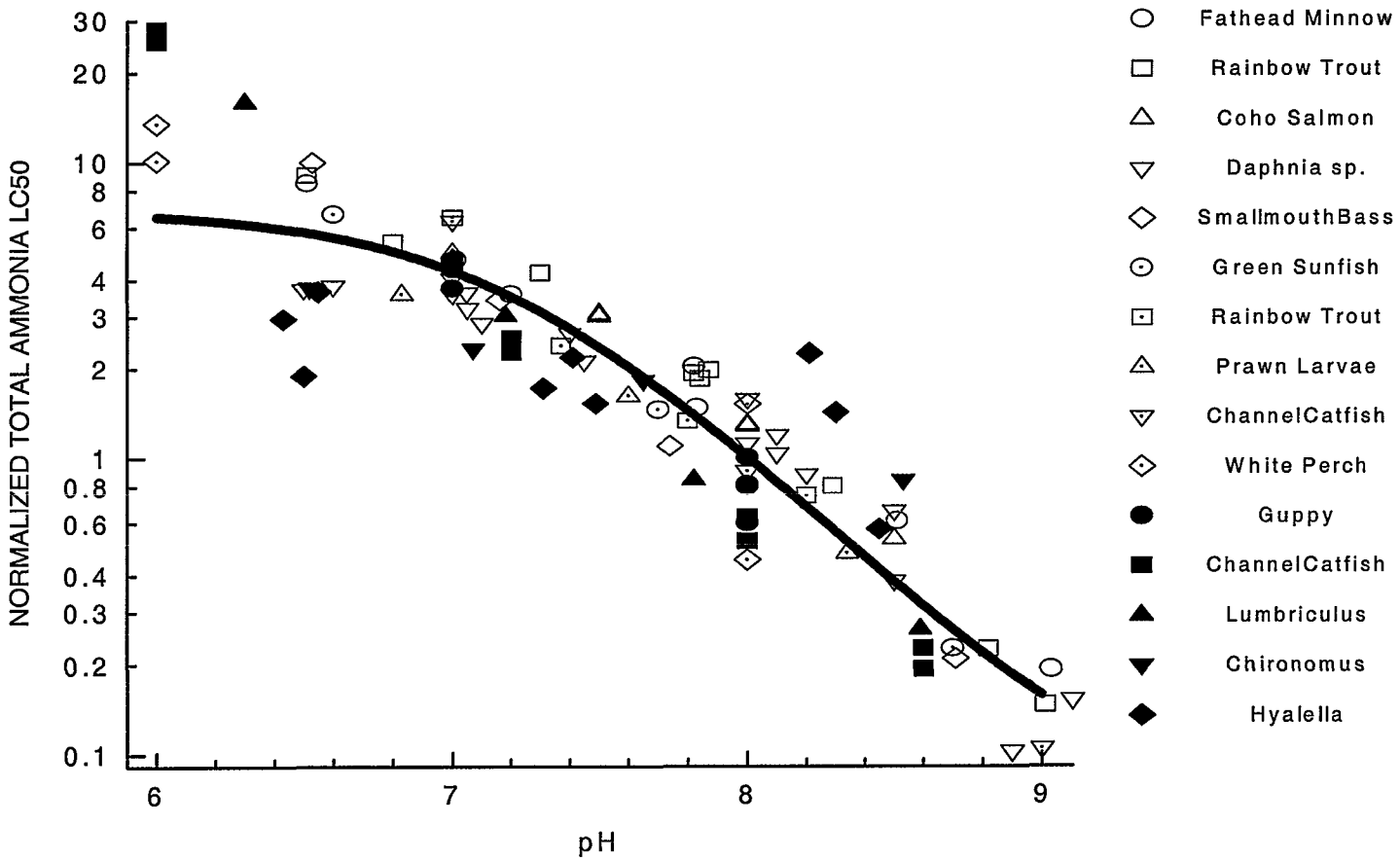
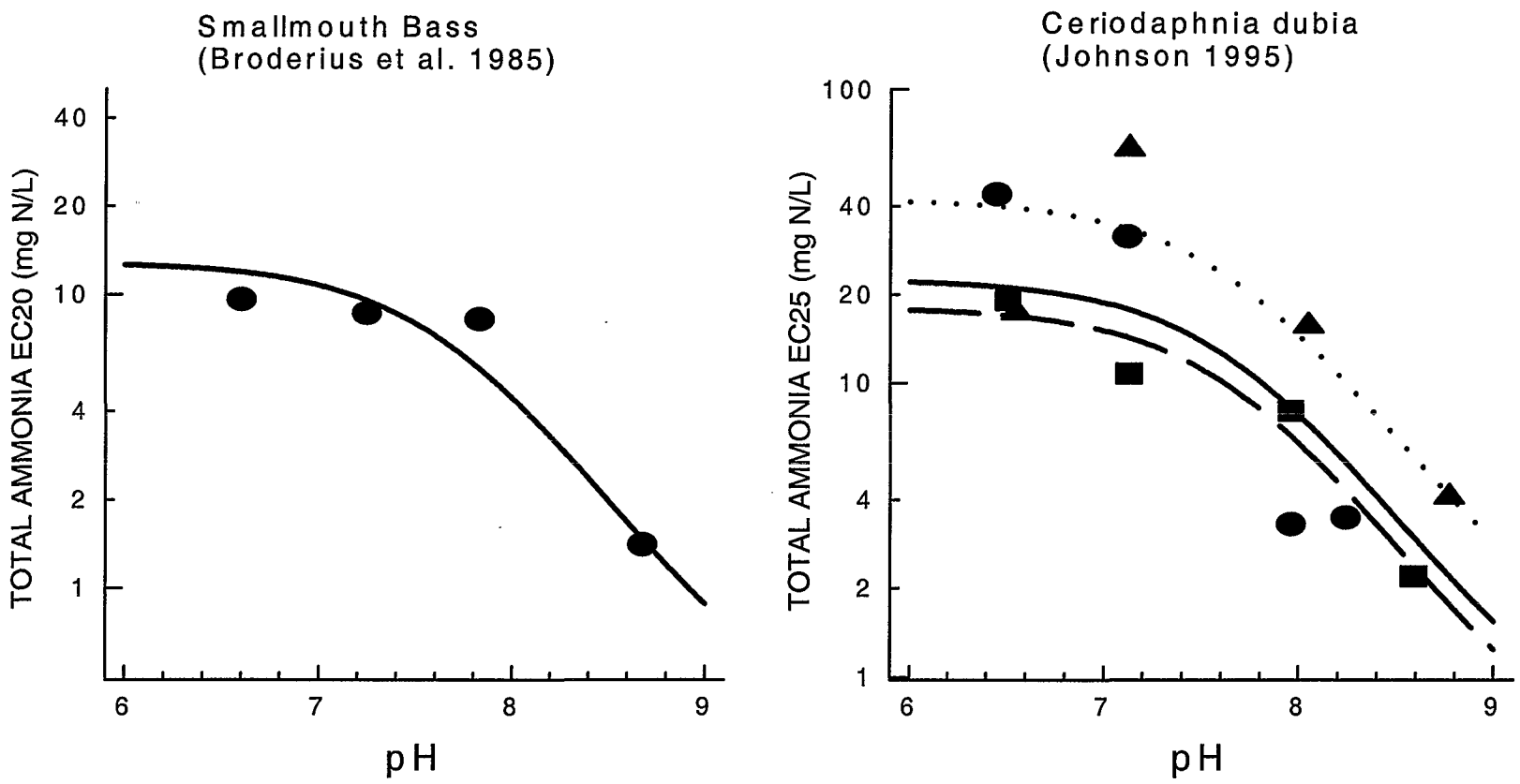


Figure 8. The effect of pH on chronic ammonia toxicity in terms of total ammonia. Symbols denote chronic effect concentrations and lines denote regressions of effect concentrations versus pH. For *C. dubia*, different symbols denote different test water formulations.



in the 1984/1985 criteria document regarding the pH dependence of acute and chronic toxicity. Tests by Borgmann (1994) on the chronic toxicity of ammonia to *Hyalella azteca* and by Armstrong et al. (1978) on the 6-day toxicity of ammonia to *Macrobrachium rosenbergii* also support a lower slope for total ammonia chronic toxicity versus pH at pH<8. The dependence of chronic ammonia toxicity on pH appears to be sufficiently different from the dependence of acute ammonia toxicity to justify use of two equations.

By substituting the values for R and pH_T into equation 7, the following equations are obtained for describing the pH-dependence of acute values (AVs) and chronic values (CVs) expressed in terms of total ammonia nitrogen:

$$AV_t = \left(AV_{t,8}\right)\left(\frac{0.0489}{1 + 10^{7.204-pH}} + \frac{6.95}{1 + 10^{pH-7.204}}\right) \quad \textbf{(10)}$$

$$CV_t = \left(CV_{t,8}\right)\left(\frac{0.0676}{1 + 10^{7.688-pH}} + \frac{2.91}{1 + 10^{pH-7.688}}\right) \quad \textbf{(11)}$$

The range of the data used to derive these equations indicates that they should be applicable from pH=6 to 9, although considerable error 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).

DERIVATION OF THE NEW CMC

The scope of this project included a re-examination of the temperature and pH relationships underlying the 1984/1985 Criterion Maximum Concentration (CMC). Because the acute toxicity dataset contained in the 1984/1985 criteria document (U.S. EPA 1985a) is relatively large, with tests involving species in 34 genera, the scope of this project did not include a comprehensive literature search and critical review of all of the acute toxicity data now available. Thus, the derivation here relies solely on acute tests reported in Table 1 in the 1984/1985 criteria document. However, some newer studies of acute toxicity known to this effort were examined to determine whether new data might materially affect the CMC. These studies include Ankley et al. 1995; Arthur et al. 1987; Bailey et al. 1985; Bergerhouse 1992,1993; Dabrowska and Sikora 1986; DeGraeve et al. 1987; Diamond et al. 1993 (see Appendix 1); Gersich and Hopkins 1986; Goudreau et al. 1993; Gulyas and Fleit 1990; Hasan and Macintosh 1986; Henderson et al. 1961; Lee 1976; Mayes et al. 1986; Monda et al. 1995; Nimmo et al. 1989; Russo et al. 1988; Sheehan and Lewis 1986; Snell and Persoone 1989; Thomas et al. 1991; Tomasso and Carmichael 1986; Wade 1992; and Williams et al. 1986. These studies would add few new genera to the dataset and their data are generally in the range already observed and would have little impact on the four lowest Genus Mean Acute Values (GMAVs). The most significant result of these studies is that some invertebrates are acutely sensitive to ammonia at low pH and low ion concentration (Borgmann 1994; Ankley et al. 1995). Although new data are not used in the derivation of the new CMC, they are compared to the new CMC below.

All of the un-ionized ammonia acute values (LC50s and EC50s) in Table 1 of the 1984/1985 criteria document were converted to total ammonia nitrogen acute values, using the reported temperatures and pHs and using the pK relationship from Emerson et al. (1975). These total ammonia nitrogen acute values were then adjusted (see Appendix 3) to pH=8 using the pH relationship developed above, with no adjustment for temperature. These adjusted total ammonia nitrogen acute values (see Appendix 4) were then averaged to determine Species Mean Acute Values (SMAVs) and GMAVs at pH=8 (Table 1) using the procedure described in the 1985 Guidelines (U.S. EPA 1985b). (The same genera are in Table 1 in this 1998 Update as are in Table 3 in the 1984/1985 criteria document and the SMAVs and GMAVs in both tables are based on the test results in Table 1 in the criteria document. The GMAVs in the two tables are different because (a) pH and temperature are addressed differently in the two sets of calculations, (b) the

Table 1. Ranked Genus Mean Acute Values

Rank	Genus Mean Acute Value (mg N/L[a])	Species	Species Mean Acute Value (mg N/L[a])
34	388.8	Caddisfly, Philarctus quaeris	388.8
33	246.0	Crayfish, Orconectes immunis	1466.
		Crayfish, Orconectes nais	41.27
32	210.6	Isopod, Asellus racovitzai	210.6
31	189.2	Mayfly, Ephemerella grandis	189.2
30	115.5	Mayfly, Callibaetis skokianus	175.6
		Mayfly, Callibaetis sp.	75.93
29	113.2	Beetle, Stenelmis sexlineata	113.2
28	108.3	Amphipod, Crangonyx pseudogracilis	108.3
27	97.82	Tubificid worm, Tubifex tubifex	97.82
26	93.52	Snail, Helisoma trivolvis	93.52
25	77.10	Stonefly, Arcynopteryx parallela	77.10
24	73.69	Snail, Physa gyrina	73.69
23	51.73	Mottled sculpin, Cottus bairdi	51.73
22	51.06	Mosquitofish, Gambusia affinis	51.06
21	43.55	Fathead minnow, Pimephales promelas	43.55
20	38.11	White sucker, Catostomus commersoni	45.82

Rank	Genus Mean Acute Value (mg N/L[a])	Species	Species Mean Acute Value (mg N/L[a])
		Mountain sucker, Catostomus platyrhynchus	31.70
19	36.82	Cladoceran, Daphnia magna	35.76
		Cladoceran, Daphnia pulicaria	37.91
18	36.39	Brook trout, Salvelinus fontinalis	36.39
17	35.65	Clam, Musculium transversum	35.65
16	34.44	Channel catfish, Ictalurus punctatus	34.44
15	33.99	Cladoceran, Simocephalus vetulus	33.99
14	33.14	Guppy, Poecilia reticulata	33.14
13	32.82	Flatworm, Dendrocoelum lacteum	32.82
12	30.89	White perch, Morone americana	30.89
11	26.97	Stoneroller, Campostoma anomalum	26.97
10	26.50	Smallmouth bass, Micropterus dolomieu	35.07
		Largemouth bass, Micropterus salmoides	20.03
9	26.11	Walleye, Stizostedion vitreum	26.11
8	25.78	Cladoceran, Ceriodaphnia acanthina	25.78
7	25.60	Red shiner, Notropis lutrensis	45.65
		Spotfin shiner, Notropis spilopterus	19.51
		Steelcolor shiner, Notropis whipplei	18.83

Rank	Genus Mean Acute Value (mg N/L[a])	Species	Species Mean Acute Value (mg N/L[a])
6	23.74	Brown trout, Salmo trutta	23.74
5	23.61	Green sunfish, Lepomis cyanellus	30.27
		Pumpkinseed, Lepomis gibbosus	18.05
		Bluegill, Lepomis macrochirus	24.09
4	21.95	Golden trout, Oncorhynchus aquabonita	26.10
		Cutthroat trout, Oncorhynchus clarki	25.80
		Pink salmon, Oncorhynchus gorbuscha	42.07
		Coho salmon, Oncorhynchus kisutch	20.26
		Rainbow trout, Oncorhynchus mykiss	11.23[b]
		Chinook salmon, Oncorhynchus tshawytscha	17.34
3	17.96	Orangethroat darter, Etheostoma spectabile	17.96
2	14.67	Golden shiner, Notemigonus crysoleucas	14.67
1	12.11	Mountain whitefish, Prosopium williamsoni	12.11

[a] All values are total ammonia nitrogen at pH=8.
[b] Thurston and Russo (1983) conducted numerous acute toxicity tests with larval, juvenile, yearling, and larger rainbow trout and demonstrated that large rainbow trout were measurably more sensitive than other life stages. The average adjusted total ammonia nitrogen acute value for large rainbow trout was 11.23 mg N/L. Therefore, this SMAV was lowered to 11.23 mg N/L in order to protect large rainbow trout, as per the 1985 Guidelines (U.S. EPA 1985b).

golden trout, cutthroat trout, and rainbow trout are now in a different genus, and (c) and the new GMAVs are expressed in terms of total ammonia nitrogen; the order of the genera is different mostly because no temperature adjustment is used in either the criteria document or this 1998 Update for invertebrates even though Table 3 in the 1984/1985 criteria document is based on un-ionized ammonia whereas Table 1 in this 1998 Update is based on total ammonia nitrogen.) The Final Acute Value (i.e., the fifth percentile) at pH=8 was calculated from this set of adjusted total ammonia GMAVs to be 14.32 mg N/L.

The SMAV for rainbow trout is 11.23 mg N/L, and so the FAV is lowered to this value, as per the 1985 Guidelines (U.S. EPA 1985b), comparable to what was done in the 1984/1985 ammonia criteria document. The CMC at pH=8 equals one-half of this FAV. Substitution of this CMC at pH=8 for $AV_{t,8}$ in equation 10 results in the following equation for expressing the CMC as a function of pH:

$$CMC = \frac{0.275}{1 + 10^{7.204-pH}} + \frac{39.0}{1 + 10^{pH-7.204}} \qquad \textbf{(12)}$$

If the four genera (*Oncorhynchus*, *Prosopium*, *Salmo*, and *Salvelinus*) in the family Salmonidae are excluded from the dataset in Table 1, the fifth percentile FAV with salmonids absent is 16.8 mg N/L and the CMC is 8.4 mg N/L at pH=8; substitution into equation 10 gives the CMC as a function of pH:

$$CMC = \frac{0.411}{1 + 10^{7.204-pH}} + \frac{58.4}{1 + 10^{pH-7.204}} \qquad \textbf{(13)}$$

Figure 9 shows the ranked GMAVs, the CMC with salmonids present, and the CMC with salmonids absent, all at pH=8. The GMAVs represent LC50s, whereas the CMCs represent concentrations that are lethal to substantially less than 50 percent of the individuals in either the fifth percentile genus or a sensitive important species.

FAVs and CMCs are plotted in Figure 10, along with all of the individual total ammonia acute values, unadjusted for pH, used in the calculations. The FAVs show good correspondence with the lower range of the acute values. As discussed above, more recent acute data are also in general accordance with the FAVs, except that the *Hyalella azteca* LC50 from Ankley et al. (1995) at low ion concentration and pH=6.5 is more than a factor of two below the FAV. Although some toxicity data are expected to be below

Figure 9. Ranked Genus Mean Acute Values (GMAVs) with Criterion Maximum Concentrations (CMCs).

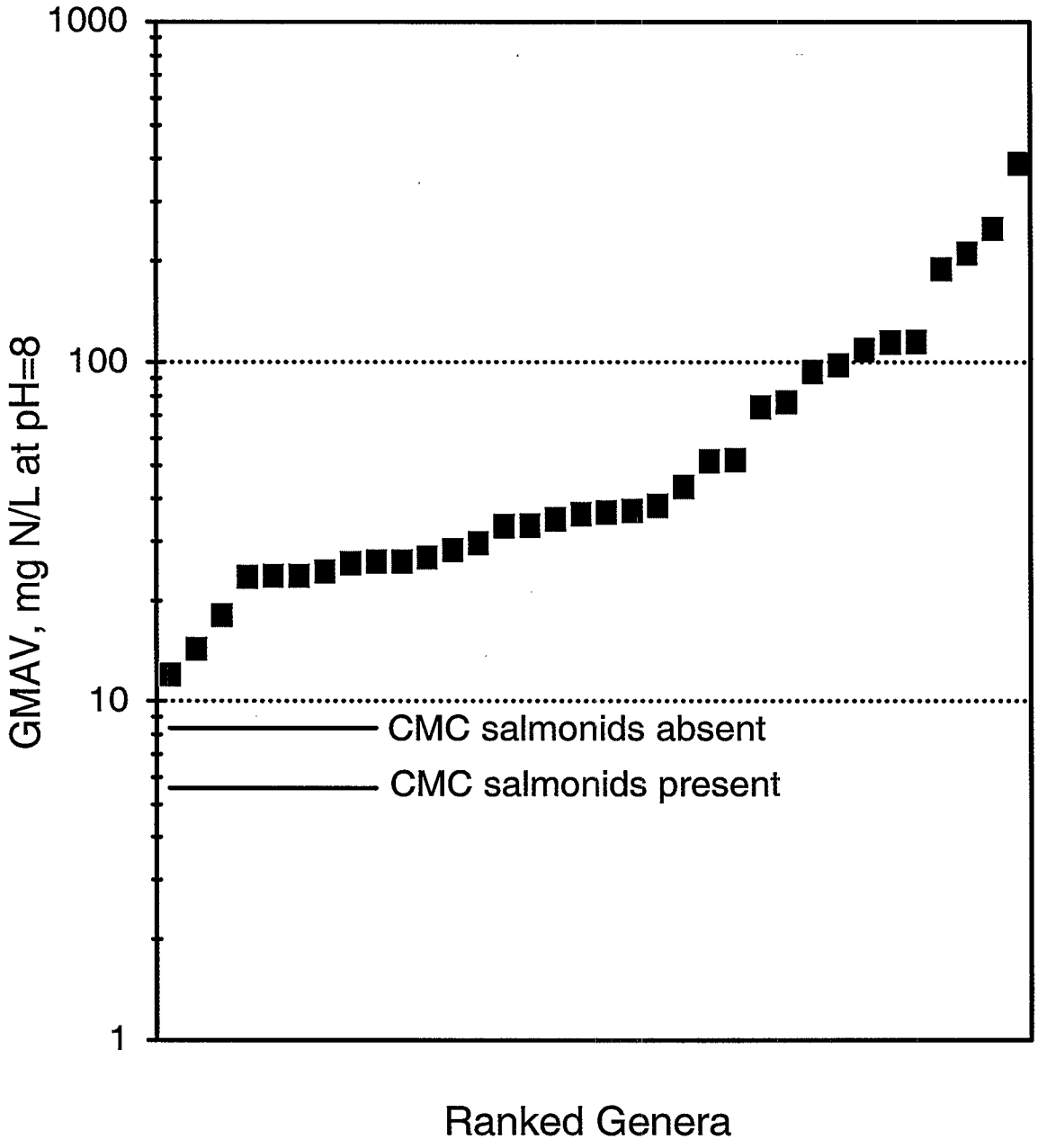
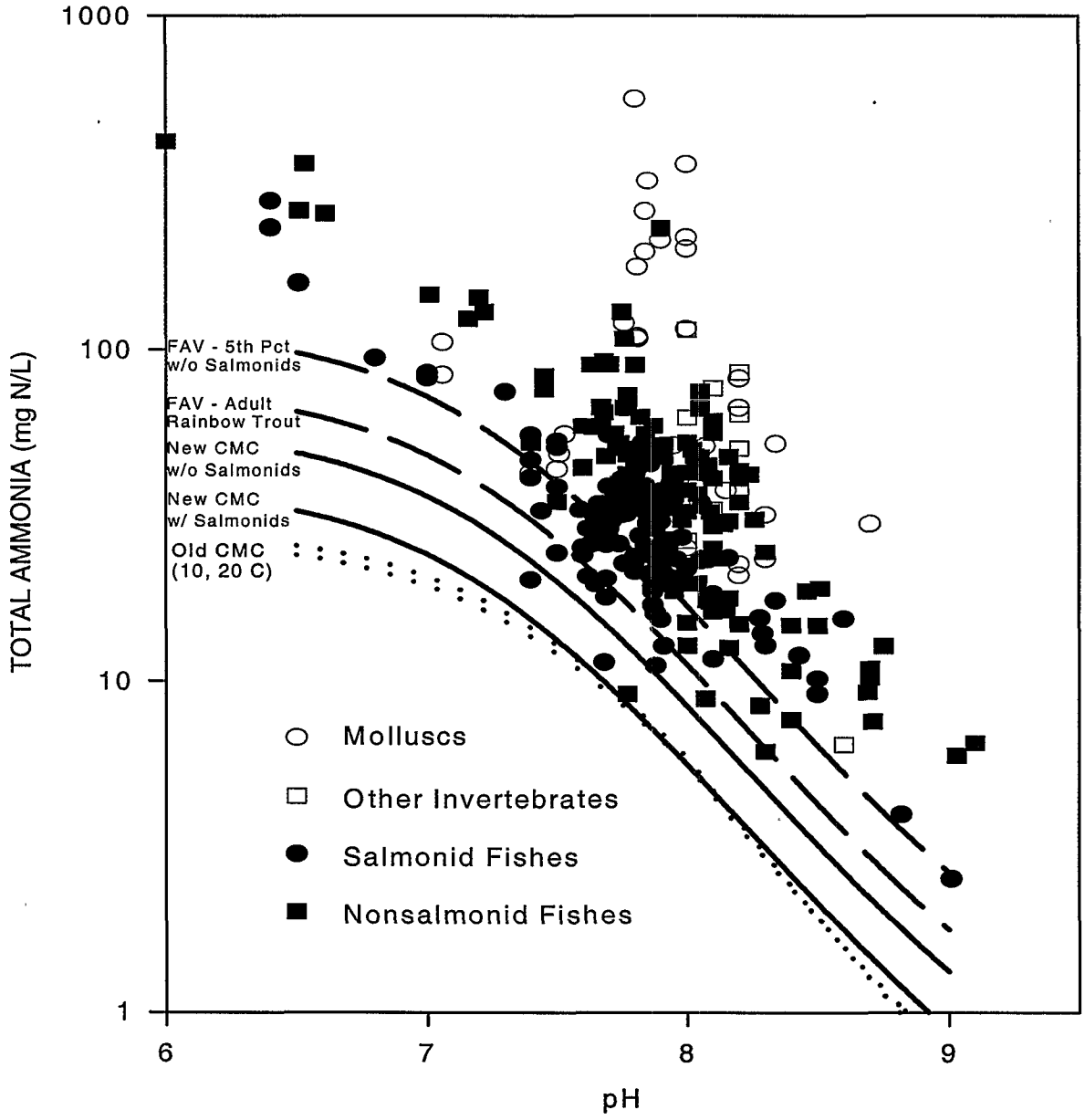


Figure 10. Acute LC50s used in criteria derivation in relationship to Final Acute Values (FAVs) and Criterion Maximum Concentrations (CMCs).



the FAV, inclusion of this genus in the calculation would have resulted in a lower CMC, but only under these extreme water quality conditions and only if the effects of both pH and ionic composition were described for each individual genus, which is not possible with the data that are currently available.

REVIEW AND ANALYSIS OF CHRONIC DATA

Due to the magnitudes of the acute-chronic ratios (ACRs) for ammonia, the ammonia CCC is sufficiently low relative to the CMC that the CCC generally will be the determining factor for permit limits. In the 1984/1985 ammonia criteria document, the CCC is more uncertain than the CMC because (1) the CCC was calculated by dividing the FAV by an ACR (thus including the uncertainties of both the FAV and the ACR) and (2) fewer acceptable chronic toxicity tests were available and not all of them could be used to derive ACRs. Additionally, depending on how they were derived, the individual chronic values could differ with respect to the nature and degree of the toxic effects they represented. To reduce this variability, all of the chronic data used in the 1984/1985 criteria document and newer chronic data known to the authors or suggested by reviewers were reviewed and analyzed to produce a more extensive and consistent set of Chronic Values (CVs) that could be used to directly calculate a CCC rather than to calculate it using ACRs. This procedure also has some limitations because (a) the criterion usually decreases as the number of genera used in the calculation of the 95th percentile decreases and (2) chronic tests have been conducted with a larger proportion of the species that are acutely sensitive to ammonia than those that are acutely resistant to ammonia.

The first two parts of this section describe how the chronic tests on ammonia were reviewed and how the CVs were calculated. The third part discusses each chronic test of which this project was aware and presents the relevant results.

Review of Chronic Data

Each chronic dataset was subjected to the following two-step review process. The first step was to determine whether the test methodology was acceptable for providing information about a CV. A test was considered acceptable if the dilution water, control mortality, experimental design, loading, etc., were consistent with ASTM Standards E1193, E1241, and E1295 (ASTM 1997a,b,c). The concentration of dissolved oxygen was also reviewed on the basis of U.S. EPA (1986).

Reviewing the concentration of dissolved oxygen (DO) was difficult because (a) ASTM Standards E1193, E1241, and E1295 (ASTM 1997a,b,c) express limits on high and low concentrations of DO in terms of percent saturation, whereas U.S. EPA (1986) expresses limits on low concentrations of DO in terms of the concentration itself, and (b) neither specifies the limits in a

way that can be used directly to interpret the kinds of information that are given in most reports of the results of toxicity tests. Therefore, the following rationale was used. The mean DO concentration needs to be within an acceptable range, but limits expressed as long-term averages can allow excessively low or high concentrations for too long a period. Conversely, a limit that must be satisfied at all times can unnecessarily penalize investigators who make more than the minimum number of measurements and ignores the fact that organisms can tolerate extreme concentrations for brief periods of time. Therefore, limits were placed on the mean and the fifth and ninety-fifth percentiles of the DO concentrations. Use of limits that are expressed in terms of the mean and the fifth and ninety-fifth percentiles is straightforward when the mean and standard deviation are reported or when all of the individual measurements are reported, but not when only the range is reported. If the measured concentration of DO during a chronic test was reported as a range, the lowest and highest values were considered to be concentrations that existed for at least 5 percent of the time during the test.

The limits used were:

1. A chronic test was considered questionable if either (a) the mean DO concentration was below 60 or above 100 percent of saturation or (b) the concentration of DO was below 50 or above 105 percent of saturation more than 5 percent of the time during the test. These limits are similar to, but different from, the limits given in ASTM Standards E1193, E1241, and E1295 (ASTM 1997a,b,c).

   It is clear that 60 percent of saturation is the desirable lower limit in Section 11.2.1 of ASTM Standard E729 (ASTM 1997d); for practical reasons, this section allows the concentration of DO to be between 40 and 60 percent of saturation during the last 48 hours of 96-hr static acute tests. Because test organisms and BOD utilize oxygen, when the concentration of DO is above 100 percent of saturation, it is quite possible that the concentration of dissolved nitrogen is even more supersaturated, which increases the possibility of gas bubble disease.
2. A chronic test was considered questionable if either (a) the mean measured DO concentration was below the mean given below or (b) the DO concentration was below the lower limit given below for more than 5 percent of the time during the test:

	Mean (mg/L)	Lower Limit (mg/L)
Salmonids:	6.5	5.0
Warmwater fishes		
Early life stages	6.0	5.0
Other life stages	5.5	4.0
Invertebrates	6.0	5.0

The first three means are presented on page 34 of U.S. EPA (1986) and are 0.5 mg/L above the concentrations given for "slight production impairment" on page 31. U.S. EPA (1986) does not give a "mean" for invertebrates on page 34 and so the last mean given above is 1 mg/L higher than the concentration given for "some production impairment" on page 31. The lower limits are concentrations given on page 31 for "moderate production impairment" or "some production impairment".

Regardless of how limits on the DO concentration are expressed, it is sometimes difficult to apply them to the information that is reported concerning toxicity tests.

If there was no reason to believe that the test methodology was unacceptable, the second step of the review process was to determine whether the test satisfied one of the definitions given in the 1985 Guidelines for life-cycle, partial life-cycle, and early life-stage test. By definition, life-cycle tests can be conducted with either a fish species or an invertebrate species, but partial life-cycle and early life-stage tests can only be conducted with a fish species. The considerations that excluded the most tests were that (a) tests that did not include the newly hatched life stage cannot be acceptable life-cycle, partial life-cycle, or early life-stage tests, and (b) tests that did not study reproduction cannot be acceptable life-cycle or partial life-cycle tests. Each test that satisfied one of the definitions could provide one of three kinds of information:

1. If all of the tested concentrations of the toxicant were so high that all of them caused unacceptable effects, the test will probably provide an upper limit on a CV, i.e., the CV will be lower than the lowest tested concentration.
2. If all of the tested concentrations were so low that none of them caused an unacceptable effect, the test will probably provide a lower limit on a CV, i.e., the CV will be higher than the highest tested concentration.
3. If the low tested concentrations did not cause unacceptable effects but the high tested concentrations did, the test will probably provide a CV.

If the test did not satisfy the requirements for any of the three kinds of tests, it was necessary to determine whether the toxicant caused an unacceptable reduction in (a) survival, reproduction, and/or hatchability over any period of at least

seven days, or (b) growth over a period of at least 90 days. If it caused either kind of unacceptable reduction, the test will probably provide an upper limit on a CV or it might lower a CV from an early life-stage test. If it did not cause either kind of unacceptable reduction, the test cannot provide a CV or an upper or lower limit on a CV, but the test might provide other useful information. Because the test is not an acceptable life-cycle, partial life-cycle, or early life-stage test, an upper limit on a CV can be based on a reduction in survival, reproduction, and/or hatchability over any period of at least seven days, but it cannot be based on a reduction in weight gain for fewer than 90 days because such a reduction might be temporary; such a test cannot provide a lower limit on a CV because some other life stage might be more sensitive. Although some CVs were based on histopathological effects in the 1984/1985 ammonia criteria document, this current effort could find no justification for equating histopathological effects with effects on survival, growth, and reproduction (see Appendix 5).

Calculation of Chronic Values

Chronic values used in aquatic life criteria documents have traditionally been based on analysis of data to determine the highest tested concentration at which no relevant toxicological variable had a value that was statistically significantly different from the value for the control treatment (highest no observed adverse effect concentration, HNOAEC) and the lowest concentration at which the value for at least one of the relevant toxicological variables was significantly different from the value for the control treatment (lowest observed adverse effect concentration, or LOAEC). When endpoints are defined on the basis of such hypothesis testing of each tested concentration against the control treatment, the CV is set equal to the geometric mean of the HNOAEC and the LOAEC. Such a procedure has the disadvantage of resulting in marked differences between the magnitudes of the effects corresponding to the individual CVs, due to variation in the power of the statistical tests used, the concentrations tested, and the size and variability of the samples used (Stephan and Rogers 1985). For example, the CVs reported in the 1984/1985 ammonia criteria document corresponded to reductions from the control treatment of just a few percent to more than fifty percent.

To make CVs reflect a uniform level of effect, regression analysis was used here both to demonstrate that a significant concentration-effect relationship was present and to estimate CVs with a consistent level of effect. Use of regression analysis is provided for on page 39 of the 1985 Guidelines (U.S. EPA 1985b). The most precise estimates of effect concentrations can generally

be made for 50 percent reduction (EC50); however, such a major reduction is not necessarily consistent with criteria providing adequate protection. In contrast, a concentration that caused a low level of reduction, such as an EC5 or EC10, is rarely statistically significantly different from the control treatment. As a compromise, the EC20 is used here as representing a low level of effect that is generally significantly different from the control treatment across the useful chronic datasets that are available for ammonia.

Regression analysis was performed on a chronic dataset only if the dataset met the following conditions: (1) it contained a control treatment to anchor the curve at the low end, (2) it contained at least four concentrations of ammonia to provide at least two error degrees of freedom when the three-parameter equation is fit to a set of data, (3) the highest tested concentration of ammonia caused >50 percent reduction relative to the control treatment to anchor the curve at the high end, and (4) at least one tested concentration of ammonia caused <20 percent reduction relative to the control treatment to ensure that the EC20 was bracketed by tested concentrations of ammonia.

For life-cycle and partial life-cycle tests, the toxicological variables used in these regression analyses were survival, embryo production, and embryo hatchability. For early life-stage tests, the variables used were embryo hatchability, fry survival, and fry growth; if ammonia apparently reduced both survival and growth, the product of these variables (biomass) was analyzed, rather than analyzing them separately. For other acceptable chronic tests, the toxicological variable analyzed was survival, reproduction, hatchability, and/or growth as appropriate, based on the requirements stated above concerning acceptability of chronic tests.

The regression model used was based on the logistic equation:

$$T = \frac{T_0}{1 + A \cdot C^B} \qquad \textbf{(14)}$$

This equation produces an "S-shaped" curve, with the toxicological variable of interest (T) being at a control value (T_0) at low concentrations, zero at high concentrations, and declining at intermediate concentrations; the location and steepness of this decline are determined by the parameters A and B, respectively. It is not argued that this equation embodies a mechanistic description of chronic toxicity, but rather that this is a useful equation that incorporates the major features commonly observed in concentration-effect relationships.

Application of various forms and extensions of this equation to toxicological data have been discussed by various authors, most recently by Moore and Caux (1997).

To make the equation more directly interpretable with respect to effect concentrations and to assist in determining confidence limits for such effect concentrations as the EC20, the equation was reformulated to:

$$T = \frac{T_0}{1 + \left(\frac{p}{100-p}\right)\left(10^{B(\log C - \log ECp)}\right)} \qquad \textbf{(15)}$$

where logECp (i.e., the logarithm of the concentration causing T to be reduced by p percent from T_0) is a parameter rather than A. This equation was applied to each dataset using nonlinear least-squares regression analysis (Draper and Smith 1981), with p=20%. Software used for determining the least-squares solution was written in FORTRAN using nonlinear search routines based on the Newton-Raphson method (Dahlquist and Bjorck 1974).

Either transformation or weighting was applied to each dataset to improve the homogeneity of the variance:

1. When T was a percentage, the regression analysis was conducted on a transformation T_i^* of each data point T_i as follows (Draper and Smith 1981):

$$T_i^* = \arcsin(\sqrt{T_i/100}) \qquad \textbf{(16)}$$

   The regression equation was similarly transformed and the parameter T_0 was formulated to be the transformed effect.
2. When T was count data, the regression analysis was conducted on the square root transformation of T_i and the regression equation was similarly transformed (Draper and Smith 1981).
3. When T was weight or biomass, no transformation was used, but each datum was weighted by the inverse of its variance (Draper and Smith 1981). For weight data, these weighting factors were based on standard errors (SEs) or standard deviations (SDs) divided by $N^{1/2}$ as reported by the authors. For biomass [B = product of proportion survival (P) and weight (W) in early life-stage tests], the variance was estimated as follows:

$$VAR(B) \approx W^2 \cdot SE_P^2 + P^2 \cdot SE_W^2 \qquad \textbf{(17)}$$

where SE_P is the SE of P as reported by the authors or calculated as $(P(1-P)/N)^{1/2}$, and SE_W is the SE of W as reported by the authors or calculated from their data.

In addition to the dataset-specific transformation or weighting described above, all regression analyses used a general weighting scheme to make the analyses more appropriate for calculating EC20s. When this type of regression analysis is used to calculate such low-effect concentrations as an EC20, lack of fit of the model at high-effect concentrations can perturb the fit of the model at low-effect concentrations. If the form of the regression equation is known to be completely accurate, such perturbation is appropriate; in this case, however, the equation is not expected to describe the exact form of the concentration-effect curve over the whole range of T. Because high effect concentrations contain useful information about the nature of the curve, they should not be excluded, but they should not be allowed to unduly influence the fit in the range from 0 to 50 percent reduction. Consequently, normal weights were given to data points up to the first concentration with a 50% or greater reduction relative to the control treatment and points at higher concentrations were weighted by half. An alternative was to use a more complicated form of the logistic equation (e.g., Moore and Caux 1997), but such equations introduce their own uncertainties, especially for small datasets, and their main effect on calculation of the EC20 is to reduce the influence of data points at high effects, with much the same results as the weighting scheme used here.

SEs of the regression parameters were calculated based on the variance/covariance matrix of the linearized model at the least-squares solution (Draper and Smith 1981) and 95% confidence limits for the parameters were calculated by multiplying these SEs by the applicable t-statistic. Simulations showed that this procedure produces confidence levels that are near or greater than 95%. The EC20 and its confidence limits were computed by taking the antilog of the calculated logEC20 and its confidence limits. Confidence limits on effect concentrations for percentages other than 20 and on values for T at concentrations other than 0 were estimated by reformulating the regression equation to use these values rather than EC20 and T_0 as parameters, and then recomputing the variance/covariance matrix at the least-squares solution to determine the SEs of the new parameters.

Evaluation of the Chronic Data Available for Each Species

The following presents a species-by-species discussion of each chronic test on ammonia evaluated by this project. For each

species, the available chronic tests are discussed in the following order: life-cycle tests, partial life-cycle tests, early life-stage tests, other laboratory tests, and then results from a field study. Also presented are the results of regression analysis of each dataset that was from an acceptable chronic test and contained sufficient acceptable data. For each such dataset, Appendix 6 contains a figure that presents the data and regression line. All analyses were conducted in terms of total ammonia nitrogen, either as reported by the authors or as converted by us from the reported values for un-ionized ammonia, pH, and temperature using the speciation relationship of Emerson et al. (1975). When an EC20 could be determined, it is first reported as calculated by regression analysis of the data at the pH and temperature of the test. Then, to facilitate comparisons of sensitivities within and between species, each EC20 is adjusted to pH=8 using the relationship between chronic toxicity and pH derived above on the basis of Broderius et al. (1985) and Johnson (1995). Species Mean Chronic Values (SMCVs) were derived when justified by the data, and then Genus Mean Chronic Values (GMCVs) were derived when justified by the SMCVs. All of the EC20s, SMCVs, and GMCVs that were derived are tabulated in Table 2, which is located at the end of this section.

Musculium transversum (Sphaerium transversum) (Fingernail clam)

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 so similar that the data were pooled for analysis. The lowest mean measured DO concentration in any treatment was 6.5 mg/L (77 percent of saturation) and the lowest individual measured concentration was 5 mg/L (60 percent of saturation). Survival in the control treatment and low ammonia concentrations (<5.1 mg N/L) ranged from 79 to 90%, but decreased to zero at 18 mg N/L. Regression analysis of the survival data using an arcsine transformation resulted in a calculated EC20 of 5.82 mg N/L at 23.5°C and pH=8.15. The EC20 is 7.30 mg N/L when adjusted to pH=8.

Sparks and Sandusky (1981) conducted a test similar to that of Anderson et al. (1978) with field-collected juvenile clams whose average length was 2.1 mm. Although this test used a better food, the test was conducted in the same laboratory and used test organisms from the same pool in the Mississippi River as Anderson et al. (1978); Sparks participated in both studies. The lowest mean measured DO concentration in any treatment was 6.4 mg/L (73 percent of saturation) and the lowest individual measured concentration was 5.0 mg/L (57 percent of saturation). Survival in the control treatment was

92% and decreased with increasing concentration of ammonia to 17% at 18 mg N/L. Effects on survival were evident at lower concentrations, resulting in an EC20 of 1.23 mg N/L at 21.8°C and pH=7.80. The EC20 adjusted to pH=8 is 0.94 mg N/L. Although this EC20 is substantially lower than that obtained by Anderson et al. (1978), the difference is less than a factor of 10.

Zischke and Arthur (1987) studied fingernail clam growth, survival, and reproduction in enclosures placed in experimental streams for periods of 4 to 10 weeks during a 16-month field study of the effects of ammonia (Hermanutz et al. 1987). Experiments during the first year showed reductions in survival of clams in a stream in which the concentration of total ammonia nitrogen was approximately 2 mg N/L during the test period (Hermanutz et al. 1987), but not in a stream in which the concentration was 0.7 mg N/L. The daily mean stream temperature ranged from 20 to 25°C and pH ranged from 7.4 to 7.8 during this test period. During the second year of the study, substantial effects occurred on reproduction of clams at 1 mg N/L (the lowest tested concentration of ammonia) at 24 to 26°C and pH=7.8 to 8.2 during the test period. Adjusted to pH=8, both years showed effects at about 1 mg N/L. These results are not included in Table 2 because results of field tests are not used in the derivation of Final Chronic Values (U.S. EPA 1985b).

The SMCV at pH=8 is ≤2.62 mg N/L. This concentration is the geometric mean of the adjusted EC20s for the two laboratory studies and is an upper limit on the SMCV because the EC20s are based on survival of juveniles, which might not be as sensitive to ammonia toxicity as early life stages. This SMCV is uncertain due to the difference between the results of the two chronic tests. However, the experimental stream data suggest that the SMCV should be close to 1 mg N/L. The GMCV is also ≤2.62 mg N/L.

Ceriodaphnia acanthina

Mount (1982) conducted a life-cycle test that started with <1-day-old organisms and proceeded until most of the control organisms produced three broods. The DO concentration ranged from 5.7 to 6.4 mg/L (68 to 77 percent of saturation). Total offspring production per treatment was unaffected at concentrations ≤21 mg N/L, but reproduction was virtually absent at concentrations ≥77 mg N/L. Regression analysis using a square root transformation resulted in an EC20 of 44.9 mg N/L at pH=7.15 and 24.5°C. The EC20 adjusted to pH=8 is 19.8 mg N/L, which is the SMCV.

Ceriodaphnia dubia

Willingham (1987) conducted a 7-day life-cycle test starting with <1-day-old organisms. The lowest mean measured DO concentration in any treatment was 6.04 mg/L (74 percent of saturation) and the lowest calculated fifth percentile of the DO concentrations was 5.62 mg/L (69 percent of saturation). Production of young during the third brood was unaffected at concentrations up to 2.8 mg N/L, but was reduced at higher concentrations and was absent at 43 mg N/L. The EC20 calculated using regression analysis was 5.80 mg N/L at pH=8.57 and 26.0°C. Adjusted to pH=8, the EC20 is 14.6 mg N/L.

Nimmo et al. (1989) conducted a 7-day life-cycle test at 25°C and pH=7.8 in water from the St. Vrain River. The DO concentration was reported to be low in some other tests that were conducted during this study, but it was not reported to be low in this test. Based on the average number of neonates per original female, the EC20 calculated using regression analysis and a square root transformation was 15.2 mg N/L. Adjusted to pH=8, the EC20 is 11.6 mg N/L.

As stated above in the discussion of the effect of pH on the toxicity of ammonia, Johnson (1995) conducted twelve chronic tests on ammonia with *C. dubia* at four pHs and three hardnesses. The lowest reported mean concentration of DO was 6.9 mg/L (82 percent of saturation). When adjusted to pH=8, the mean EC25s are 9.03, 7.46, and 17.1 mg N/L at average hardnesses of 42, 86, and 170 mg/L, respectively. These mean adjusted EC25s are similar to the adjusted EC20s obtained by Willingham (1987) and Nimmo et al. (1989). These EC25s are not included in Table 2 because they are not EC20s and were calculated using a different regression-type approach.

Adjusted to pH=8, the two EC20s for *C. dubia* are 14.6 and 11.6 mg N/L, which gives a SMCV of 13.0 mg N/L. For *C. acanthina* at pH=8, the SMCV is 19.8 mg N/L, which gives a GMCV of 16.0 mg N/L.

Daphnia magna

Gersich et al. (1985) and Gersich and Hopkins (1986) reported results of a life-cycle test that was conducted in water from the Tittabawassee River. This water was probably an acceptable dilution water because it was apparently collected upstream of all known point discharges (Alexander et al. 1986; James Grant, Michigan Department of Environmental Quality, personal communication). The lowest and highest measured DO concentrations were 8.8 and 9.2 mg/L (96 and 101 percent of

saturation). No significant effects were found at concentrations up to 4.2 mg N/L at pH=8.45 and 19.8°C, but progressively larger reductions were found at concentrations of 9 to 36 mg N/L. The EC20 calculated from regression analysis was 7.37 mg N/L.

In another life-cycle test, Reinbold and Pescitelli (1982a) found little reduction in reproduction at 20 mg N/L, but a large reduction at 33 mg N/L. The measured DO concentrations averaged 88 to 91 percent of saturation. The EC20 is 21.7 mg N/L at pH=7.92 and 20.1°C.

Gulyas and Fleit (1990) conducted a 9-day chronic test to study the effect of ammonia on development and growth. Concentrations that caused more than fifty percent reduction compared to the controls were considered toxic. The "no effect level" was reported to be 0.1 mg/L. No results from this test are included in Table 2 because neither survival nor reproduction was studied.

Adjusted to pH=8, the respective EC20s are 15.1 and 19.4 mg N/L. The SMCV for this species is 17.1 mg N/L, which is the geometric mean of the two adjusted EC20s; this is also the GMCV.

Crangonyx spp. (amphipod)

The available data for this species are not used for the reason(s) given in Appendix 1.

Hyalella azteca (amphipod)

Borgmann (1994) conducted three tests that began with <1-week-old organisms, all of which utilized weekly renewals and dechlorinated tap water originating from Lake Ontario. One of the three tests lasted four weeks, but the other two lasted ten weeks and produced data concerning both survival and reproduction. The results of these last two tests were sufficiently similar that the results were analyzed together. No information was reported concerning the DO concentration. Sufficient raw data were obtained from the author so that each test chamber could be plotted as a separate point for the combined regression analysis. Survival over the ten weeks in the control treatment averaged 66.3 percent and reproduction per chamber averaged 48 offspring. The 33.7% mortality in the control treatment is considered acceptable in a 10-week test because ASTM Standard E1706 (ASTM 1997e) allows 10% mortality of *H. azteca* in a 4-day test (see Tables 10 and 11) and allows 20% mortality in a 10-day test (see Table 15). In addition, although ASTM Standard E1706 allows 20% mortality in a 10-day

test, Table 3 in Borgmann (1994) indicates that only 11.6% of the controls died in four weeks.

At the lowest tested concentration, survival was reduced 25 percent relative to the control treatment and reproduction was reduced 55 percent. Regression analysis produced an EC20 of 0.88 mg N/L based on reproduction, but this EC20 is below the lowest tested concentration because the dataset does not contain a concentration that caused <20 percent reduction relative to the control treatment. However, the confidence limits on the regression analysis indicate that the 55 percent reduction in reproduction caused by the lowest tested concentration is statistically significant. Based on the raw data, the concentration of ammonia in the lowest tested concentration was 1.58 mg N/L and the mean pH of this treatment was 7.94. Therefore, the EC20 is <1.58 mg N/L at pH=7.94 and 25°C. Adjusted to pH=8, the EC20 is <1.45 mg N/L. Even though chronic survival appeared to be less sensitive than reproduction in this test, slightly more than 20% mortality occurred at the lowest tested concentration; therefore, the LC50 for chronic survival is <≈1.45 mg N/L.

Because the test solutions were renewed once a week, the pH dropped and the concentration of total ammonia increased between renewals; the average of the weekly measured initial and final values was used for both pH and total ammonia. The pH measured at the end of each week averaged 0.54 lower than the pH measured at the beginning of each week in the control test chambers, and averaged 0.78 lower in the two test chambers at the lowest tested concentration of ammonia. Even though the average pH drop in the control test chambers for the second test was 0.21 and was 0.87 in the control test chambers for the third test, survival and reproduction were both higher in the control test chambers for the third test; therefore, the pH variation probably did not reduce survival or reproduction. The pH-adjustment was based on the average measured pH in the lowest tested concentration of ammonia. The SMCV and the GMCV are <1.45 mg N/L.

Procambarus clarkii (crayfish)

The available data for this species are not used for the reason(s) given in Appendix 1.

Pteronarcella badia (stonefly)

Thurston et al. (1984a) studied the effect of ammonia on the survival and emergence of nymphs from two sources for 30 and 24 days. When expressed in terms of total ammonia nitrogen adjusted to pH=8, the 30-day LC50 for nymphs from the Gallatin

River was about 170 mg N/L, whereas the 24-day LC50 for nymphs from Rocky Creek was about 70 mg N/L. The degree of development of the nymphs at the beginning of each test was not determined and there is no reason to believe that the tested life stage is the one that is most sensitive to ammonia. In addition, it is not possible to interpret the data concerning emergence from either test. The test with nymphs from the Gallatin River might have been ended before emergence was complete in the control or any other treatment. In the test with nymphs from Rocky Creek, 25 percent of the nymphs in the control treatment neither died nor emerged, whereas this percentage was 5 to 15 in the treatments that contained ammonia. These tests do not allow derivation of a SMCV for this species, but they imply that this species is resistant to ammonia.

Carassius auratus (goldfish)

Marchetti (1960) exposed fish for 90 minutes and then observed mortality and histological effects for up to 42 days, whereas Reichenbach-Klinke (1967) studied the effects of a one-week exposure on gills and blood. Neither study provided useful information concerning the SMCV for the goldfish.

Pimephales promelas (fathead minnow)

Thurston et al. (1986) reported similar results from two life-cycle tests that started with 3 to 5-day-old fry and ended with 60-day-old offspring. The lowest mean measured DO concentration in any treatment was 6.08 mg/L (72 percent of saturation) and the lowest calculated fifth percentile of the DO concentrations was 5.16 mg/L (61 percent of saturation). At the highest tested un-ionized ammonia concentration of 0.93 mg NH_3/L, significant mortality occurred throughout the development of the parental generation. The most sensitive effect was reduction in egg hatching and the highest concentration that reportedly did not cause a significant reduction in egg hatching was 0.19 mg NH_3/L, but this concentration caused 33 and 55% reductions in percent hatch. For the purpose of regression analysis of percent hatch, the tested concentrations and results were so similar in the two tests that the data were analyzed as replicates of the test concentrations. In terms of total ammonia nitrogen, the EC20 based on percent hatch was 1.97 mg N/L at 24.2°C and pH=8.0. However, there are concerns about this test:

1. Effects on survival and weight of F1 fry were uncertain due to high mortality attributed to handling during cleaning.
2. The eggs were dipped in malachite green daily.
3. Hatchability of the controls was about 50 percent.

4. There was a large difference between the replicate test chambers in the control-adjusted percent hatch at 0.09 mg NH_3/L.

Swigert and Spacie (1983) conducted a 30-day early life-stage test starting with 10 to 18-hour-old embryos. The fifth percentile of the measured DO concentrations was 6.5 mg/L (79 percent of saturation) and the highest measured DO concentration was 7.96 mg/L (97 percent of saturation). Both survival and weight gain were reduced at 30 days and the product of these two (i.e., biomass) was analyzed using regression analysis. The resulting EC20 was 3.73 mg N/L at 25.1°C and pH=7.82, which would be 2.92 mg N/L at pH=8.

Mayes et al. (1986) conducted a 28-day early life-stage test in water from the Tittabawassee River. This water was probably an acceptable dilution water because it was apparently collected upstream of all known point discharges (Alexander et al. 1986; James Grant, Michigan Department of Environmental Quality, personal communication). The lowest and highest measured DO concentrations were 5.0 and 8.5 mg/L (59 and 101 percent of saturation). Adverse effects were observed on 28-day survival, but only the highest tested concentration reduced weight. Regression analysis of the survival data resulted in an EC20 of 5.12 mg N/L at 24.8°C and pH=8.0.

As stated above in the discussion of the effect of temperature on the toxicity of ammonia, DeGraeve et al. (1987) studied the effect of ammonia on 30-day survival of juvenile fathead minnows at several temperatures. The tests at 15 and 20°C did not have concentrations sufficiently high to cause effects, but survival was significantly decreased at the higher concentrations of ammonia in the tests run at 6, 10, 25, and 30°C. At 30°C, the mean measured DO concentration in most of the treatments was below 5.5 mg/L, but it was above 60% of saturation in all treatments. EC20s based on survival were calculated to be 11.9, 13.8, 39, and 39 mg N/L at temperatures of 6.0, 10.0, 25.4, and 30.2°C and pHs of 7.83, 7.73, 7.35, and 7.19, respectively. When adjusted to pH=8, the EC20s are 9.45, 9.72, 19.35, and 17.54 mg N/L, respectively. Although these EC20s were used to assess the effect of temperature on the chronic toxicity of ammonia, they are not included in Table 2 and are not used in the derivation of the SMCV because they indicate that 30-day survival of juveniles is not as sensitive to ammonia as the life-cycle and early life-stage tests discussed above.

The study of Smith (1984) concerned histopathological examination of lesions on the test fish and cannot be used to calculate an EC20.

Hermanutz et al. (1987) studied the survival, growth, and reproduction of fathead minnows in experimental streams. (See the section below titled "A Field Study Relevant to the CCC" and associated figures and table.) Two generations were each exposed for periods of approximately two months, during which pH averaged 7.5 to 7.7 and temperature averaged 19.6°C. Deleterious effects on biomass were not apparent at or below the highest tested concentration of ammonia, which was 3.92 mg N/L when adjusted to pH=8. These results are not included in Table 2 because they are from a field study.

In the 1985 Guidelines (U.S. EPA 1985b), results of early life-stage tests are used as predictors of results of life-cycle and partial life-cycle tests; comparisons of these kinds of chronic tests had been reported by McKim (1977) and Macek and Sleight(1977). Because early life-stage tests are only predictors, results of such tests are not used when results of life-cycle or partial life-cycle tests are available. In the present case, however, because of the concerns about the life-cycle test, the SMCV for the fathead minnow at pH=8 is set equal to 3.09 mg N/L, which is the geometric mean of the three EC20s from Thurston et al. (1986), Swigert and Spacie (1983), and Mayes et al. (1986); the range of the three EC20s is only a factor of 2.6.

Catostomus commersoni (white sucker)

Reinbold and Pescitelli (1982a) conducted a 31-day early life-stage test starting with 3-day-old embryos. The concentration of DO averaged 68 to 74 percent of saturation (6.3 to 6.9 mg/L). No effect on growth or survival was observed at concentrations of total ammonia nitrogen up to 2.9 mg N/L at pH=8.32 and 18.6°C, which is equivalent to 4.79 mg N/L at pH=8. As measured by time-to-swimup, development of larvae was delayed, suggesting that slightly higher concentrations would have affected growth and/or survival. The results of this test do not provide sufficient data to allow regression analysis, but the data indicate that the EC20 would be greater than 4.79 mg N/L if an EC20 could be calculated.

Hermanutz et al. (1987) studied survival and growth of juvenile white suckers in experimental streams. (See the section below titled "A Field Study Relevant to the CCC" and associated figures and table.) Two separate tests were started with individuals whose average weight was 10 g and

lasted 88 and 183 days. The average temperatures in the two tests were 18 and 21°C. The two highest tested concentrations caused a slight reduction in biomass. However, juveniles might not be as sensitive to ammonia toxicity as early life stages. These results are not included in Table 2 because they are from a field study.

The value of ">4.79 mg N/L" is included in Table 2 and is the GMCV; even though it is a "greater than" value, it can be used in the calculation of the FCV because it is not one of the four lowest GMCVs.

Ictalurus punctatus (channel catfish)

Swigert and Spacie (1983) conducted a 30-day exposure starting with newly hatched larvae that were fewer than 3 hours old. The mean measured DO concentration was 5.66 mg/L (70 percent of saturation) but the lowest individual measured concentration was 3.5 mg/L (45 percent of saturation). Reduced growth was found at total ammonia concentrations of 5.8 mg N/L and above and reduced survival at concentrations of 21 to 22 mg N/L. In separate tests, they determined that survival and hatching of embryos were more resistant than survival and growth of fry. Regression analysis of biomass at the end of the 30-day exposure produced an EC20 of 11.5 mg N/L at pH=7.76 and 26.9°C. The EC20 adjusted to pH=8 is 8.38 mg N/L. This EC20 is questionable because the lowest measured DO concentration was below 5.0 mg/L and was below 50 percent of saturation.

Reinbold and Pescitelli (1982a) conducted a 30-day exposure starting with <36-hour old embryos. The concentration of DO averaged 70 to 76 percent of saturation (5.7 to 6.2 mg/L). No effect on either percent hatch or fry survival was found at concentrations up to 11 mg N/L, but reduced growth was found at 5.2 mg N/L and above, as well as a delay in swimup at concentrations as low as 1 mg N/L. The EC20 for growth is 12.2 mg N/L at pH=7.80 and 25.8°C. Adjusted to pH=8, this EC20 is 9.33 mg N/L. However, the percent reduction at the highest tested concentration was less than 50%, as specified above in the data requirements.

Colt and Tchobanoglous (1978) and Colt (1978) exposed juveniles for 31 days to total ammonia nitrogen concentrations ranging from 1.6 to 14.4 mg N/L. The mean measured DO concentration was 7.6 mg/L (97 percent of saturation) and the calculated fifth percentile of the DO concentrations was 7.27 mg/L (93 percent of saturation); the calculated 95th percentile of the DO concentrations was 7.93 mg/L (101 percent

of saturation). Biomass in the control treatment increased tenfold during the test, but the increases were smaller at ammonia concentrations as low as 1.6 mg N/L. Because this was a test with juveniles that lasted only 31 days, only the data concerning mortality will be used. The concentration of 6.81 mg N/L killed 83%, whereas the higher concentration killed 100%. A range is reported for the concentration of 5.71 mg N/L and so the mean percent mortality is between 28 and 45%. It was reported that the lower concentrations killed 9 of 400 organisms, and so it is likely that the concentration of 5.02 mg N/L killed no more than 5%. Therefore, the EC20 at pH=8.35 and 27.9°C is between 5.02 and 5.71 mg N/L; adjusted to pH=8, the EC20 is between 8.7 and 9.9 mg N/L. Although this EC20 is included in Table 2, it is not used in the derivation of the SMCV and GMCV because it is based on survival of juveniles in a 31-day test and therefore is an upper limit on the SMCV because juveniles might not be as sensitive to ammonia toxicity as early life stages.

In several tests, each of which consisted of one concentration of ammonia and a control, Robinette (1976) studied the effect of ammonia on growth of 25 to 30-g channel catfish for about thirty days at 23 to 26°C. No information was reported concerning survival of the test fish. A concentration of total ammonia nitrogen of 2.7 mg N/L at pH=7.6 caused fish to gain weight faster than the control fish. In contrast, concentrations of 3.5 and 3.6 mg N/L at pH=7.8 caused fish to lose weight while the controls were gaining weight. Adjusted to pH=8, these concentrations would be 1.7, 2.7, and 2.8 mg N/L, respectively. Because these tests studied growth of juveniles for only 30 days, the results are not included in Table 2.

Bader (1990) and Bader and Grizzle (1992) reported that ammonia reduced growth, but the concentration of ammonia in the controls was substantial. DeGraeve et al. (1987) studied the effect of ammonia on survival and growth of juveniles for thirty days. 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. Mitchell and Cech (1983) reported that ammonia did not damage gills unless residual chlorine was present. Soderberg et al. (1984) studied the culture of channel catfish in ponds and found that the ambient concentration of ammonia caused gill lesions, but did not affect survival or growth. Results of these tests are not included in Table 2.

Hermanutz et al. (1987) studied survival and growth of juvenile channel catfish in experimental streams. (See the section below titled "A Field Study Relevant to the CCC" and associated figures and table.) 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 in Table 2 because they are from a field study.

Although there are problems with the early life-stage tests by Swigert and Spacie (1983) and Reinbold and Pescitelli (1982a), the EC20s are similar. Therefore, the channel catfish SMCV at pH=8 is 8.84 mg N/L, which is the geometric mean of the two EC20s. The data of Colt and Tchobanoglous (1978) and Robinette (1976) support a SMCV of this magnitude. The GMCV is also 8.84 mg N/L.

Oncorhynchus clarki (cutthroat trout)

Thurston et al. (1978) obtained 29-day LC50s of 16.4 and 15.9 mg N/L with fish whose average weights were 3.3 and 3.4 g, respectively; the 96-hr LC50s were 1.2 and 1.7 times higher than the 29-day LC50s. In two other tests they obtained 36-day LC50s of 23.7 and 24.4 mg N/L with fish whose average weight was 1.0 g; no fish died after day 29. The tests were conducted at 12.2 to 13.1°C and all four of the LC50s are expressed as total ammonia nitrogen at pH=8.0. The mean measured DO concentrations for the various tests ranged from 8.2 to 8.6 mg/L (77 to 82 percent of saturation). The lowest and highest measured DO concentrations were 7.4 and 9.2 mg/L (70 and 87 percent of saturation). EC20s cannot be calculated, but would be lower than the geometric mean of 19.7 mg N/L. The SMCV might be substantially lower than 19.7 mg N/L because this test was not conducted with an early life stage. In all four of the tests, there was a negative correlation between the concentration of ammonia and weight gain, but this might have been a temporary effect. Histological examinations were performed at the end of the tests. The EC20 of <19.7 mg N/L is included in Table 2, but this value cannot be used in the calculation of a SMCV.

Oncorhynchus gorbuscha (pink salmon)

Rice and Bailey (1980) exposed embryos and alevins of pink salmon for 61 days to concentrations of total ammonia nitrogen

ranging from 0.07 to 13.6 mg/L at pH=6.4 and 4°C. The only chronic test began sometime after hatch and ended when the alevins emerged (i.e., at the beginning of swimup); 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. At the higher tested concentrations, the weight of emerging alevins was significantly reduced, relative to the controls, by as much as 22% at 11.2 mg/L. This would be equivalent to about 4.1 mg N/L at pH=8. Size at emergence was said to be important because smaller fry are less capable of surviving in the environment because they have less swimming endurance and are selectively preyed upon by larger predators. This test did not provide data concerning survival and is not an early life-stage test because it began after hatch; therefore, this test did not provide a useful EC20 and is not included in Table 2.

Oncorhynchus kisutch (coho salmon)

Buckley et al. (1979) exposed fish whose average wet weight was 3.4 g for 91 days to study effects of ammonia on blood. The highest tested concentration of 47 mg N/L killed only three percent of the fish. The EC20 is >47 mg N/L, but this not useful information about the SMCV because there is no reason to believe that the tested life stage is the one that is most sensitive to ammonia. This test is not included in Table 2 because it does not provide useful information concerning the SMCV for this species.

Oncorhynchus mykiss (Salmo gairdneri) (rainbow trout)

Many investigators have reported results of chronic tests conducted on ammonia with rainbow trout, but the most ambitious chronic test was the five-year test conducted by Thurston et al. (1984b). In this test the initial fish were exposed through growth, maturation and reproduction, the next generation through hatch, growth, maturation, and reproduction, and the third generation through hatch and survival of the young. The mean measured DO concentration was 7.43 mg/L (65 percent of saturation) and the lowest calculated fifth percentile of the measured DO concentrations in the various treatments was 5.9 mg/L (51 percent of saturation). Measured temperatures ranged from 7.5 to 10.5°C and the tested concentrations of total ammonia nitrogen ranged from 1.1 to 8.0 mg N/L at pH=7.7. When adjusted to pH=8, the range is 0.77 to 5.4 mg N/L. All of the fish used to start the test came from one pair of adults of the Ennis strain. In addition, the important data for each life stage are so variable that it is not possible to discern whether there is a

concentration-effect curve. Despite the variability, it can be inferred that the EC20 cannot be much lower than the highest tested concentration because severe effects were not apparent at any tested concentration; if the EC20 was much lower than the highest tested concentration, this concentration would have caused severe effects.

Also using fish from the Ennis strain, Burkhalter (1975) and Burkhalter and Kaya (1977) reported a 21-day LC50 of 39.6 mg N/L for embryos and sac fry and interpolation off a graph indicates a 42-day LC50 of 33.6 mg N/L, based on total ammonia nitrogen, at 9.5 to 12.5°C and pH=7.5, assuming either no control mortality or adjustment for control mortality. When adjusted to pH=8, the LC50s would be 22.0 and 18.7 mg N/L, respectively, but LC20s would be lower than LC50s. The measured DO concentrations were all above 8 mg/L (72 percent of saturation). The test began within 24 hours of fertilization, continued to the beginning of feeding, and found retardation of development and growth of very young fish, similar to the tests discussed above with the pink salmon (Rice and Bailey 1980). Thurston et al. (1984b) speculated that they did not observe the reduced growth reported by Burkhalter and Kaya (1977) because of compensation during the next several months of the longer exposure. Indeed, Burkhalter and Kaya (1977) reported compensation at the lowest tested concentration.

Contrasting information concerning EC20s is provided by the early life-stage tests conducted by Solbe and Shurben (1989) and Calamari et al. (1977,1981). Both tests began within 24 hours after fertilization and lasted for 72 to 73 days until the fry had been feeding for about 30 days.

1. Solbe and Shurben (1989) reported that the dry weight of the test organisms varied little between treatments. The test was conducted at pH=7.52 and an average temperature of 14.9°C. The DO concentration equaled or exceeded 76 to 95 percent of saturation during various portions of the test. The four highest concentrations of ammonia killed 78 to 99 percent. The fifth and lowest tested concentration of total ammonia nitrogen was 2.55 mg N/L and it reduced survival by 67 percent; this would correspond to 1.44 mg N/L at pH=8, and the LC20 would be lower. These authors demonstrated that exposure to ammonia should begin soon after fertilization. When exposure began within 24 hours after fertilization, 26 mg N/L killed 98 percent of the embryos, whereas when exposure began 24 days after fertilization, 26 mg N/L killed only 3 percent of the embryos and killed only 40 percent in a 49-day exposure.

2. Calamari et al. (1977,1981) conducted an early life-stage test, but did not report any information concerning weight, although, as stated above, Solbe and Shurben (1989) reported no effect on weight during their early life-stage test. The DO concentration was over 80 percent of saturation. For total ammonia nitrogen at pH=7.4, Calamari et al. (1977,1981) obtained a 72-day LC50 of 8.2 mg N/L at 14.5°C. They also reported that adjusted mortalities were 15 and 23 percent at 1.5 and 3.7 mg N/L, respectively, and that higher tested concentrations killed more than 50 percent of the test organisms. Because Calamari et al. did not report the actual percentage killed at the higher tested concentrations, regression analysis could not be applied; semilog interpolation between 1.5 and 3.7 mg N/L produced an LC20 of 2.6 mg N/L, which would correspond to 1.34 mg N/L at pH=8.

Both Calamari et al. (1977,1981) and Solbe and Shurben (1989) found that longer exposures of embryos and fry resulted in much lower LC50s than 96-hour exposures.

Several investigators reported results concerning the effect of total ammonia nitrogen on long-term survival:

1. Thurston and Russo (1983) reported five 35-day LC50s that were determined using fish whose average initial weights were 0.7 to 10 g. The 35-day LC50s were 27.9 and 36.1 mg N/L for fish whose average weights were 3.7 and 9.7 g, respectively. The 35-day LC50s were 32.4, 34.5, and 37.0 mg N/L for fish whose average weights were 0.7 to 3.3 g; when adjusted to pH=8, the geometric mean of these three 35-day LC50s with the smaller fish was 26.4 mg N/L.
2. Broderius and Smith (1979) reported that 16.2 mg N/L killed 30 percent of fry in 30 days at 10°C and pH=7.95, which corresponds to 15.1 mg N/L at pH=8.
3. Daoust and Ferguson (1984) reported that 23.3 mg N/L did not kill any fingerlings in 90 days at pH=7.93, which would correspond to 21.1 mg N/L at pH=8. However, some of the fish that exhibited clinical signs during the exposure were removed for examination during the test. The swimming and feeding of some fish were affected for a while, but the fish recovered.

This variety of results might be due to differences in the size or age of the test organisms.

Several other chronic tests did not provide information that could be used in the derivation of a SMCV. Fromm (1970), Reichenback-Klinke (1967), and Smart (1976) exposed fish to study the effects of ammonia on gills and blood. In a test reported by Smith and Piper (1975), exposed fish had abnormal tissues, but fish placed in clean water for 45 days at the end

of the test had normal tissues. When Soderberg et al. (1983) studied the culture of rainbow trout in ponds, parasitic epizootics caused mortalities. The Ministry of Technology (1968) reported the effect of ammonia on percent survival in a 90-day test, but did not report the age or size of the fish or the temperature or the pH of the water. Samylin (1969) conducted tests in water from the Vyg River, with some of the exposures being conducted in Petri dishes. Schulze-Wiehenbrauck (1976) found that growth of juveniles at 10°C and pH=8 was reduced during two-week exposures to a total ammonia nitrogen concentration of 2.26 mg N/L, but the decrease was completely compensated for during the next three or four weeks. Smith (1972) reported that as long as the DO concentration was maintained at 5 mg/L or greater, growth of rainbow trout was not significantly reduced until average total ammonia concentrations reached 1.6 mg/L.

Hermanutz et al. (1987) studied survival and growth of juvenile rainbow trout in experimental streams. (See the section below titled "A Field Study Relevant to the CCC" and associated figures and table.) Three separate tests were conducted with individuals whose average initial weights were 7 to 11 g. The tests lasted from 28 to 237 days, with the 237-day test including an entire winter. Average temperatures in the three tests ranged from 5.9 to 10.6°C, whereas pH averaged 7.7 to 8.4. Reductions in biomass were consistently observed at concentrations greater than or equal to 2.29 mg N/L when adjusted to pH=8. However, juveniles might not be as sensitive to ammonia toxicity as early life stages. These results are not included in Table 2 because they are from a field study.

The early life-stage test by Calamari et al. (1977,1981) produced a total ammonia nitrogen LC20 of 1.34 mg N/L at pH=8, whereas Solbe and Shurben (1989) indicate that the LC20 might be lower. In contrast, both Thurston et al. (1984a) and Burkhalter and Kaya (1977) found no indication of severe mortality of young fish at higher concentrations. Exposure was continuous for several generations in the test of Thurston et al. (1984b), whereas exposure began within 24 hours of fertilization in the other three tests. Because of the concerns about some of the tests, the differences among the results, and the fact that some of the results are either "greater than" or "less than" values, even though the various results are included in Table 2, a SMCV is not derived for rainbow trout; instead, the results of the chronic tests will be used to assess the appropriateness of the CCC.

Oncorhynchus nerka (sockeye salmon)

Rankin (1979) exposed embryos of sockeye salmon for 62 days from fertilization to hatch; the tested concentrations of total ammonia nitrogen ranged from 2.13 to 87 mg N/L at 10°C. The DO concentration was reported to be at saturation. This test ended as soon as the embryos hatched, and so hatchability was the only toxicological variable studied. The percentage of the embryos that hatched was 63.3% in the controls, but was 49% at the lowest tested concentration (2.13 mg N/L) and was 0% at 8.1 mg N/L and above. The concentration of 2.13 mg N/L at pH=8.42 corresponds to 4.16 mg N/L at pH=8. Thus the EC20 at pH=8 is less than 4.16 mg N/L. Because the effects on newly hatched fish were not studied, the SMCV is <4.16 mg N/L.

Oncorhynchus tshawytscha (chinook salmon)

Burrows (1964) exposed fingerlings for six weeks at 6 and 14°C to three concentrations of ammonia and a control treatment to study effects on gills at pH=7.8. There was no recovery in three weeks in clean water at 6°C, but there was recovery at 14°C. At both temperatures, no significant mortality occurred during exposure to the highest tested concentration of 0.57 mg N/L or for three weeks afterward in clean water. No information is given concerning the DO concentration during the exposures, and there is no reason to believe that the tested life stage is the one that is most sensitive to ammonia.

Tests conducted by Sousa et al. (1974) suggest that chinook salmon tolerate higher concentrations of ammonia when pH is decreased and salinity is increased. However, there was no control treatment, no information was given concerning the DO concentration, temperature was not controlled, and the fish were given an antibiotic.

These tests are not included in Table 2 because they do not provide useful information concerning the SMCV for this species.

A GMCV is not derived for *Oncorhynchus* because the available data do not provide an adequate basis for a useful conclusion concerning the GMCV.

Salmo trutta (brown trout)

Carline et al. (1987) exposed brown trout for twelve months to dilutions of effluent from a sewage treatment plant. Survival, growth, swimming performance, and degree of damage to gills were studied, but no information was obtained concerning effects on embryos, newly hatched fish, or

reproduction. No data from this test are included in Table 2 because this test does not provide useful information concerning the SMCV for this species.

Lepomis cyanellus (green sunfish)

Reinbold and Pescitelli (1982a) conducted a 31-day early life-stage 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 EC20 because the fish were heavier in 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 to the end of the test was reduced at total ammonia nitrogen concentrations of 6.3 mg N/L and above and regression analysis of the survival data calculated an EC20 of 5.84 mg N/L at pH=8.16 and 25.4°C. Adjusted to pH=8, the EC20 is 7.44 mg N/L.

McCormick et al. (1984) conducted a 44-day early life-stage test, starting with <24-hour-old embryos. The mean measured DO concentration was 7.9 mg/L (91 percent of saturation) and the calculated fifth percentile of the measured DO concentrations was 7.7 mg/L (88 percent of saturation). No effect was found on percent hatch, but reduced survival and growth occurred at concentrations of 14 mg N/L and above. Although survival in one control test chamber and in the low concentrations of ammonia averaged about 40 percent and was only 10 percent in the other control chamber, the concentration-effect curve was well defined. Regression analysis of biomass calculated an EC20 of 5.61 mg N/L at pH=7.9 and 22.0°C. This EC20 was obtained with the 10 percent used in the regression analysis. An EC20 of 5.51 mg N/L was obtained if the 10 percent was not used; the two EC20s are similar partly because the weight given to each treatment was inversely related to the variance for the treatment, which meant that the control treatment was given a low weight in the regression analysis. Adjusted to pH=8, the EC20 calculated using all of the data is 4.88 mg N/L.

Jude (1973) found that growth of juveniles weighing 4 to 16 g each for 40 days was proportional to temperature at 13, 22, and 28°C. In a second test, the effect of ammonia on survival and growth of 10 to 14-g juveniles was studied for 20 days.

Too few fish died to allow calculation of an EC20. Neither of these tests provided results that can be included in Table 2.

Adjusted to pH=8, the EC20 of 7.44 mg N/L from Reinbold and Pescitelli (1982a) agrees quite well with the EC20 of 4.88 mg N/L from McCormick et al. (1984). 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. Even though there were experimental problems with both tests, the results of the tests agree well and therefore the geometric mean (6.03 mg N/L) of the two EC20s is used as the SMCV.

Lepomis macrochirus (bluegill)

Smith et al. (1984) conducted a 30-day early life-stage test, starting with <28-hour-old embryos. No information was reported concerning the DO concentration, but the flow-rate was high. The values reported in Table 1 as standard deviations on the pH appear excessively large; it is likely that they were not calculated correctly, because, as explained in footnote d, the mean pH was calculated by conversion of pH to H^+ (i.e., hydrogen ion) concentration. Other tests conducted on ammonia in the same laboratory at about the same time reported much less variation in pH. For example, McCormick et al. (1984) reported that the 95% confidence interval on the experiment-wide pH was 7.8 to 8.0. Broderius et al. (1985) calculated average pH by converting to hydrogen ion concentration, but reported small standard deviations and ranges for four acute tests and four chronic tests.

Smith et al. (1984) found no significant reduction in percent hatch up to a total ammonia nitrogen concentration of 37 mg N/L, but hatched larvae were deformed at this concentration and died within six days. At the end of the test, survival and growth at 1.64 mg N/L were near values for the controls, but were greatly reduced at 3.75 to 18 mg N/L. Regression analysis of biomass calculated an EC20 of 1.85 mg N/L at pH=7.76 and 22.5°C. The EC20 adjusted to pH=8 is 1.35 mg N/L.

Diamond et al. (1993) conducted two chronic tests. The test at 12°C is discussed in Appendix 1. The data sheets for the test at 20°C indicate that this test studied the effect of ammonia on survival and growth of bluegills for 21 days. (The durations of the chronic tests with the bluegill at 12 and 20°C are switched in Table 1 in the publication.) The test at 20°C was started with bluegills that were less than 98-days old, were less than 1 inch (2.5 cm), and averaged 0.11 to 0.15 g. The highest tested concentration of total ammonia nitrogen

was 64 mg N/L, which caused 30% mortality at the test pH of 7.3; most of the deaths occurred in the last two days of the test. Adjusted to pH=8, the highest tested concentration was 31 mg N/L as total ammonia nitrogen, which is in the range of the adjusted 96-hr LC50s reported in Table 1 of the 1984/1985 ammonia criteria document. This test is not very useful because it lasted for only 21 days and mortality began occurring near the end of the test. Neither of these tests provides results that can be included in Table 2.

Hermanutz et al. (1987) studied survival and growth of the juvenile bluegills in experimental streams. (See the section below titled "A Field Study Relevant to the CCC" and associated figures and table.) The individual weights averaged 2.2 g at the beginning and the test duration was 90 days. The mean pH and temperature were 8.2 and 21.1°C, respectively. A substantial effect on biomass was apparent only at the highest concentration, which was 9.5 mg N/L when adjusted to pH=8. These juvenile bluegills were not particularly sensitive compared to older life stages of other species tested during this study. However, juveniles apparently are not as sensitive to ammonia toxicity as the early life stages tested by Smith et al. (1984). These results are not included in Table 2 because they are from a field study.

The SMCV for the bluegill is 1.35 mg N/L, and the GMCV of 2.85 mg N/L for *Lepomis* is calculated as the geometric mean of the two SMCVs (6.03 and 1.35 mg N/L).

Micropterus dolomieu (smallmouth bass)

As stated above in the discussion of the effect of pH on the toxicity of ammonia, Broderius et al. (1985) conducted 32-day early life-stage tests at four pHs at 22.3°C, starting with embryos near hatch. The mean measured DO concentration was 7.72 mg/L (89 percent of saturation); the lowest and highest measured DO concentrations were 7.1 and 8.3 mg/L (81 and 96 percent of saturation). Survival of embryos and fry within the first week was not affected by ammonia, except at the highest concentration at the highest pH, although effects on these life stages might have been reduced due to the exposure not starting until just prior to hatch. In all tests, growth and survival of older fry were reduced at higher concentrations and regressions of biomass resulted in EC20s of 9.61, 8.62, 8.18, and 1.54 mg N/L at pHs of 6.60, 7.25, 7.83, and 8.68, respectively. Adjusted to pH=8, these EC20s are 3.57, 4.01, 6.50, and 4.65 mg N/L, with a geometric mean of 4.56 mg N/L, which is the SMCV and the GMCV.

Stizostedion vitreum (walleye)

Reinbold and Pescitelli (1982a) could not conduct a successful early life-stage test because only 20% of the newly hatched fish survived.

Hermanutz et al. (1987) studied survival and growth of juvenile walleyes in experimental streams. (See the section below titled "A Field Study Relevant to the CCC" and associated figures and table.) A 46-day test was conducted at an average temperature of 24°C and was started with yearlings averaging 100 g initial weight. A second test at an average temperature of 17°C was started with young-of-year averaging 19 g initial weight and lasted 43 days. Adjusted to pH=8, concentrations of 2.0 to 3.7 mg N/L somewhat reduced walleye biomass, whereas concentrations of 9.5 to 13.3 mg N/L completely eliminated walleye from the streams. However, juveniles might not be as sensitive to ammonia toxicity as early life stages. These results are not included in Table 2 because they are from a field study.

Rana pipiens (leopard frog)

The available data for this species are not used for the reason(s) given in Appendix 1.

Hyla crucifer (spring peeper)

The available data for this species are not used for the reason(s) given in Appendix 1.

Table 2. EC20s from Acceptable Chronic Tests[a]

Species	Reference	Test and Effect[b]	Temp. (C)	pH	EC20[c] at test pH (mg N/L)	EC20[c] at pH=8 (mg N/L)	SMCV[c] at pH=8 (mg N/L)	GMCV[c] at pH=8 (mg N/L)
Musculium transversum	Anderson et al. 1978	42-d Juv Survival	23.5	8.15	5.82	7.30	≤2.62	≤2.62
	Sparks and Sandusky 1981	42-d Juv Survival	21.8	7.80	1.23	0.94		
Ceriodaphnia acanthina	Mount 1982	LC Reproduction	24.5	7.15	44.9	19.8	19.8	16.0
Ceriodaphnia dubia	Willingham 1987	7-d LC Reproduction	26.0	8.57	5.80	14.6	13.0	
	Nimmo et al. 1989	7-d LC Reproduction	25.	7.8	15.2	11.6		
Daphnia magna	Gersich et al. 1985	21-d LC Reproduction	19.8	8.45	7.37	15.1	17.1	17.1
	Reinbold and Pescitelli 1982a	21-d LC Reproduction	20.1	7.92	21.7	19.4		
Hyalella azteca	Borgmann 1994	10-wk LC Reproduction	25.	7.94	<1.58 (EC50)	<1.45	<1.45	<1.45
Pimephales promelas	Thurston et al. 1986	LC Hatchability	24.2	8.0	1.97	1.97	3.09	3.09
	Swigert and Spacie 1983	30-d ELS Biomass	25.1	7.82	3.73	2.92		
	Mayes et al. 1986	28-d ELS Survival	24.8	8.0	5.12	5.12		

Species	Reference	Test and Effect[b]	Temp. (C)	pH	EC20[c] at test pH (mg N/L)	EC20[c] at pH=8 (mg N/L)	SMCV[c] at pH=8 (mg N/L)	GMCV[c] at pH=8 (mg N/L)
Catostomus commersoni	Reinbold and Pescitelli 1982a	30-d ELS Biomass	18.6	8.32	>2.9	>4.79	>4.79	>4.79
Ictalurus punctatus	Swigert and Spacie 1983	30-d ELS Biomass	26.9	7.76	11.5	8.38	8.84	8.84
	Reinbold and Pescitelli 1982a	30-d ELS Weight	25.8	7.80	12.2	9.33		
	Colt and Tchobanoglous 1978	30-d Juv Survival	27.9	8.35	≤5.02-≤5.71	≤8.7-≤9.9[d]		
Oncorhynchus clarki	Thurston et al. 1978	29-d Juv Survival	12.2-13.1	8.0	<19.7	<19.7[d]	---	---
Oncorhynchus mykiss	Thurston et al. 1984b	5-year LC	7.5-10.5	7.7	>≈8.0	>≈5.4[d]		
	Burkhalter and Kaya 1977	42-d ELS Survival	9.5-12.5	7.5	<33.6	<18.7[d]		
	Solbe and Shurben 1989	73-d ELS Survival	14.9	7.52	<2.55	<1.44[d]		
	Calamari et al. 1977,1981	72-d ELS Survival	14.5	7.4	2.6	1.34[d]		
Oncorhynchus nerka	Rankin 1979	62-d Embryos Hatchability	10.	8.42	<2.13	<4.16	<4.16[e]	

Species	Reference	Test and Effect[b]	Temp. (C)	pH	EC20[c] at test pH (mg N/L)	EC20[c] at pH=8 (mg N/L)	SMCV[c] at pH=8 (mg N/L)	GMCV[c] at pH=8 (mg N/L)
Lepomis cyanellus	Reinbold and Pescitelli 1982a	30-d ELS Survival	25.4	8.16	5.84	7.44	6.03	2.85
	McCormick et al. 1984	30-d ELS Biomass	22.0	7.9	5.61	4.88		
Lepomis macrochirus	Smith et al. 1984	30-d ELS Biomass	22.5	7.76	1.85	1.35	1.35	
Micropterus dolomieu	Broderius et al. 1985	32-d ELS Biomass	22.3	6.60	9.61	3.57	4.56	4.56
	Broderius et al. 1985	32-d ELS Biomass	22.3	7.25	8.62	4.01		
	Broderius et al. 1985	32-d ELS Biomass	22.3	7.83	8.18	6.50		
	Broderius et al. 1985	32-d ELS Biomass	22.3	8.68	1.54	4.65		

[a] An EC20 is assumed for a stonefly but is not given in this table (see text concerning calculation of the CCC).
[b] Juv = juvenile; LC = life cycle; ELS = early life stage.
[c] Total ammonia nitrogen.
[d] Not used in the derivation of a SMCV (see text).
[e] Not used in the derivation of a GMCV (see text).

DERIVATION OF THE NEW CCC

Nine Genus Mean Chronic Values (GMCVs) are presented in Table 2. The five lowest total ammonia nitrogen GMCVs at pH=8 are <1.45 mg N/L for *Hyalella*, ≤2.62 mg N/L for *Musculium*, 2.85 mg N/L for *Lepomis*, 3.09 mg N/L for *Pimephales*, and 4.56 mg N/L for *Micropterus*. The more resistant genera with GMCVs greater than 4.7 mg N/L are *Catostomus*, *Ictalurus*, *Ceriodaphnia*, and *Daphnia*. Although Table 2 contains chronic data for the genus *Oncorhynchus*, no GMCV is derived because of the large range in the EC20s; rather these chronic data will be used to evaluate whether the FCV poses a risk to this genus.

Although Table 2 does not contain data for an insect genus, available information concerning a stonefly (Thurston et al. 1984a) indicates that at least one species is relatively resistant to ammonia. Therefore, calculations based on the GMCVs in Table 2 should adequately reflect the intent of the 1985 Guidelines. Use of the GMCVs for *Hyalella*, *Musculium*, *Lepomis*, and *Pimephales* in the fifth percentile calculation procedure described in the 1985 Guidelines results in a FCV of <1.27 mg N/L at pH=8. N=10 is used in this calculation because a GMCV for an insect is assumed to be greater than 4.7 mg N/L. This FCV is a "less than" value because the lowest two GMCVs are "less than" values. Because no GMCV for a salmonid species is used in the calculation of the FCV, it is not possible to calculate FCVs with salmonids present and absent, as was done above for the FAV. The CCC is set to 1.27 mg N/L at pH=8. Figure 11 shows the ranked GMCVs and the CCC, all at pH=8.

Substitution of this CCC at pH=8 for $CV_{t,8}$ in equation 11 results in the following equation for expressing the new CCC as a function of pH:

$$CCC = \frac{0.0858}{1 + 10^{7.688-pH}} + \frac{3.70}{1 + 10^{pH-7.688}} \qquad \textbf{(18)}$$

This equation is plotted in Figure 12, along with the old CCC and the EC20s from Table 2. The new CCC is near the old CCC in the range of pH from about 7.5 to 8, but is increasingly higher than the old CCC at lower and higher values of pH. At pH=8, the new CCC corresponds to acute-chronic ratios of (14.4 mg N/L)/(1.27 mg N/L) = 11.3 using the calculated FAV when salmonids are present (but not lowered to protect large rainbow trout) and (16.8 mg N/L)/(1.27 mg N/L) = 13.2 using the FAV when salmonids are absent. These are in the range of the ACRs that can be derived

Figure 11. Ranked Genus Mean Chronic Values (GMCVs) with the Criterion Continuous Concentration (CCC).

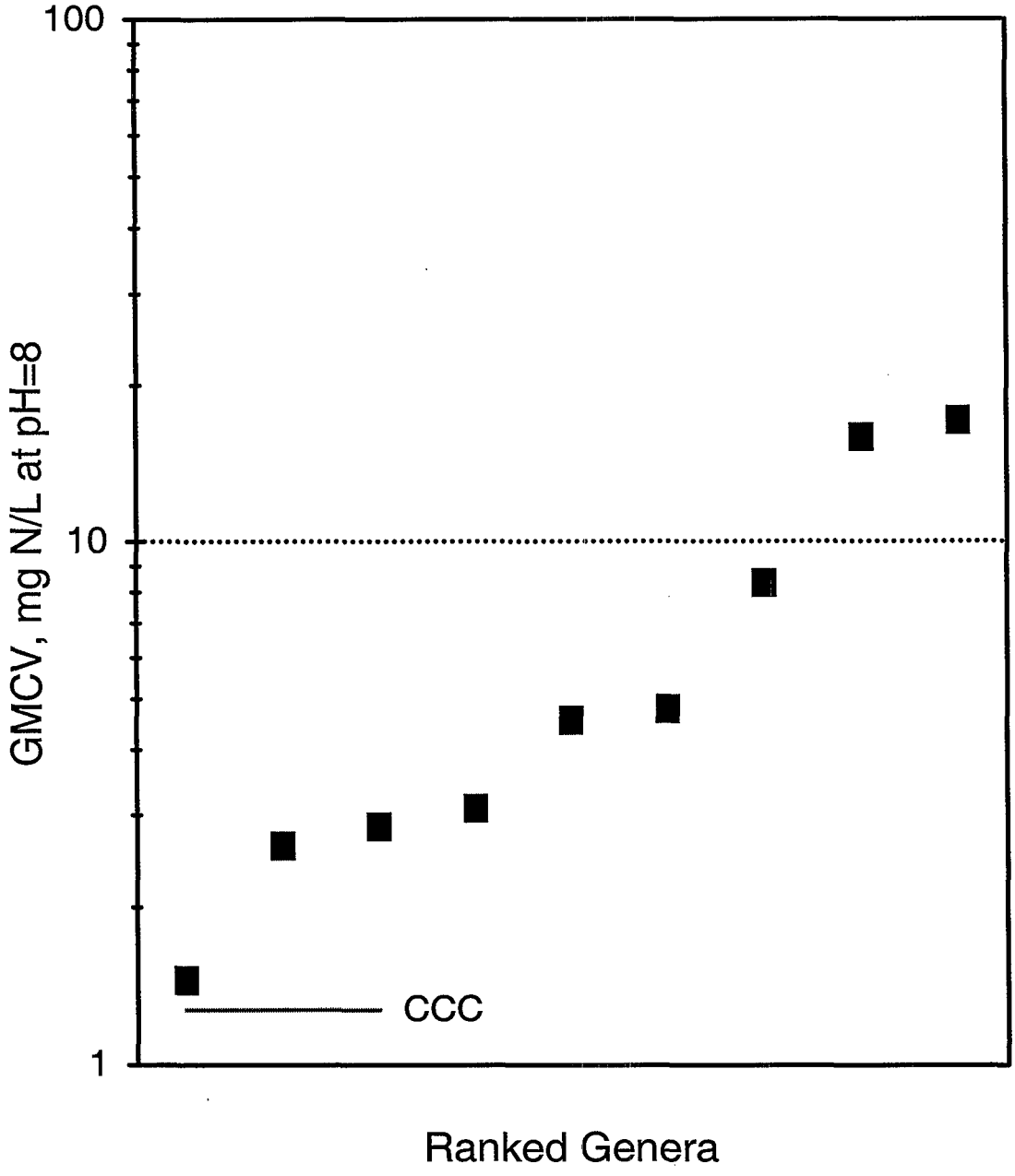
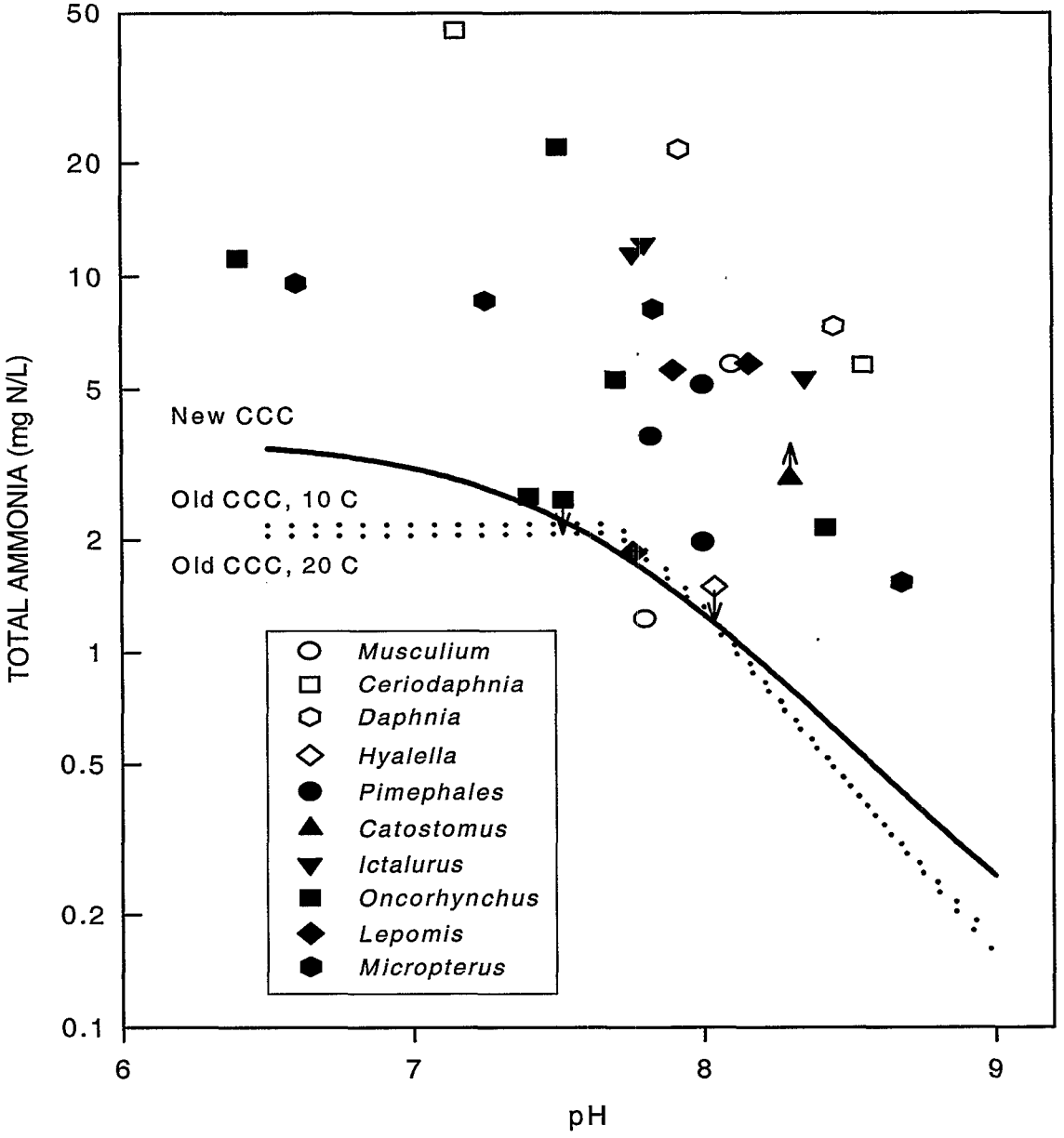


Figure 12. Chronic EC20s used in criteria derivation in relationship to Criterion Continuous Concentrations (CCCs).



from the EC20s in Table 2 (see Appendix 7). The ACR used to calculate the old CCC was 13.5 (Heber and Ballentine 1992).

Several points should be noted concerning the CCC:

a. The two lowest GMCVs are "less than" values. The CCC would be lower if a point estimate, rather than a "less than" value, could have been derived from the Borgmann (1994) study with *Hyalella*, the most sensitive genus. The CCC also might be lower if a point estimate, rather than a "less than" value, could have been derived from the studies with the fingernail clam.
b. Any substantial increase in the CCC derived with the procedures in this 1998 Update would require a higher GMCV for *Hyalella* and a higher SMCV for the recreationally important bluegill.
c. Because acutely resistant taxa are under-represented in the chronic dataset in Table 2, it could be argued that n, the number of genera used in the calculation of the FCV, should be increased from 10 to a higher value. A reasonable increase in n would not have a large effect, however. For example, adding three resistant genera would only raise the CCC to 1.37 mg N/L at pH=8 (although then the CCC would be lowered to equal the SMCV for the bluegill).
d. The available chronic EC20s for salmonids, even though not used directly in the calculation of the CCC, indicate that these species would probably be protected by the CCC, although the data suggest that there might be important differences between strains of rainbow trout.
e. Some of the laboratory and field data for the fingernail clam, which might be considered to have special ecological importance at some sites, indicate that this species would be affected at concentrations below the CCC, although other data indicate that it might not be affected by such concentrations and at most sites the intermittency of exposures would probably reduce risk.
f. When 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.
g. Partly for statistical reasons, the CCC is based on a 20 percent reduction in survival, growth, and/or reproduction. Whether the maximum acceptable percent reduction should be lower or higher than 20 percent under a set of conditions is a risk management decision.
h. If it had been derived using available acute-chronic ratios (see Appendix 7), the CCC would be greater than 2 mg N/L, which would be inappropriate because (1) it would be above one of the GMCVs in a dataset for which n is only ten, (2) it

would not appear to protect early life stages of the recreationally important bluegill, and (3) it might not protect the fingernail clam.

COLD-WEATHER CONDITIONS

Dischargers that use biological treatment of ammonia are likely to find it most difficult to meet water quality-based discharge limits for ammonia when the temperature is the lowest. This has raised questions about whether criteria based on toxicity tests conducted mainly at warm temperatures appropriately define concentrations that should be met under cold-weather conditions. Considerable data indicate that toxicity of total ammonia does not vary significantly with temperature, but this is based on a few kinds of tests conducted with fishes. Furthermore, if criteria are based on endpoints for invertebrates, there is a question of whether the endpoints might in fact be less sensitive at colder temperatures. Even if the *toxicity* of total ammonia is independent of temperature for all endpoints, *criteria* should not necessarily be independent of temperature unless the endpoints upon which they are based are relevant during all portions of the year.

The CMC is appropriate during all portions of the year because the organisms (i.e., juvenile and adult fish) and effects (i.e., survival) on which it is based are relevant during all portions of the year and because available data indicate that these endpoints are largely independent of temperature. The CCC, however, is based in part on endpoints that might not be of concern during cold-weather conditions (fish early life stages, *Hyalella* reproduction) and in part on endpoints that might be less sensitive under colder temperatures (fingernail clam survival). Therefore, it is necessary to consider to what extent and under what conditions the CCC can be higher during cold-weather conditions.

An important consideration regarding raising the CCC during cold-weather conditions is whether early life stages of fishes are absent, which is not necessarily true for many waters. For example, salmonids can spawn in cold temperatures in late fall or early spring, so that early life stages can be present throughout cold-weather conditions in such waters. Similarly, perch spawn during cold-weather conditions in some waters, and early life stages of some warmwater species are present during cold-weather conditions in some southern waters. Furthermore, in some situations, it might be necessary to limit the concentration of ammonia in a discharge before spawning begins in order to ensure that the concentration of ammonia is acceptably low at the site soon enough in the reproductive cycle.

Nevertheless, it is likely that there are bodies of water for which some of the endpoints upon which the CCC is based are not relevant during cold-weather conditions, and there is thus some potential for the CCC to be raised. Unfortunately, a good determination of how high the CCC can be in such situations is not possible because few data are available concerning the chronic sensitivities of the relevant life stages at the relevant temperatures. The data that are needed are the results of toxicity tests that are sufficiently long, are conducted at appropriately low temperatures, and determine the effects of ammonia on survival of life stages that are present during cold-weather conditions.

In the absence of such data, however, there are ways in which available data can be used to provide some indication of how different the CCC can be during cold-weather conditions.

Fish

If it is assumed that the toxicity of total ammonia to fish is independent of temperature for each endpoint, the CCC at cold temperatures can be based on chronic tests conducted at warm temperatures *if the results are based on sensitive chronic endpoints that are relevant during cold-weather conditions*. Therefore, when early life stages of fish are not present, the best indication of what the CCC should be under cold-weather conditions would be chronic survival tests, at any temperature, with juvenile and adult fishes.

The only chronic survival tests conducted over a range of temperatures are those of DeGraeve et al. (1987), which studied 30-day survival of juvenile fathead minnows. When expressed in terms of total ammonia and adjusted to pH=8, the EC20s were 9.6, 12.6, 19.3, and 15.9 mg N/L at 6, 10, 25, and 30°C respectively. In the life-cycle fathead minnow test by Thurston et al. (1986), parental generation mortality over several months exposure at 24°C was not significant at 7 mg N/L but exceeded 90% at 14 mg N/L, suggesting an EC20 close to 10 mg N/L for long-term survival. This result is somewhat more sensitive than the warmwater tests by DeGraeve et al. (1987), but is still less sensitive than the SMCV by about three-fold.

However, in contrast to early life stages being more sensitive than juvenile and adult fathead minnows, results obtained with channel catfish by Colt and Tchobanoglous (1978) and Robinette (1976) suggest that growth and survival of juveniles is as or more sensitive than early life stages, based on the EC20s from Swigert and Spacie (1983) and Reinbold and Pescitelli

(1982a,c) in Table 2. Colt and Tchobanoglous (1978) incompletely reported mortality data for juvenile channel catfish, but the available information indicates that the EC20 at 28°C is between 8.7 and 9.9 mg N/L, when adjusted to pH=8.

For a 21-day exposure of juvenile bluegills at pH=7.3, Diamond et al. (1993) reported 30% mortality at 64 mg N/L, which is 31 mg N/L when adjusted to pH=8. Although this might seem to suggest considerable resistance relative to early life-stage bluegills, this was a short test and the raw data indicate that mortality was just starting during the last few days of the test. The LC20 for more extended exposures would almost certainly be no higher than half of this concentration, and quite likely lower than that.

Although the absence of early life stages during cold-weather conditions will generally not be an issue for salmonids, the chronic sensitivities of juvenile and adult trout can be useful in estimating what criteria should be in the absence of early life stages. When exposures began after sensitive embryo stages of rainbow trout, Solbe and Shurben (1989) did not observe mortality significantly above control values until 26 mg N/L total ammonia (15 mg N/L adjusted to pH=8), at which the control-corrected mortality was 30% after a 49-day exposure. As discussed earlier, Broderius and Smith (1979) reported 30% mortality of rainbow trout during a 30-day exposure to 15.1 mg N/L (adjusted to pH=8). Based on three tests by Thurston and Russo (1983) in which the concentration of DO was always above 60 percent of saturation, the average 35-day LC50 for rainbow trout in the 0.6 to 10 g range is 26.5 mg N/L at pH=8. If the average slope of the chronic regressions is used, this would correspond to an LC20 of about 15 mg N/L. For juvenile cutthroat trout, Thurston et al. (1978) reported LC50s which averaged 19.7 mg N/L when adjusted to pH=8, which would correspond to an LC20 of about 11 mg N/L.

The above data suggest that juveniles and adults of some fish species have chronic LC20s in the range of 9 to 15 mg N/L (at pH=8). This is in contrast to GMCVs in the range of 3 to 8 mg N/L in Table 2. It should be noted, however, that most of the juvenile and adult tests cited above were relatively short compared to the duration of cold-weather conditions of concern. Also, they do not address to what extent ammonia effects that are not directly lethal will affect survival under field conditions in which food availability and other stresses are less favorable than in the laboratory (Lemly 1996), especially considering that ammonia is more persistent and therefore more widespread during cold-weather conditions. Furthermore, any cold-weather criterion derived from these

data should lie below the lowest GMCV because of the small number of genera with which tests have been conducted. Therefore, a criterion on the order of 9 mg N/L at pH=8 would not likely provide adequate protection. There is no clear evidence for how much lower this number should be; setting a cold-weather criterion must involve some site-specific risk management considerations.

Invertebrates

Of the two chronically sensitive invertebrates, the fingernail clam chronic value is already based on long-term survival of juveniles so it is a relevant endpoint for cold-weather conditions. For *Hyalella,* long-term survival is almost as sensitive as reproduction, and the *Hyalella* GMCV based on survival would be <≈1.45 mg N/L. Therefore, the CCC would not change. However, a few data are available concerning the temperature-dependence of ammonia toxicity to invertebrates and so there is a possibility that survival is less sensitive under cold-weather conditions and that the CCC could consequently be raised.

Based on toxicity tests by Arthur et al. (1987) during different seasons, the 96-hr LC50 for the fingernail clam, when expressed in terms of total ammonia nitrogen and adjusted to pH=8, is a factor of 1.9 higher at 15°C than at 21°C, and a factor of 2.7 higher at 5°C. For an amphipod (*Crangonyx pseudogracilis*), Arthur et al. (1987) reported that LC50s were about 6-fold higher at 12 to 13°C and 8-fold higher at 4°C than at 25°C. The effect of temperature on the rate of biochemical processes might, however, affect the results of acute (i.e., short-term) tests more than the results of chronic (i.e., long-term) tests. Furthermore, these tests might be confounded by effects other than temperature because they were performed during different seasons. Nevertheless, they still indicate that these invertebrates are more resistant to ammonia at colder temperatures and/or during colder seasons.

The above discussion is not intended to provide a definitive value for relaxation of the CCC during cold-weather conditions, but rather to indicate what types of data would be useful for determining this and how much relaxation might conceivably occur. The degree of relaxation is uncertain because the available data do not directly address the endpoints of concern during long-term exposures under cold-weather conditions. Deciding whether a cold-weather CCC is justified and what the value should be is highly site specific and the information provided here should be considered to provide only suggestions as to how it might be

derived. Careful consideration is needed regarding what data here, and from other sources, are most relevant to the site in question and what uncertainty factors should be applied. Until more relevant data are available, application of available information to development of a site-specific cold-weather CCC requires a degree of risk management, after consideration of biological and climatic conditions at the site, but incorporating an explicit relationship concerning season or temperature into the national criterion would require further research.

CCC AVERAGING PERIOD

The averaging period for a CCC often needs to be shorter than the length of the tests upon which it is based for two main reasons. First, concentrations in the field are typically much more variable than concentrations in laboratory tests, and variable concentrations of ammonia have been shown to be more toxic than constant concentrations when the comparisons are based on average concentrations during the exposure (Thurston et al. 1981a). By shortening the averaging period to which the CCC applies, the average concentration over the entire exposure will be below the CCC, increasingly so as the variability of the concentration increases. Second, chronic tests generally encompass different life stages, which might have different sensitivities, so that effects might be elicited only, or disproportionately, during the fraction of the test in which a sensitive life stage is present, rather than cumulatively over the whole test. The 1984/1985 ammonia criteria document specified a CCC averaging period of 4 days as recommended in the 1985 Guidelines (U.S. EPA 1985b), except that an averaging period of 30 days could be used when exposure concentrations were shown to have "limited variability". The purpose of this section is to better define when a 30-day averaging period is acceptable.

Tests having different durations and/or starting with organisms of different ages can indicate how restrictive the averaging period needs to be. The best information available is for the fathead minnow. Based on 7-day tests, EC20s of 7.08 mg N/L at pH=8.34 and 5.25 mg N/L at pH=8.42 were calculated from the data of Willingham (1987) and CVs of 8.37 mg N/L at pH=8 and 3.87 mg N/L at pH=8.5 were reported by Camp Dresser and McKee (1997). Adjusted to pH=8, these concentrations are 12.1, 10.25, 8.37, and 8.65 mg N/L, respectively, with a geometric mean of 9.7 mg N/L. This is approximately 2.5 times the geometric mean EC20 for the 30-day early life-stage tests conducted by Swigert and Spacie (1983) and Mayes et al. (1986) as discussed above. This suggests that the CCC averaging period could be 30 days, as long as excursions above the CCC are restricted sufficiently to not exceed the mean EC20 from the 7-day tests. A rigorous definition of this excursion restriction is not possible with the limited data available, especially because no information is available concerning the effects of variations within the 7-day period. It is convenient, 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 and because it provides a substantial limitation of variability relative to the 7-day EC20s. It is uncertain how much higher than the CCC the 4-day

average can be, but based on these fathead minnow test results, two-fold higher concentrations should pose little risk.

Some other data support the use of a longer averaging period. For example, the studies of Anderson et al. (1978) and Sparks and Sandusky (1981) with fingernail clams showed that effects gradually accumulated during exposures, suggesting that longer averaging periods are acceptable. Also, in the field study at Monticello, time variations in pH yielded time variations in the applicable CCC. Analysis of the data presented by Zischke and Arthur (1987) for the fingernail clam indicated that limiting the highest 4-day average concentration to two times the CCC would protect this species, whereas application of a 30-day average without this stipulation would allow substantial effects on this species. In addition, Calamari et al. (1977,1981) and Solbe and Shurben (1989) found that longer exposures of embryos and fry resulted in much lower LC50s than 96-hr exposures.

In contrast, some other studies suggest possible risks from longer averaging periods under variable concentrations. For channel catfish, Bader (1990) reported a 24% reduction in growth at 2.4 mg N/L in 7-day tests with young fry at pH=8.2; this corresponds to just 3.3 mg N/L at pH=8, which is lower than the adjusted EC20s reported from longer early-life stage tests and juvenile tests in Table 2. This suggests that a short averaging period is advisable, but such a conclusion is very uncertain because it involves interlaboratory comparisons with very few data and because Bader (1990) also found similar sensitivity with older fry, so his results might represent a high sensitivity of the test stock rather than factors relevant to the averaging period. A short averaging period might also be inferred by the fact that the fathead minnow life-cycle test (Thurston et al. 1986) showed an EC20 of 2.0 mg N/L for embryo hatchability, substantially lower than for early life-stage tests. It is possible that this greater sensitivity might be due to exposures starting earlier in the life-cycle tests than in the early life-stage tests. The importance of early exposure to embryos was demonstrated by Solbe and Shurben (1989) for rainbow trout. However, they dealt with a one-week delay in exposures rather than <1 day and there are other possibilities for the more sensitive results of Thurston et al. (1986).

Based on the fathead minnow early life-stage data, a 30-day averaging period is justified with the restriction that the highest 4-day average within the 30 days is no greater than twice the CCC. The data of Bader (1990) and Thurston et al. (1986) suggest a potential risk from long averaging periods during fish spawning season, but the evidence is weak and, even if variability within long averaging periods produces short

exposures that are sufficiently high to affect young embryos, only a small fraction of total reproduction would generally be affected. A high priority should be given to research to resolve how to better address different time-series of exposure.

WATER-EFFECT RATIOS

Although the current guidance concerning Water-Effect Ratios (WERs) mainly concerns their use with metals (U.S. EPA 1994), the U.S. EPA allows the determination and use of WERs for ammonia. Because pH is the factor that has been shown to substantially affect the toxicity of total ammonia in fresh water and the freshwater criterion for ammonia is adjusted for pH, EPA expects that WERs for ammonia will usually be close to 1. Indeed, most experimentally determined WERs for ammonia have been close to 1:

a. Gersich and Hopkins (1986) and Mayes et al. (1986) reported that the acute and chronic toxicity of ammonia in Tittabawassee River water was about the same as reported by other investigators in laboratory dilution waters.
b. When Nimmo et al. (1989) compared a river water with a well water, the four WERs ranged from 0.84 to 1.3; the four WERs obtained in comparisons of a wastewater with the well water ranged from 0.5 to 1.5.
c. Diamond et al. (1993) obtained WERs of 1.1 and 2.0 with the fathead minnow and *Daphnia magna*, respectively, using a well water and a pH-adjusted laboratory water.
d. In comparisons of a sewage effluent (pH=7.86 to 7.94) and a well water (pH=8.15 to 8.17), Monda et al. (1995) found WERs of 0.83 and 0.62 with a chironomid.
e. Using five species and waters from eight rivers, Willingham (1996) obtained nineteen WERs that ranged from 0.57 to 1.47; one other WER was 3.
f. Acute and chronic tests with the fathead minnow and *Ceriodaphnia dubia* produced four WERs that ranged from about 0.73 to 1.07 for Lake Mead (Willingham 1987).
g. Camp Dresser and McKee (1997) reported a WER of 2.5 with the fathead minnow, but the test in site water lasted for seven days, whereas the tests in laboratory dilution waters lasted for 30 and 350 days.

Although some of these WERs were not determined according to the guidance presented in U.S. EPA (1984) and some might not have been adjusted for a pH difference in the waters, they do illustrate that experimentally-determined WERs for ammonia are likely to be close to 1.

It is possible that WERs for ammonia might be substantially different from 1 if there is an interaction with other pollutants or if there is a substantial difference in ionic composition, possibly in conjunction with a difference in pH or hardness (Ankley et al. 1995; Borgmann 1994; Borgmann and Borgmann 1997; Russo et al. 1988). WERs might also be different from 1 if they are used to derive criteria for ammonia at pH<6.5 or pH>9.0. The

pH of each of the waters used in the determination of the WERs given above was between 7.3 and 8.7, except that pH was not reported by Willingham (1996). Even though it appears that most WERs for ammonia will usually be close to 1.0, dischargers may determine and use WERs to derive site-specific criteria for ammonia whenever they want, as long as sufficient WERs are determined in an acceptable manner (U.S. EPA 1994).

A FIELD STUDY RELEVANT TO THE CCC

Hermanutz et al. (1987) and Zischke and Arthur (1987) reported the effects of different concentrations of ammonia on fishes and invertebrates in various tests at the Monticello, MN, outdoor experimental stream facility. The study involved essentially constant dosing of total ammonia into four parallel streams (three concentrations of ammonia and a control treatment). The approximate average concentrations of total ammonia nitrogen were:

0.08 mg N/L in the control stream
0.66 mg N/L in the low concentration stream
2.0 mg N/L in the medium concentration stream, and
7.1 mg N/L in the high concentration stream.

Although the streams were physically identical, the different concentrations of ammonia caused chemical and microbiological differences among the streams. Higher ammonia concentrations yielded lower pH, and, as a result of higher nitrifying bacterial activity, higher nitrite and nitrate concentrations and lower concentrations of dissolved oxygen, particularly in the lower reaches of the streams containing added ammonia. For example, in the lower reaches of the high concentration stream, dissolved oxygen regularly dropped to 2 mg/L at night during summer. Although these differences between streams reflect real-world phenomena usually accompanying ammonia enrichment, they confound interpreting some of the results in terms of the toxicity of ammonia. Six of the thirteen tests with fishes, however, either did not use the lower reaches of the streams or did not take place during the summer. For these tests the confounding influences of nitrifier activity should not be of much concern.

The study began in June 1983 and ended in November 1984, but all of the tests with the various taxa were of shorter durations. Macroinvertebrate tests lasted for two months, whereas the durations of the fish tests were 28 to 237 days. During all of the tests, the organisms were left to forage on naturally occurring flora and fauna, except that the walleyes were fed fathead minnows.

As reported by Hermanutz et al. (1987), densities of individual macroinvertebrate taxa, sampled approximately 1 to 2 months after the start of the dosing, differed somewhat among the streams. Cladoceran and protozoan densities might have been inhibited by elevated ammonia concentrations (or accompanying changes), rotifer densities might have been somewhat stimulated, and copepod densities showed little effect. However, concentration-

effect patterns were generally inconsistent, and the results do not support any overall conclusion of either stimulatory or inhibitory effects. Because laboratory toxicity tests indicate that these types of macroinvertebrates are generally substantially more resistant to ammonia than fishes, absence of effects might not be viewed as unexpected.

Tests with fishes included two tests with the fathead minnow, one with the bluegill, three with the channel catfish, two with the white sucker, two with the walleye, and three with the rainbow trout. Hermanutz et al. (1987) studied percent survival, fish length, fish weight, and final fish biomass, and identified those treatments and variables that were significantly different than the control stream for individual species. The Technical Support Document for Water Quality-based Toxics Control, EPA (1991) attempted a subjective summarization of these results, relative to the CCC defined in U.S. EPA (1985a).

The fingernail clam data of Zischke and Arthur (1987) were also evaluated. These investigators selected this species for study because it is an important component of many freshwater communities and because it was reported to be highly sensitive to ammonia (Anderson et al. 1978; Sparks and Sandusky 1981).

The intent of this new analysis is to provide a quantitative graphical portrayal of the results of the thirteen tests with fishes and the two tests with the fingernail clam. Recognizing that field and macrocosm data involve a substantial amount of variability, this analysis is intended to determine whether any pattern emerged from the noise.

To integrate the results as much as possible, this analysis used biomass at the end of each test with fish, which Hermanutz et al. (1987) determined from the number of surviving individuals multiplied by the individual mean weight. For the fathead minnow, this measure combines survival, growth, and reproduction. For the other tested fish species, this measure combines survival and growth. Biomass was not available from the data on the fingernail clam. In its place, the product of survival and mean organism length was used.

Concentrations of ammonia were normalized to account for the dependence of ammonia toxicity on pH. The exposure metric used was the concentration of ammonia in the stream divided by the CCC.

Because both 4-day and 30-day averaging periods are used in the criteria statement, this analysis considered whether the maximum 4-day or the maximum 30-day average was significantly different

than the long-term average concentration. Although the concentration of total ammonia varied little over the duration of the Monticello tests, the pH, and therefore ammonia toxicity, varied somewhat over time, particularly in the longer tests. In this case, the CCC varies over time, while the concentration of total ammonia is more constant. The CCC calculated from the maximum 4-day mean pH would be lower than the CCC calculated from the maximum 30-day mean pH. Both would be lower than the CCC calculated from the long-term mean pH. Because the original data books for these tests are no longer available, this analysis relied on data published by Hermanutz et al. (1987) and Zischke and Arthur (1987), which precluded any attempt to estimate the day-by-day exposure.

For tests of 28 to 90 days (that is, up to threefold greater than the 30-day averaging period), the applicable CCC applied with a 30-day averaging period was calculated from the mean pH for the test. For the longer tests within this range, use of the mean pH probably causes a slight bias toward underestimating the excursion of the CCC.

For tests of 91 to 237 days (more than threefold greater than the 30-day averaging period), the applicable CCC applied with a 30-day averaging period was calculated from the highest 30-day mean pH occurring during the test. For the high ammonia stream, this mean pH was estimated directly from the published graph of pH-time variability in this stream. For the other streams, which lacked published graphs on the time course of pH variations, the maximum 30-day mean pH was estimated from the test mean pH for the stream, coupled with the variation about the mean observed in the high treatment stream. That is, the degree of pH variability was assumed to be the same in all of the streams.

For the fish tests, the applicable CCC applied with a 4-day averaging period was estimated from the maximum weekly mean pH, estimated from the published graphs, or from the expected pH variability, in the manner described in the preceding paragraph. For the fingernail clam tests, the maximum 4-day mean pH was taken to be the maximum weekly mean pH published by Zischke and Arthur (1987) for their tests, which is likely to be lower than the actual maximum 4-day mean pH.

Table 3 presents the fish data from Hermanutz et al. (1987) and the fingernail clam data from Zischke and Arthur (1987). The results of the analysis are presented in Figure 13, which show the biological effect, relative to the control treatment, on the vertical axis, and the exposure concentration, relative to the new CCC of 1.27 mg N/L, on the horizontal axis.

Table 3. Data for Fishes and Clams in the Monticello Study[a]

Test	Duration (Days)	Mean temp. (C)	Mean pH	Est. Max pH 30-d mean	Est. Max pH 4-d mean	Est. Total Ammonia N Criterion[b] (mg N/L)	Est. Total Ammonia N est. ave. exp. conc (mg N/L)	Rel. Conc.[c]	Biomass Final (g)	Biomass Rel.[d]
Fathead minnow	63	19.6	7.8	7.8	8.5	1.14[e]	0.08	0.07	81	
1st generation			7.7	7.7	8.4	1.35[e]	0.64	0.47	90	1.11
Start 5/18/83			7.6	7.6	8.3	1.59[e]	1.98	1.24	86	1.07
in lower reach			7.5	7.5	8.2	1.88[e]	7.04	3.75	70	0.87
Fathead minnow	63	19.6	7.8	7.8	8.5	1.14[e]	0.08	0.07	377	
2nd generation			7.7	7.7	8.4	1.35[e]	0.64	0.47	726	1.93
End 8/19/83			7.6	7.6	8.3	1.59[e]	1.98	1.24	263	0.70
in lower reach			7.5	7.5	8.2	1.88[e]	7.04	3.75	2437	6.46
Bluegill	90	21.1	8.3	8.3	8.5	0.80	0.08	0.10	1237	
6/27/84-9/25/84			8.1	8.1	8.3	1.10	0.64	0.58	1489	1.20
in lower reach			8.2	8.2	8.4	0.94	1.98	2.11	1118	0.90
			8.2	8.2	8.4	0.94	7.04	7.50	803	0.65
Channel catfish	177	18.2	8.1	8.5	8.7	0.57	0.08	0.14	5138	
1983			7.9	8.4	8.6	0.67	0.64	0.95	4981	0.97
5/25/83-11/18/83			7.5	8.0	8.2	1.27	1.98	1.55	4385	0.85
in lower reach			7.5	8.0	8.2	1.27	7.04	5.53	3238	0.63
Channel catfish	36	16.8	8.1	8.1	8.3	1.10	0.08	0.08	2108	
1984A			8.0	8.0	8.2	1.27	0.64	0.50	2030	0.96
5/7/84-6/12/84			7.7	7.7	7.9	1.87	1.98	1.06	2202	1.04
in lower reach			7.6	7.6	7.8	2.08	7.04	3.39	1921	0.91
Channel catfish	89	21.1	8.3	8.3	8.5	0.80	0.08	0.10	2923	
1984B			8.1	8.1	8.3	1.10	0.64	0.58	2377	0.81
6/28/84-9/25/84			8.1	8.1	8.3	1.10	1.98	1.80	1204	0.41
in lower reach			8.2	8.2	8.4	0.94	7.04	7.50	1037	0.35

White sucker	183	18.2	8.4	8.7	8.8	0.41	0.08	0.20	2313	
1983			7.9	8.4	8.6	0.67	0.64	0.95	4287	1.85
5/19/83-11/18/83			7.5	8.0	8.2	1.27	1.98	1.55	3010	1.30
in lower reach			7.5	8.0	8.2	1.27	7.04	5.53	5854	2.53
White sucker	88	21.1	8.3	8.3	8.5	0.80	0.08	0.10	4319	
1984			8.1	8.1	8.3	1.10	0.64	0.58	3866	0.90
6/29/84-9/25/84			8.1	8.1	8.3	1.10	1.98	1.80	3034	0.70
in lower reach			8.2	8.2	8.4	0.94	7.04	7.50	3366	0.78
Walleye yearling	46	24.1	8.2	8.2	8.4	0.94	0.08	0.09	2958	
6/29/84-8/14/84			8.1	8.1	8.3	1.10	0.64	0.58	2731	0.92
in upper reach			8.0	8.0	8.2	1.27	1.98	1.55	2092	0.71
			8.2	8.2	8.4	0.94	7.04	7.50	0	0.00
Walleye young	43	16.7	8.4	8.4	8.5	0.67	0.08	0.12	3056	
8/20/84-10/2/84			8.3	8.3	8.4	0.80	0.64	0.80	2678	0.88
in upper reach			8.4	8.4	8.5	0.67	1.98	2.93	2178	0.71
			8.4	8.4	8.5	0.67	7.04	10.44	0	0.00
Rainbow trout	237	5.9	8.3	8.6	8.6	0.48	0.08	0.17	5305	
1983-1984			8.1	8.4	8.4	0.67	0.64	0.95	4514	0.85
10/19/83-6/12/84			7.8	8.1	8.1	1.10	1.98	1.80	5487	1.03
in lower reach			7.7	8.0	8.0	1.27	7.04	5.53	3630	0.68
Rainbow trout	69	10.6	8.3	8.3	8.5	0.80	0.08	0.10	1781	
1984A			8.2	8.2	8.4	0.94	0.64	0.68	1971	1.11
9/6/84-11/14/84			8.1	8.1	8.3	1.10	1.98	1.80	948	0.53
in lower reach			8.4	8.4	8.6	0.67	7.04	10.44	0	0.00
Rainbow trout	28	5.9	8.1	8.1	8.3	1.10	0.08	0.08	403	
1984B			7.9	7.9	8.1	1.46	0.64	0.44	420	1.04
10/16/84-11/13/84			8.1	8.2	8.3	1.10	1.98	1.80	252	0.63
in lower reach			8.4	8.4	8.6	0.67	7.04	10.44	201	0.50

Fingernail clam A	7.9	8.7	0.81[e]	0.11	0.13	25[f]	
6/6/83-8/1/83	7.9	8.5	1.14[e]	0.60	0.53	25[f]	1.01
	7.9	8.6	0.96[e]	2.06	2.14	12[f]	0.48
	7.9	8.5	1.14[e]	7.82	6.87	0[f]	0.00
Fingernail clam B	7.7	8.5	1.14[e]	0.11	0.09	11[f]	
6/13/83-7/11/83	7.7	8.3	1.59[e]	0.60	0.38	14[f]	1.31
	7.7	8.4	1.35[e]	2.06	1.53	2.4[f]	0.22
	7.7	8.3	1.59[e]	7.82	4.91	0[f]	0.00

[a] The data are from Hermanutz et al. (1987) and Zischke and Arthur (1987). All concentrations are total ammonia nitrogen and are expressed as mg N/L.

[b] The tabulated criterion is the lower of (1) the CCC calculated from the estimated maximum 30-day average pH or (2) two times the CCC calculated from the estimated maximum 4-day average pH. Footnote e indicates where the latter condition controlled the result.

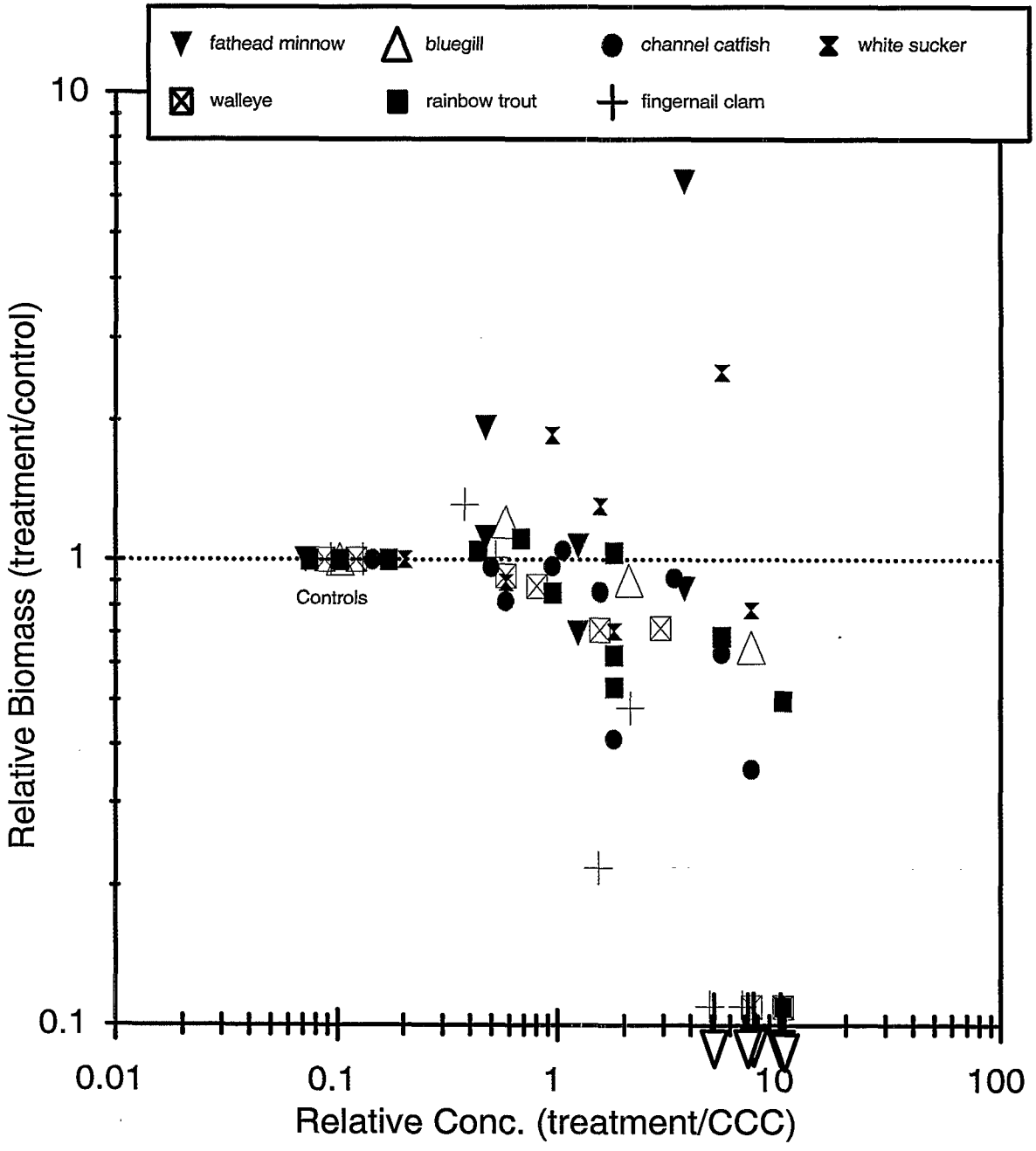
[c] Relative concentration = (treatment concentration/CCC calculated from the estimated maximum 30-day average pH).

[d] Relative biomass = (treatment biomass/control biomass).

[e] For the fathead minnow and the fingernail clam, two times the CCC calculated from the estimated maximum 4-day average pH was less than the CCC calculated from the estimated maximum 30-day average pH.

[f] For the fingernail clam, number of survivors times mean length is tabulated instead of biomass.

Figure 13. Monticello data compared with the new CCC statement



Uncertainties exist in the vertical and horizontal locations of points in Figure 13. Biological measurements on side-by-side macrocosms generally show substantial inherent variability. The frequent occurrence of inversions in the concentration-effect curves suggests that an overly specific or overly literal interpretation of each individual data point might not be well founded. With regard to the exposure concentration associated with the effect, uncertainties are introduced by the time variability of the concentration of ammonia during the tests, and by longitudinal gradients in the streams during some of the tests. Horizontal placement of points is subject to uncertainties caused by the time variability of pH, and might be subject to a slightly low bias in some cases. Finally, the elevated concentrations of ammonia yielded other changes (e.g., depressed concentration of dissolved oxygen) that confound the attribution of effects solely to ammonia toxicity, although many of the data points appear to have little potential to be affected by such other changes.

Some patterns can nevertheless be recognized in the data in Figure 13. Considering the inherent variability, concentrations of ammonia below the CCC appear to yield no significant effects relative to the control treatment. At concentrations above the CCC applied as a 30-day average, many species experienced substantial stress, although certain species might flourish under the conditions associated with such concentrations of ammonia. Concentrations more than fourfold above the CCC applied as a 30-day average appeared to yield conditions intolerable to many tested species.

Tests with two species, the fathead minnow and the fingernail clam, occurred during a time period when the pH was so variable that the CCC applied as a 4-day average was substantially different than the CCC applied as a 30-day average. If applied simply as a 30-day average, the CCC would have allowed substantial effects on the fingernail clam. However, this species, which appeared to be the most sensitive tested species in the study, would be protected by the additional limitation, which is expressed in the criterion statement, that the 4-day average concentration cannot be more than two times the CCC.

THE NATIONAL CRITERION FOR AMMONIA IN FRESH WATER

The available data for ammonia, evaluated using the procedures described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses", indicate that, except possibly where a very sensitive species is important at a site, freshwater aquatic life should be protected if both of the following conditions are satisfied:

1. The one-hour average concentration of total ammonia nitrogen (in mg N/L) does not exceed, more than once every three years on the average, the CMC calculated using the following equation:

$$CMC = \frac{0.275}{1 + 10^{7.204-pH}} + \frac{39.0}{1 + 10^{pH-7.204}}$$

   In situations where salmonids do not occur, the CMC may be calculated using the following equation:

$$CMC = \frac{0.411}{1 + 10^{7.204-pH}} + \frac{58.4}{1 + 10^{pH-7.204}}$$

2. The thirty-day average concentration of total ammonia nitrogen (in mg N/L) does not exceed, more than once every three years on the average, the CCC calculated using the following equation:

$$CCC = \frac{0.0858}{1 + 10^{7.688-pH}} + \frac{3.70}{1 + 10^{pH-7.688}}$$

   and the highest four-day average within the 30-day period does not exceed twice the CCC.

The numeric values of the CMC with salmonids present and absent and the CCC are:

pH	CMC with salmonids present	CMC with salmonids absent	CCC
6.5	32.6	48.8	3.48
6.6	31.3	46.8	3.42
6.7	29.8	44.6	3.36
6.8	28.1	42.0	3.28

6.9	26.2	39.1	3.19
7.0	24.1	36.1	3.08
7.1	22.0	32.8	2.96
7.2	19.7	29.5	2.81
7.3	17.5	26.2	2.65
7.4	15.4	23.0	2.47
7.5	13.3	19.9	2.28
7.6	11.4	17.0	2.07
7.7	9.65	14.4	1.87
7.8	8.11	12.1	1.66
7.9	6.77	10.1	1.46
8.0	5.62	8.40	1.27
8.1	4.64	6.95	1.09
8.2	3.83	5.72	0.935
8.3	3.15	4.71	0.795
8.4	2.59	3.88	0.673
8.5	2.14	3.20	0.568
8.6	1.77	2.65	0.480
8.7	1.47	2.20	0.406
8.8	1.23	1.84	0.345
8.9	1.04	1.56	0.295
9.0	0.885	1.32	0.254

Several points should be noted concerning the criterion:

1. The two lowest GMCVs are "less than" values. The CCC would be lower if a point estimate, rather than a "less than" value, could have been derived from the Borgmann (1994) study with *Hyalella*, the most sensitive genus. The CCC also might be lower if a point estimate, rather than a "less than" value, could have been derived from the studies with the fingernail clam.
2. The available chronic EC20s for salmonids, even though not used directly in the calculation of the CCC, indicate that these species would probably be protected by the CCC, although the data suggest that there might be important differences between strains of rainbow trout.
3. Some of the laboratory and field data for the fingernail clam, which might be considered to have special ecological importance at some sites, indicate that this species would be affected at concentrations below the CCC, although other data indicate that it might not be affected by such concentrations and at most sites the intermittency of exposures would probably reduce risk.
4. When 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.

5. Partly for statistical reasons, the CCC is based on a 20 percent reduction in survival, growth, and/or reproduction. Whether the maximum acceptable percent reduction should be lower or higher than 20 percent under a set of conditions is a risk management decision.

Because the chronic values for two of the four most chronically sensitive genera are based on tests with early life stages of fish, there is some uncertainty in applying the CCC during conditions, such as during cold-weather conditions, when such life stages are not present. Furthermore, although the data for the two most sensitive genera (i.e., *Hyalella* and fingernail clam) do not involve this life-stage issue, the acute toxicity data for these taxa indicate that they probably become more resistant to total ammonia as the temperature decreases. Nevertheless, without exercising a degree of risk management that is beyond the scope of this 1998 Update, the available data do not allow a determination of how much higher the CCC could be during a period during which the temperature is low and early life stages of fishes are absent.

The Recalculation Procedure, the WER Procedure, and the Resident Species Procedure may be used to derive site-specific criteria for ammonia, but most WERs that have been determined for ammonia are close to 1.

The CMC, CCC, and CCC averaging period presented above supersede those given in previous guidance concerning the aquatic life criterion for ammonia in fresh water. This 1998 Update does not address or alter the past recommendation of a one-hour averaging period for the CMC or the past recommendation of a once-in-three years on the average allowable frequency for exceeding the CMC or CCC. Many issues concerning the implementation of aquatic life criteria are discussed in the "Technical Support Document for Water Quality-based Toxics Control" (U.S. EPA 1991).

Because the ammonia criterion is a function of pH, calculation of the appropriate weighted average pH is complicated. For some purposes, calculation of an average pH can be avoided. For example, if samples are obtained from a receiving water over a period of time during which pH is not constant, the pH and the concentration of total ammonia in each sample should be determined. For each sample, the criterion should be determined at the pH of the sample, and then the concentration of total ammonia nitrogen in the sample should be divided by the criterion to determine a quotient. If the geometric mean of the quotients is less than 1 over an appropriate period of time, there is no evidence that the criterion has been exceeded.

REFERENCES

Alexander, H.C., P.B. Latvaitis, and D.L. Hopkins. 1986. Site-Specific Toxicity of Un-ionized Ammonia in the Tittabawassee River at Midland, Michigan: Overview. Environ. Toxicol. Chem. 5:427-435.

Anderson, K.B., R.E. Sparks, and A.A. Paparo. 1978. Rapid Assessment of Water Quality, using the Fingernail Clam, Musculium transversum. WRC Research Report No. 133, University of Illinois, Water Resources Center, Urbana, IL.

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. 52:2078-2083.

Armstrong, D.A., D. Chippendale, A.W. Knight, and J.E. Colt. 1978. Interaction of Ionized And Un-ionized Ammonia on Short-term Survival and Growth of Prawn Larvae, *Macrobrachium rosenbergii*. Biol. Bull. 154:15-31.

Arthur, J.W., C.W. West, K.N. Allen, and S.F. Hedtke. 1987. Seasonal Toxicity of Ammonia to Five Fish and Nine Invertebrate Species. Bull. Environ. Contam. Toxicol. 38:324-331.

ASTM. 1997a. Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with *Daphnia magna*. Standard E1193 in Vol. 11.05 of the Annual Book of ASTM Standards. American Society for Testing and Materials, West Conshohocken, PA.

ASTM. 1997b. Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fishes. Standard E1241 in Vol. 11.05 of the Annual Book of ASTM Standards. American Society for Testing and Materials, West Conshohocken, PA.

ASTM. 1997c. Standard Guide for Conducting Three-Brood, Renewal Toxicity Tests with *Ceriodaphnia dubia*. Standard E1295 in Vol. 11.05 of the Annual Book of ASTM Standards. American Society for Testing and Materials, West Conshohocken, PA.

ASTM. 1997d. Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians. Standard E729 in Vol. 11.05 of the Annual Book of ASTM Standards. American Society for Testing and Materials, West Conshohocken, PA.

ASTM. 1997e. Standard Test Methods for Measuring the Toxicity of Sediment-Associated Contaminants with Fresh Water Invertebrates. Standard E1706 in Vol. 11.05 of the Annual Book of ASTM Standards. American Society for Testing and Materials, West Conshohocken, PA.

Bader, J.A. 1990. Growth-Inhibiting Effects and Lethal Concentrations of Un-ionized Ammonia for Larval and Newly Transformed Juvenile Channel Catfish (Ictalurus punctatus). M.S. Thesis, Auburn University, Auburn, AL.

Bader, J.A., and J.M. Grizzle. 1992. Effects of Ammonia on Growth and Survival of Recently Hatched Channel Catfish. J. Aquatic Animal Health 4:17-23.

Bailey, H.C., D.H.W. Liu, and H.A. Javitz. 1985. Time/Toxicity Relationships in Short-Term Static, Dynamic, and Plug-Flow Bioassays. In: Aquatic Toxicology and Hazard Assessment: Eighth Symposium. R.C. Bahner and D.J. Hansen, Eds. ASTM STP 891. American Society for Testing and Materials, Philadelphia, PA. pp. 193-212.

Bergerhouse, D.L. 1992. Lethal Effects of Elevated pH and Ammonia on Early Life Stages of Walleye. N. Amer. J. Fish. Manage. 12:356-366.

Bergerhouse, D.L. 1993. Lethal Effects of Elevated pH and Ammonia on Early Life Stages of Hybrid Striped Bass. J. Appl. Aquacult. 2:81-100.

Bianchini, A., W. Wasielesky, Jr., and K.C.M. Filho. 1996. Toxicity of Nitrogenous Compounds to Juveniles of Flatfish *Paralichthys orbignyanus*. Bull. Environ. Contam. Toxicol. 56:453-459.

Borgmann, U. 1994. Chronic Toxicity of Ammonia to the Amphipod *Hyalella azteca*; Importance of Ammonium Ion and Water Hardness. Environ. Pollut. 86:329-335.

Borgmann, U., and A.I. Borgmann. 1997. Control of Ammonia Toxicity to *Hyalella azteca* by Sodium, Potassium and pH. Environ. Pollut. 95:325-331.

Box, G.E.P., and N.R. Draper. 1965. The Bayesian Estimation of Common Parameters from Several Responses. Biometrika 52:355-365.

Box, G.E.P., W.G. Hunter, J.F. MacGregor, and J. Erjavec. 1973. Some Problems Associated with the Analysis of Multiresponse Data. Technometrics 15:33-51.

Broderius, S.J., and L.L. Smith, Jr. 1979. Lethal and Sublethal Effects of Binary Mixtures of Cyanide and Hexavalent Chromium, Zinc, or Ammonia to the Fathead Minnow (*Pimephales promelas*) and Rainbow Trout (*Salmo gairdneri*). J. Fish. Res. Bd. Can. 36:164-172.

Broderius, S., R. Drummond, J. Fiandt, and C. Russom. 1985. Toxicity of Ammonia to Early Life Stages of the Smallmouth Bass at Four pH Values. Environ. Toxicol. Chem. 4:87-96.

Buckley, J.A., C.M. Whitmore, and B.D. Liming. 1979. Effects of Prolonged Exposure to Ammonia on the Blood and Liver Glycogen of Coho Salmon (*Oncorhynchus kisutch*). Comp. Biochem. Physiol. 63C:297-303.

Burkhalter, D.E. 1975. Effects of Prolonged Exposure to Ammonia on Rainbow Trout (*Salmo gairdneri*) Eggs and Sac Fry. M.S. Thesis, Montana State University, Boseman, MT. 67 pp.

Burkhalter, D.E., and C.M. Kaya. 1977. Effects of Prolonged Exposure to Ammonia on Fertilized Eggs and Sac Fry of Rainbow Trout (*Salmo gairdneri*). Trans. Amer. Fish. Soc. 106:470-475.

Burrows, R.E. 1964. Effects of Accumulated Excretory Products on Hatchery-Reared Salmonids. Research Report 66, U.S. Fish and Wildlife Service, Washington, DC.

Calamari, D., R. Marchetti, and G. Vailati. 1977. Effects of Prolonged Treatments with Ammonia on Stages of Development of *Salmo gairdneri*. (English translation used.) Nuovi Ann. Ig. Microbiol. 28:333-345.

Calamari, D., R. Marchetti, and G. Vailati. 1981. Effects of Long-term Exposure to Ammonia on the Developmental Stages of Rainbow Trout (*Salmo gairdneri* Richardson). Rapp. P.-v. Reun. Cons. int. Explor. Mer. 178:81-86.

Camp Dresser and McKee. 1997. Site-Specific Limits for Un-ionized Ammonia - Red River of the North. Report to the City of Moorhead, MN.

Carline, R.F., A.J. Benson, and H. Rothenbacher. 1987. Long-term Effects of Treated Domestic Wastewater on Brown Trout. Wat. Res. 21:1409-1415.

Cary, G.A. 1976. A Report on the Assessment of Aquatic Environmental Impact of Union Carbide's Uravan Operations. Aquatic Environmental Sciences, Tarrytown, NY. 67 pp.

Chipman, W.A., Jr. 1934. The Role of pH in Determining the Toxicity of Ammonium Compounds. Ph.D. Thesis. University of Missouri, Columbia, MO. 153 pp.

Colt, J.E. 1978. The Effects of Ammonia on the Growth of Channel Catfish, Ictalurus punctatus. Ph.D. Thesis, University of California, Davis, CA. 185 pp.

Colt, J., and G. Tchobanoglous. 1976. Evaluation of the Short-term Toxicity of Nitrogenous Compounds to Channel Catfish, *Ictalurus punctatus*. Aquaculture 8:209-224.

Colt, J., and G. Tchobanoglous. 1978. Chronic Exposure of Channel Catfish, *Ictalurus punctatus*, to Ammonia: Effects on Growth and Survival. Aquaculture 15:353-372.

Dabrowska, H., and H. Sikora. 1986. Acute Toxicity of Ammonia to Common Carp (*Cyprinus carpio* L.) Pol. Arch. Hydrobiol. 33:121-128.

Dahlquist, G., and A. Bjorck. 1974. Numerical Methods. Translated by N. Anderson. Prentice-Hall, Englewood Cliffs, NJ. 573 pp.

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.

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 Un-ionized Ammonia to Fathead Minnows and Channel Catfish. Final Report to U.S. EPA by Battelle, Columbus, OH. 39 pp.

Diamond, J.M., D.G. Mackler, W.J. Rasnake, and D. Gruber. 1993. Derivation of Site-Specific Ammonia Criteria for an Effluent-Dominated Headwater Stream. Environ. Toxicol. Chem. 12:649-658.

Draper, N.R., and H. Smith. 1981. Applied Regression Analysis. Second Edition. Wiley, New York. 709 pp.

Emerson, K., R.C. Russo, R.E. Lund, and R.V. Thurston. 1975. Aqueous Ammonia Equilibrium Calculations: Effect of pH and Temperature. J. Fish. Res. Bd. Can. 32:2379-2383.

Erickson, R.J. 1985. An Evaluation of Mathematical Models for the Effects of pH and Temperature on Ammonia Toxicity to Aquatic Organisms. Water Res. 19:1047-1058.

Fromm, P.O. 1970. Toxic Action of Water Soluble Pollutants on Freshwater Fish. EPA Water Pollution Control Research Series. 18050 DST 12/70. PB 201650, National Technical Information Service, Springfield, VA. 56 pp.

Gersich, F.M., and D.L. Hopkins. 1986. Site-Specific Acute and Chronic Toxicity of Ammonia to *Daphnia magna* Straus. Environ. Toxicol. Chem. 5:443-447.

Gersich, F.M., D.L. Hopkins, S.L. Applegath, C.G. Mendoza, and D.P. Milazzo. 1985. The Sensitivity of Chronic Endpoints Used in *Daphnia magna* Straus Life-Cycle Tests. In: Aquatic Toxicology and Hazard Assessment: Eighth Symposium. R.C. Bahner and D.J. Hansen, Eds. ASTM STP 891. American Society for Testing and Materials, Philadelphia, PA. pp. 245-252.

Goudreau, S.E., R.J. Neves, and R.J. Sheehan. 1993. Effects of Wastewater Treatment Plant Effluents on Freshwater Molluscs in the Upper Clinch River, Virginia, USA. Hydrobiologia 252:211-230.

Gulyas, P., and E. Fleit. 1990. Evaluation of Ammonia Toxicity on *Daphnia magna* and Some Fish Species. Aquacultura Hungarica (Szarvas) 6:171-183.

Hasan, M.R., and D.J. Macintosh. 1986. Acute Toxicity of Ammonia to Common Carp Fry. Aquaculture 54:97-107.

Hazel, C.R., W. Thomsen, and S.J. Meith. 1971. Sensitivity of Striped Bass and Stickleback to Ammonia in Relation to Temperature and Salinity. Calif. Fish Game 57:138-153.

Heber, M., and K. Ballentine. 1992. Memorandum to Water Quality Standards Coordinators. July 30.

Henderson, C., Q.H. Pickering, and A.E. Lemke. 1961. The Effect of Some Organic Cyanides (Nitriles) on Fish. Industrial Waste Conf. 15:120-130.

Hermanutz, R.O., S.F. Hedtke, J.W. Arthur, R.W. Andrew, and K.N. Allen. 1987. Ammonia Effects on Microinvertebrates and Fish in Outdoor Experimental Streams. Environ. Pollut. 47:249-283.

Iwama, G.K., J.C. McGeer, P.A. Wright, M.P. Wilkie, and C.M. Wood. 1997. Divalent Cations Enhance Ammonia Excretion in Lahontan Cutthroat Trout in Highly Alkaline Water. J. Fish Biol. 50:1061-1073.

Johnson, C.G. 1995. Effects of pH and Hardness on Acute and Chronic Toxicity of Un-ionized Ammonia to *Ceriodaphnia dubia*. M.S. Thesis. University of Wisconsin, Stevens Point, WI. 69 pp.

Jude, D.J. 1973. Sublethal Effects of Ammonia and Cadmium on Growth of Green Sunfish. Ph.D. Thesis. Michigan State University, East Lansing, MI. 193 pp.

Knoph, M.B. 1992. Acute Toxicity of Ammonia To Atlantic Salmon (*Salmo salar*) Parr. 101C:275-282.

Lee, D.R. 1976. Development of an Invertebrate Bioassay to Screen Petroleum Refinery Effluents Discharged into Freshwater. Thesis. Virginia Polytechnic Institute and State University. 105 pp.

Lemly, A.D. 1996. Wastewater Discharges may be most Hazardous to Fish during Winter. Environ. Pollut. 93:169-174.

Lloyd, R. 1980. Toxicity Testing with Aquatic Organisms: A Framework for Hazard Assessment and Pollution Control. In: Biological Effects of Marine Pollution and the Problems of Monitoring. A.D. McIntyre and J.B. Pearce, Eds. Rapp. P.-v. Reun. Cons. int. Explor. Mer. 179:339-341.

Lloyd, R., and D.W.M. Herbert. 1960. The Influence of Carbon Dioxide on the Toxicity of Un-ionized Ammonia to Rainbow Trout (*Salmo gairdnerii* Richardson). Ann. Appl. Biol. 48:399-404.

Macek, K.J., and B.H. Sleight, III. 1977. Utility of Toxicity Tests with Embryos and Fry of Fish in Evaluating Hazards Associated with the Chronic Toxicity of Chemicals to Fishes. In: Aquatic Toxicology and Hazard Evaluation. F.L. Mayer and J.L. Hamelink, Eds. ASTM STP 634. American Society for Testing and Materials, Philadelphia, PA. pp. 137-146.

Malins, D.C. 1982. Alterations in the Cellular and Subcellular Structure of Marine Teleosts and Invertebrates Exposed to Petroleum in the Laboratory and Field: A Critical Review. Can. J. Fish. Aquat. Sci. 39:877-889.

Marchetti, R. 1960. New Studies on the Toxicology of Fish with Respect to Control of Waste Waters. (English translation used.) Ann. Sta. Centr. Hydrobiol. Appl. 8:107-124.

Mayes, M.A., H.C. Alexander, D.L. Hopkins, and P.B. Latvaitis. 1986. Acute and Chronic Toxicity of Ammonia to Freshwater Fish: A Site-Specific Study. Environ. Toxicol. Chem. 5:437-442.

McCormick, J.H., S.J. Broderius, and J.T. Fiandt. 1984. Toxicity of Ammonia to Early Life Stages of the Green Sunfish *Lepomis cyanellus*. (with erratum) Environ. Pollut. (Series A) 36:147-163.

McKim, J.M. 1977. Evaluation of Tests with Early Life Stages of Fish for Predicting Long-Term Toxicity. J. Fish. Res. Board Can. 34:1148-1154.

Ministry of Technology. 1968. Effects of Pollution on Fish. In: Water Pollution Research 1967. H.M. Stationery Office, London. pp. 56-65.

Mitchell, S.J., and J.J. Cech, Jr. 1983. Ammonia-Caused Gill Damage in Channel Catfish (*Ictalurus punctatus*): Confounding Effects of Residual Chlorine. Can. J. Fish. Aquat. Sci. 40:242-247.

Monda, D.P., D.L. Galat, S.E. Finger, and M.S. Kaiser. 1995. Acute Toxicity of Ammonia (NH_3-N) in Sewage Effluent to *Chironomus riparius*: II. Using a Generalized Linear Model. Arch. Environ. Contam. Toxicol. 28:385-390.

Moore, D.R.J., and P.-Y. Caux. 1997. Estimating Low Toxic Effects. Environ. Toxicol. Chem. 16:794-801.

Mount, D.I. 1982. Memorandum to R.C. Russo. August 6.

Mount, D.R., D.D. Gulley, J.R. Hockett, T.D. Garrison, and J.M. Evans. 1997. Statistical Models to Predict the Toxicity of Major Ions to *Ceriodaphnia dubia, Daphnia magna* and *Pimephales promelas* (Fathead Minnows). Environ. Toxicol. Chem. 16:2009-2019.

Nimmo, D.W.R., D. Link, L.P. Parrish, G.J. Rodriguez, W. Wuerthele, and P.H. Davies. 1989. Comparison of On-Site and Laboratory Toxicity Tests: Derivation of Site-Specific Criteria for Un-ionized Ammonia in a Colorado Transitional Stream. Environ. Toxicol. Chem. 8:1177-1189.

Rankin, D.P. 1979. The Influence of Un-ionized Ammonia on the Long-term Survival of Sockeye Salmon Eggs. Technical Report No. 912, Fisheries and Marine Service, Nanaimo, British Columbia, Canada. 17 pp.

Reichenbach-Klinke, H.-H. 1967. Investigations on the Influence of the Ammonia Content on the Fish Organism. (English translation used.) Archiv fur Fischereiwissenschaft 17:122-132.

Reinbold, K.A., and S.M. Pescitelli. 1982a. Effects of Exposure to Ammonia on Sensitive Life Stages of Aquatic Organisms. Report to U.S. EPA by Illinois Natural History Survey, Champaign, IL.

Reinbold, K.A., and S.M. Pescitelli. 1982b. Acute Toxicity of Ammonia to the White Sucker. Report to U.S. EPA by the Illinois Natural History Survey, Champaign, IL.

Reinbold, K.A., and S.M. Pescitelli. 1982c. Acute Toxicity of Ammonia to Channel Catfish. Report to U.S. EPA by the Illinois Natural History Survey, Champaign, IL.

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. 78:641-648.

Robinette, H.R. 1976. Effect of Selected Sublethal Levels of Ammonia on the Growth of Channel Catfish (*Ictalurus punctatus*). Prog. Fish-Cult. 38:26-29.

Robinson-Wilson, E.F., and W.K. Seim. 1975. The Lethal and Sublethal Effects of a Zirconium Process Effluent on Juvenile Salmonids. Water Resour. Bull. 11:975-986.

Roseboom, D.P., and D.L. Richey. 1977. Acute Toxicity of Residual Chlorine and Ammonia to Some Native Illinois Fishes. Report of Investigation 85, Illinois State Water Survey, Urbana, IL. 42 pp.

Russo, R.C., D.J. Randall, and R.V. Thurston. 1988. Ammonia Toxicity and Metabolism in Fishes. In: Protection of River Basins, Lakes, and Estuaries. R.C. Ryans, Ed. American Fisheries Society, Bethesda, MD. pp. 159-173.

Samylin, A.F. 1969. Effect of Ammonium Carbonate on the Early Stages of Development of Salmon. (English translation used.) Uch. Zap. Leningr. Gos. Pedagog. Inst. Im. A.I. Gertsena 422:47-62.

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. 14:713-717.

Schulze-Wiehenbrauck, H. 1976. Effects of Sublethal Ammonia Concentrations on Metabolism in Juvenile Rainbow Trout (*Salmo gairdneri* Richardson). Ber. dt. wiss. Kommn. Meeresforsch. 24:234-250.

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. Amer. Fish. Soc. 115:891-899.

Smart, G. 1976. The Effect of Ammonia Exposure on Gill Structure of the Rainbow Trout (*Salmo gairdneri*). J. Fish Biol. 8:471-475.

Smith, C.E. 1972. Effects of Metabolic Products on the Quality of Rainbow Trout. Amer. Fish. Trout News. 17:7,8,21,22.

Smith, C.E. 1984. Hyperplastic Lesions of the Primitive Meninx of Fathead Minnows, *Pimephales promelas*, Induced by Ammonia: Species Potential for Carcinogen Testing. Natl. Cancer Inst. Monogr. 65:119-125.

Smith, C.E., and R.G. Piper. 1975. Lesions Associated with Chronic Exposure to Ammonia. In: The Pathology of Fishes. W.E. Ribelin and G. Migaki, Eds. U. Wisc. Press, Madison, WI. pp. 497-514.

Smith, W.E., T.H. Roush, and J.T. Fiandt. 1984. Toxicity of Ammonia to Early Life Stages of Bluegill (Lepomis macrochirus). EPA-600/x-84-175. In-house report, U.S. EPA, Duluth, MN.

Snell, T.W., and G. Persoone. 1989. Acute Toxicity Bioassays Using Rotifers. II. A Freshwater Test with *Brachionus rubens*. Aquatic Toxicol. 14:81-91.

Soderberg, R.W., and J.W. Meade. 1991. The Effects of Ionic Strength on Un-ionized Ammonia Concentration. Prog. Fish-Cult. 53:118-120.

Soderberg, R.W., and J.W. Meade. 1992. Effects of Sodium and Calcium on Acute Toxicity of Un-ionized Ammonia to Atlantic Salmon and Lake Trout. J. Appl. Aquaculture 1:83-92.

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. Amer. Fish. Soc. 112:448-451.

Soderberg, R.W., M.V. McGee, and C.E. Boyd. 1984. Histology of Cultured Channel Catfish, *Ictalurus punctatus* (Rafinesque). J. Fish Biol. 24: 683-690.

Solbe, J.F.L.G., and D.G. Shurben. 1989. Toxicity of Ammonia to Early Life Stages of Rainbow Trout (*Salmo gairdneri*). Water Res. 23:127-129.

Sousa, R.J., T.L. Meade, and R.E. Wolke. 1974. Reduction of Ammonia Toxicity by Salinity and pH Manipulation. IN: Proc. Fifth Annual Workshop, World Mariculture Society 5:343-354.

Sparks, R.E., and M.J. Sandusky. 1981. Identification of Factors Responsible for Decreased Production of Fish Food Organisms in the Illinois and Mississippi Rivers. Final Report for Project No. 3-291-R, Illinois Natural History Survey, River Research Laboratory, Havana, IL. 63 pp.

Stephan, C.E., and J.W. Rogers. 1985. Advantages of Using Regression Analysis to Calculate Results of Chronic Toxicity Tests. In: Aquatic Toxicology and Hazard Assessment: Eighth Symposium. R.C. Bahner and D.J. Hansen, Eds. ASTM STP 891. American Society for Testing and Materials, Philadelphia, PA. pp. 328-338.

Stevenson, T.J. 1977. The Effects of Ammonia, pH and Salinity on the White Perch, Morone americana. Ph.D. Thesis, University of Rhode Island, Kingston, RI. 154 pp.

Swigert, J.P. and A. Spacie. 1983. Survival and Growth of Warmwater Fishes Exposed to Ammonia Under Low Flow Conditions. PB83-257535. National Technical Information Service, Springfield, VA.

Szumski, D.S., D.A. Barton, H.D. Putnam, and R.C. Polta. 1982. Evaluation of EPA Un-ionized Ammonia Toxicity Criteria. J. Water Pollut. Control Fed. 54:281-291.

Tabata, K. 1962. Toxicity of Ammonia to Aquatic Animals with Reference to the Effect of pH and Carbonic Acid. (English translation used.) Tokai-ku Suisan Kenkyusho Kenkyu Hokoku 34:67-74.

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: Bioindicators and Environmental Management: Sixth Symposium. D.W. Jeffrey and B. Madden, Eds. Academic Press, London. pp. 353-363.

Thurston, R.V., and R.C. Russo. 1983. Acute Toxicity of Ammonia to Rainbow Trout. Trans. Amer. Fish. Soc. 112:696-704.

Thurston, R.V., R.C. Russo, and C.E. Smith. 1978. Acute Toxicity of Ammonia and Nitrite to Cutthroat Trout Fry. Trans. Amer. Fish. Soc. 107:361-368.

Thurston, R.V., C. Chakoumakos, and R.C. Russo. 1981a. Effect of Fluctuating Exposures on the Acute Toxicity of Ammonia to Rainbow Trout (*Salmo gairdneri*) and Cutthroat Trout (*S. clarki*). Water Res. 15:911-917.

Thurston, R.V., R.C. Russo, and G.A. Vinogradov. 1981b. Ammonia Toxicity to Fishes. Effect of pH on the Toxicity of the Un-ionized Ammonia Species. Environ. Sci. Technol. 15:837-840.

Thurston, R.V., R.C. Russo, and G.R. Phillips. 1983. Acute Toxicity of Ammonia to Fathead Minnows. Trans. Amer. Fish. Soc. 112:705-711.

Thurston, R.V., R.J. Luedtke, and R.C. Russo. 1984a. Toxicity of Ammonia to Freshwater Insects of Three Families. Technical Report No. 84-2, Fisheries Bioassay Laboratory, Montana State University, Bozeman, MT. 26 pp.

Thurston, R.V., R.C. Russo, R.J. Luedtke, C.E. Smith, E.L. Meyn, C. Chakoumakos, K.C. Wang, and C.J.D. Brown. 1984b. Chronic Toxicity of Ammonia to Rainbow Trout. Trans. Amer. Fish. Soc. 113:56-73.

Thurston, R.V., R.C. Russo, E.L. Meyn, R.K. Zajdel, and C.E. Smith. 1986. Chronic Toxicity of Ammonia to Fathead Minnows. Trans. Amer. Fish. Soc. 115:196-207.

Tomasso, J.R., and G.J. Carmichael. 1986. Acute Toxicity of Ammonia, Nitrite, and Nitrate to the Guadalupe Bass, *Micropterus treculi*. Bull. Environ. Contam. Toxicol. 36:866-870.

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. Amer. Fish. Soc. 109:229-234.

U.S. EPA. 1985a. Ambient Water Quality Criteria for Ammonia - 1984. EPA-440/5-85-001. National Technical Information Service, Springfield, VA.

U.S. EPA. 1985b. Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses. PB85-227049. National Technical Information Service, Springfield, VA.

U.S. EPA. 1986. Ambient Water Quality Criteria for Dissolved Oxygen. EPA 440/5-86-003. National Technical Information Service, Springfield, VA.

U.S. EPA. 1989. Ambient Water Quality Criteria for Ammonia (Saltwater) - 1989. EPA 440/5-88-004. National Technical Information Service, Springfield, VA.

U.S. EPA. 1991. Technical Support Document for Water Quality-based Toxics Control. EPA/505/2-90-001. National Technical Information Service, Springfield, VA.

U.S. EPA. 1994. Interim Guidance on Determination and Use of Water-Effect Ratios for Metals. EPA-823-B-94-001 or PB94-140951. National Technical Information Service, Springfield, VA.

U.S. EPA. 1996. "Ammonia Criteria" in the Water Quality Criteria and Standards Newsletter for January. EPA-823-N-96-001. Office of Water, Washington, DC. p. 5.

Wade, D.C. 1992. Definitive Evaluation of Wheeler Reservoir Sediments Toxicity using Juvenile Freshwater Mussels (Anodonta imbecillis Say). TVA/WR--92/25. Tennessee Valley Authority.

West, C.W. 1985. Acute Toxicity of Ammonia to 14 Freshwater Species. Internal Report. U.S. EPA, Environmental Research Laboratory, Duluth, MN.

Williams, K.A., D.W.J. Green, and D. Pascoe. 1986. Studies on the Acute Toxicity of Pollutants to Freshwater Macroinvertebrates. 3. Ammonia. Arch. Hydrobiol. 106:61-70.

Willingham, T. 1987. Acute and Short-term Chronic Ammonia Toxicity to Fathead Minnows (Pimephales promelas) and Ceriodaphnia dubia Using Laboratory Dilution Water and Lake Mead Dilution Water. U.S. EPA, Denver, CO. 40 pp.

Willingham, T. 1996. Letter to C. Stephan. November 8.

Wood, C.M. 1993. Ammonia and Urea Metabolism and Excretion. In: The Physiology of Fishes. D.H. Evans, Ed. CRC Press, Boca Raton, FL. pp. 379-425.

Wuhrmann, K., and H. Woker. 1948. Beitrage zur Toxikologie der Fische. II. Experimentelle Untersuchungen uber die Ammoniak- und Blausaurevergiftung. (Contributions to the Toxicology of Fishes. II. Experimental Investigations on Ammonia and Hydrocyanic Acid Poisoning.) Schweiz. Z. Hydrol. 11:210-244. (English translation used.)

Yesaki, T.Y., and G.K. Iwama. 1992. Survival, Acid-Base Regulation, Ion Regulation, and Ammonia Excretion in Rainbow Trout in Highly Alkaline Water. Physiol. Zool. 65:763-787.

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. 16:225-231.

Appendix 1. Review of Some Toxicity Tests

Diamond et al. (1993) reported results of a variety of acute and chronic toxicity tests on ammonia. Data sheets and reports concerning these tests were examined for additional information that would be useful in the evaluation of the tests and interpretation of the results. The most common problem was that the concentration of dissolved oxygen was too low or too high.

Water-Effect Ratios

The data sheets and reports revealed that the information in Table 2 in Diamond et al. (1993) is correct. The invertebrate used was *D. magna* as stated on page 653, not *D. pulex* as stated on page 652.

Acute toxicity at 20°C

The data sheets and reports revealed the following regarding the information in Table 3:

a. The concentration of dissolved oxygen was above 110 percent of saturation for a portion of the test with the bay silverside.
b. The highest tested concentration in the test with the bluegill killed only 40 percent of the test organisms.
c. The data sheets say that tests were conducted with two species of crayfish. Subsequently, the authors said that it was later determined that *Procambarus clarkii* was used in both tests and that all of the crayfish were obtained from the same supplier. The LC50 in the table is from a test in which the concentration of dissolved oxygen was below 44 percent of saturation for a portion of the test.
d. The LC50 given for the amphipod is a 21-day LC50. The concentration of dissolved oxygen was below 50 percent of saturation for a portion of the test.
e. The LC50 given for the spring peeper is a 9-day LC50.

Some of these tests were conducted in a laboratory dilution water and some were conducted in a well water; these were the two waters used in the determination of the Water-Effect Ratios (see above).

Chronic toxicity at 20°C

The data sheets and reports revealed the following regarding the chronic tests that are the basis of the results in Table 4:

Leopard frog (larvae-tadpole)
The concentration of dissolved oxygen was below 50 percent of saturation for a portion of the test. In addition, this test lasted for only 14 days.

Leopard frog (egg-larvae)
The concentration of dissolved oxygen was below 40 percent of saturation for a portion of the test. In addition, this test lasted for only 20 days.

Bluegill
There were no major problems with this test, which was conducted in a laboratory dilution water. The durations of the chronic tests with the bluegill in warm and cold water are switched in Table 1.

Crayfish (*Procambarus clarkii*)
The concentration of dissolved oxygen was below 40 percent of saturation for a portion of the test. In addition, this test lasted for only 21 days.

Amphipod (*Crangonyx* spp.)
The concentration of dissolved oxygen was below 40 percent of saturation for a portion of the test. In addition, this test was begun with organisms that were 8 to 42 days old and lasted for only 21 days.

Acute toxicity at 12°C

The data sheets and reports revealed the following concerning the information in Table 5:

a. The LC50 for the sheepshead minnow is a 48-hr LC50.
b. The data sheets say that the crayfish used was *Astacus pallipes*. Subsequently, the authors said that it was later determined that the crayfish used was *Procambarus clarkii*.

Some of these tests were conducted in a laboratory dilution water and some were conducted in a well water; these were the two waters used in the determination of the Water-Effect Ratios (see above).

Effect of temperature on the toxicity of ammonia

The data sheets, reports, and publication revealed the following concerning the acute values in Table 6:

1. A comparison is not possible for the dragonfly because both of the values are "greater than" values.

2. The two acute tests with the bluegill were conducted in different waters.
3. One of the chronic tests with the bluegill lasted for 14 days, whereas the other lasted for 21 days. The concentration of dissolved oxygen was below 40 percent of saturation for a portion of the 14-day test.
4. For the amphipod, the LC50 at 12°C is a 96-hr LC50, whereas the LC50 at 20°C is a 21-day LC50. In the 21-day test, the concentration of dissolved oxygen was below 50 percent of saturation for a portion of the test.
5. The two tests with crayfish were conducted in different waters. In the test at 20°C, the concentration of dissolved oxygen was below 40 percent of saturation for a portion of the test. The LC50 at 12°C was ">2.35" as reported in Table 5, not "2.35" as reported in Table 6.
6. The NOEC of 0.44 mg/L given in Table 6 for the leopard frog at 12°C is from a test with the spring peeper.
7. The concentration of dissolved oxygen was above 110 percent of saturation for a portion of one of the tests with the bay silverside.
8. The LC50 given in Table 6 for the spring peeper at 20°C is a 9-day LC50, whereas the value at 12°C is a 96-hr LC50. Because the 9-day LC50 at 20°C is greater than the 96-hr LC50 at 12°C, a qualitative comparison is possible.

Valid comparisons of 12 versus 20°C can be made only for the two amphibians.

The data sheets, reports, and publication revealed the following concerning the chronic tests that are the basis of the results in Table 6:

The three chronic tests at 20°C were addressed above.

Bluegill at 12°C:

The concentration of dissolved oxygen was below 40 percent of saturation for a portion of the test. In addition, this test was begun with juveniles and lasted for only 14 days. (The durations of the chronic tests with the bluegill in warm and cold water are switched in Table 1.)

The chronic comparison with the bluegill is based on a 21-day test and a 14-day test. In addition, the concentration of dissolved oxygen was below 40 percent of saturation during a portion of the test at 12°C.

Amphipod (*Crangonyx* spp.) at 12°C:

The concentration of dissolved oxygen was below 40 percent of saturation for a portion of the test. In addition, this test was begun with juveniles and lasted for only 21 days.

In both of the chronic tests used in the chronic comparison with the amphipod, the concentration of dissolved oxygen was below 40 percent of saturation during a portion of the test.

Leopard frog at 12°C:

This chronic test was conducted with the spring peeper, not the leopard frog. The concentration of dissolved oxygen was above 110 percent of saturation for a portion of the test. In addition, this test was begun seven days after hatch and lasted for only 21 days.

The chronic comparison with the leopard frog is based on a chronic test conducted with the leopard frog and a chronic test conducted with the spring peeper.

Appendix 2. Methods for Regression Analysis of pH Data

Analysis of the available data relating ammonia toxicity to pH using Equations 8 and 9 requires recognition that, unlike usual regression analysis with one response variable, two response variables (i.e., $LC50_u$ and $LC50_i$) are of concern here. Suitable analysis requires some assumptions about the correlations among these response variables (Box and Draper 1965; Box et al. 1973; Draper and Smith 1981). If the correlations among the data are known, Box and Draper (1965) indicate that regression analysis should involve minimization of the quantity:

$$z = \sum_{i=1}^{k} \sum_{j=1}^{k} \sigma_{ij} v_{ij}$$

$$v_{ij} = \sum_{u=1}^{n} [y_{iu} - f(x_{iu}, \theta)] [y_{ju} - f(x_{ju}, \theta)] \qquad \textbf{(22)}$$

where k is the number of dependent variables, n is the number of datapoints, y_{iu} is the observed value for the dependent variable i, and $f(x_{iu}, \theta)$ is the model prediction of the value of the dependent variable i. If correlation coefficients are zero, Equation 22 reduces to standard least squares regression techniques. However, when correlations are unknown, Box and Draper (1965) indicate that the determinant of the matrix of v_{ij}s should be minimized; this results in a formulation similar to Equation 22, but with weights calculated from relationships within the data rather than from a priori knowledge or assumptions regarding variances. If linear relationships exist among the dependent variables, further refinements are necessary (Box et al. 1973). Before using these more complicated techniques, which might have rather minimal impact on parameter estimates, consideration was first given to what could be assumed about the correlations of the errors in $LC50_u$ and $LC50_i$.

Because $LC50_u$ and $LC50_i$ are both derived from $LC50_t$ based on chemical equilibrium equations (i.e., Equation 4), it might be thought that their errors are directly correlated and proportional to that of $LC50_t$. However, uncertainty also exists in the equilibrium fractions, mainly from uncertainty in pH, and this results in errors that are inversely correlated. Lacking any definitive resolution of the degree of correlation, simulations were run to determine whether methods assuming no correlation would produce acceptable results. As mentioned

above, this assumption results in applying standard least squares regression techniques to Equations 8 and 9.

For assumed parameter values $LC50_8$=1.0, pH_T=7.5, R=0.01, and σ=0.1, four sets of 1000 simulations were run in which hypothetical datasets were randomly generated and analyzed. The four sets differed based on a 2x2 arrangement of two factors, each with two options. One factor was the size of the dataset - both small (n=5 with pH ranging from 6.5 to 8.5 at 0.5 intervals) and large (n=13 with pH ranging from 6.0 to 9.0 at 0.25 intervals) datasets were run. The other factor was the true correlation between the errors in $logLC50_u$ and $logLC50_i$: one option had the correlation coefficient = 0 (which met analysis assumptions) and the other had the correlation coefficient = 1 (which violated analysis assumptions as much as possible). Estimates of the standard errors of the parameters were based on the covariance matrix computed from the residual error and inverse Jacobian at the least squares solution; confidence limits were computed as the product of this standard error and the applicable t-statistic.

These simulations and their results are summarized in Table 4. Parameter values were found to be unbiased in all cases. When true errors were uncorrelated, as assumed in the procedure, the estimated parameter standard errors were unbiased relative to the standard deviations of the estimated parameter values, and the confidence limits were 95% using 2n-3 degrees of freedom. When true errors were correlated, the estimated parameter standard errors were biased, averaging 11 to 33% less than the observed error in the estimated parameter values, and the confidence limits were 80 to 89% rather than 95%. At the smallest sample size, the biases in the estimated errors were only 0.05 units for pK_T, 0.03 units for $log_{10}R$ (corresponding to 7% bias in the error for R), and 0.01 units for $log_{10}LC50_{t,8}$ (corresponding to only 2.5% bias in the error for $LC50_{t,8}$). Because these biases were relatively small, because the actual parameter estimates were unbiased, and because this analysis was under worst-case assumptions, standard regression methods with the assumption of no correlation of errors were adopted for the analysis of pH effects using Equations 8 and 9, rather than adopting more complicated methods.

Table 4. Results Obtained using Simulated Samples

Parameter	pK_T	$\log_{10}R$	$\log_{10}LC50_{t,8}$
True Value	7.5	-2.0	0.0
Simulations with 5 Treatments - Errors Uncorrelated			
Mean of Estimated Parameter Values	7.501	-1.994	-0.001
Standard Deviation of Estimated Parameter Values	0.104	0.123	0.050
Mean of Estimated Parameter Standard Errors	0.104	0.121	0.051
Simulated Confidence for Nominal 95% CL	95%	95%	96%
Simulations with 13 Treatments - Errors Uncorrelated			
Mean of Estimated Parameter Values	7.498	-2.000	-0.001
Standard Deviation of Estimated Parameter Values	0.057	0.068	0.030
Mean of Estimated Parameter Standard Errors	0.056	0.069	0.031
Simulated Confidence for Nominal 95% CL	94%	95%	95%
Simulations with 5 Treatments - Errors Correlated			
Mean of Estimated Parameter Values	7.499	-2.001	0.003
Standard Deviation of Estimated Parameter Values	0.145	0.146	0.058
Mean of Estimated Parameter Standard Errors	0.097	0.114	0.047
Simulated Confidence for Nominal 95% CL	80%	84%	86%
Simulations with 13 Treatments - Errors Correlated			
Mean of Estimated Parameter Values	7.501	-1.999	0.001
Standard Deviation of Estimated Parameter Values	0.079	0.079	0.034
Mean of Estimated Parameter Standard Errors	0.055	0.067	0.030
Simulated Confidence for Nominal 95% CL	82%	89%	89%

Appendix 3. Conversion of Results of Toxicity Tests

All of the acute values reported in Table 1 of the 1984/1985 ammonia criteria document (U.S. EPA 1985a) are expressed in terms of un-ionized ammonia at the pH of the toxicity test. For use in this 1998 Update, they were converted from un-ionized ammonia at the test pH to total ammonia nitrogen at pH=8. The conversion procedure is illustrated here using the data for the flatworm, *Dendrocoelum lacteum*, which is the first species in Table 1 in the 1984/1985 criteria document and is the first species in Appendix 4 in this 1998 Update:

Acute value (AV) = 1.40 mg NH_3/L
pH = 8.20
Temperature = 18.0°C

Step 1.
Equation 3 in this 1998 Update is used to calculate the pK at 18°C:

$$pK = 9.464905$$

Step 2.
Equation 2 in this update and the definitions $pK = -\log_{10}K$ and $pH = -\log_{10}[H^+]$ are used to obtain the following:

$$\frac{[NH_3]}{[NH_4^+]} = 10^{(pH-pK)} = 0.0543369$$

Step 3.
The AV in terms of total ammonia is calculated as:

$$\text{Total ammonia} = [NH_3] + [NH_4^+] = [NH_3] + \frac{[NH_3]}{0.0543369}$$

$$= 27.1652 \text{ mg total ammonia/L}$$

Step 4.
The AV in terms of total ammonia nitrogen is calculated as follows:

Total ammonia nitrogen = (27.1652 mg total ammonia/L)(14/17)
= 22.3713 mg N/L.

Step 5.
The AV in terms of total ammonia nitrogen is converted from pH=8.2 to pH=8 using equation 10 in this 1998 Update:

$$AV_{t,8} = (AV_t)/(0.681546) = 32.8244 \text{ mg N/L}$$

Because this is the only species in this genus for which data are in Table 1 in the 1984/1985 criteria document, 32.82 mg N/L is the GMAV given for the genus *Dendrocoelum* in Table 1 in this update.

Appendix 4. Acute Values[a]

Species	Un-ionized Ammonia (mg NH_3/L)	pH	Temp. (°C)	Total Ammonia (mg N/L)	Total Ammonia (mg N/L@pH8)	Reference
Dendrocoelum lacteum	1.40	8.20	18.0	22.37	32.82	Stammer 1953
Tubifex tubifex	2.70	8.20	12.0	66.67	97.82	Stammer 1953
Physa gyrina	1.59	8.00	4.0	114.93	114.87	West 1985
Physa gyrina	2.09	8.20	5.5	85.13	124.90	West 1985
Physa gyrina	2.49	8.10	12.1	76.29	92.27	West 1985
Physa gyrina	2.16	8.20	12.8	50.25	73.73	West 1985
Physa gyrina	1.78	8.00	13.3	62.39	62.36	West 1985
Physa gyrina	1.71	8.00	24.9	26.33	26.32	West 1985
Helisoma trivolvis	2.76	8.20	12.9	63.73	93.52	West 1985
Musculium transversum	0.93	8.20	5.4	38.18	56.02	West 1985
Musculium transversum	1.29	8.10	14.6	32.83	39.70	West 1985
Musculium transversum	1.10	8.60	20.5	6.43	20.38	West 1985
Ceriodaphnia acanthina	0.770	7.06	24.0	104.82	25.78	Mount 1982
Daphnia magna	2.08	8.20	25.0	20.71	30.38	Parkhurst et al. 1979,1981
Daphnia magna	2.45	7.95	22.0	51.30	46.68	Russo et al. 1985
Daphnia magna	2.69	8.07	19.6	51.09	58.33	Russo et al. 1985
Daphnia magna	2.50	8.09	20.9	41.51	49.25	Russo et al. 1985
Daphnia magna	2.77	8.15	22.0	37.44	49.86	Russo et al. 1985
Daphnia magna	2.38	8.04	22.8	38.70	41.73	Russo et al. 1985
Daphnia magna	0.75	7.51	20.1	48.32	20.72	Russo et al. 1985
Daphnia magna	0.90	7.53	20.1	55.41	24.49	Russo et al. 1985
Daphnia magna	0.53	7.40	20.6	42.31	15.48	Russo et al. 1985
Daphnia magna	0.67	7.50	20.3	43.52	18.39	Russo et al. 1985
Daphnia magna	4.94	8.34	19.7	51.92	100.02	Reinbold & Pescitelli 1982a
Daphnia pulicaria	1.16	8.05	14.0	34.50	37.91	DeGraeve et al. 1980
Simocephalus vetulus	0.613	7.06	24.0	83.45	20.52	Mount 1982
Simocephalus vetulus	2.29	8.30	17.0	31.58	56.29	West 1985
Asellus racovitzai	2.94	7.81	11.9	176.01	124.02	Thurston et al. 1983a
Asellus racovitzai	4.95	8.00	4.0	357.80	357.60	West 1985
Crangonyx pseudogracilis	2.76	8.00	4.0	199.50	199.39	West 1985

Crangonyx pseudogracilis	5.63	8.00	12.1	215.97	215.85	West 1985
Crangonyx pseudogracilis	3.56	8.20	13.0	81.60	119.73	West 1985
Crangonyx pseudogracilis	3.29	8.00	13.3	115.32	115.25	West 1985
Crangonyx pseudogracilis	1.63	8.00	24.9	25.10	25.08	West 1985
Orconectes nais	3.15	8.30	26.5	23.15	41.27	Evans 1979
Orconectes immunis	22.8	8.20	4.6	999.39	1466.35	West 1985
Callibaetis sp.	1.80	7.81	11.9	107.76	75.93	Thurston et al. 1984a
Callibaetis skokianus	4.82	7.90	13.3	211.66	175.56	West 1985
Ephemerella grandis	4.96	7.84	12.8	259.07	192.64	Thurston et al. 1984a
Ephemerella grandis	5.88	7.85	12.0	319.03	241.54	Thurston et al. 1984a
Ephemerella grandis	3.86	7.84	13.2	195.62	145.46	Thurston et al. 1984a
Arcynopteryx parallela	2.06	7.76	13.8	119.63	77.18	Thurston et al. 1984a
Arcynopteryx parallela	2.00	7.81	13.1	109.31	77.03	Thurston et al. 1984a
Philarctus quaeris	10.2	7.80	13.3	561.72	388.84	West 1985
Stenelmis sexlineata	8.00	8.70	25.0	29.69	113.17	Hazel et al. 1979
Oncorhynchus gorbuscha	0.083	6.40	4.3	230.47	38.33	Rice & Bailey 1980
Oncorhynchus gorbuscha	0.10	6.40	4.30	277.68	46.18	Rice & Bailey 1980
Oncorhynchus kisutch	0.272	7.00	15.0	82.02	19.10	Robinson-Wilson & Seim 1975
Oncorhynchus kisutch	0.280	7.00	15.0	84.43	19.66	Robinson-Wilson & Seim 1975
Oncorhynchus kisutch	0.550	7.50	15.0	52.76	22.29	Robinson-Wilson & Seim 1975
Oncorhynchus kisutch	0.528	7.50	15.0	50.65	21.40	Robinson-Wilson & Seim 1975
Oncorhynchus kisutch	0.712	8.00	15.0	22.00	21.99	Robinson-Wilson & Seim 1975
Oncorhynchus kisutch	0.700	8.00	15.0	21.63	21.62	Robinson-Wilson & Seim 1975
Oncorhynchus kisutch	0.880	8.50	15.0	9.09	23.86	Robinson-Wilson & Seim 1975
Oncorhynchus kisutch	0.55	8.10	17.2	11.59	14.02	Buckley 1978
Oncorhynchus tshawytscha	0.476	7.82	12.2	27.23	19.53	Thurston & Meyn 1984
Oncorhynchus tshawytscha	0.456	7.84	12.3	24.74	18.39	Thurston & Meyn 1984
Oncorhynchus tshawytscha	0.399	7.87	13.5	18.47	14.50	Thurston & Meyn 1984
Oncorhynchus aquabonita	0.755	8.06	13.2	23.30	26.10	Thurston & Russo 1981
Oncorhynchus clarki	0.80	7.81	13.1	43.72	30.81	Thurston et al. 1978

Oncorhynchus clarki	0.66	7.80	12.8	37.75	26.13	Thurston et al. 1978
Oncorhynchus clarki	0.62	7.80	12.4	36.55	25.30	Thurston et al. 1978
Oncorhynchus clarki	0.52	7.78	12.2	32.57	21.76	Thurston et al. 1978
Oncorhynchus mykiss	0.325	7.40	14.4	40.99	14.99	Calamari et al. 1977, 1981
Oncorhynchus mykiss	0.370	7.40	14.5	46.31	16.94	Calamari et al. 1977, 1981
Oncorhynchus mykiss	0.160	7.40	14.5	20.03	7.33	Calamari et al. 1977, 1981
Oncorhynchus mykiss	0.440	7.40	14.5	55.07	20.15	Calamari et al. 1977, 1981
Oncorhynchus mykiss	0.697	7.95	10.0	35.14	31.97	Broderius & Smith 1979
Oncorhynchus mykiss	0.40	7.50	15.0	38.37	16.21	Holt & Malcolm 1979
Oncorhynchus mykiss	0.77	8.05	14.0	22.90	25.17	DeGraeve et al. 1980
Oncorhynchus mykiss	0.436	7.90	12.7	20.03	16.61	Thurston & Russo 1983
Oncorhynchus mykiss	0.446	7.90	13.4	19.44	16.12	Thurston & Russo 1983
Oncorhynchus mykiss	0.478	7.91	13.0	20.99	17.73	Thurston & Russo 1983
Oncorhynchus mykiss	0.291	7.91	13.1	12.68	10.71	Thurston & Russo 1983
Oncorhynchus mykiss	0.232	7.88	12.8	11.07	8.85	Thurston & Russo 1983
Oncorhynchus mykiss	0.336	7.88	12.9	15.91	12.72	Thurston & Russo 1983
Oncorhynchus mykiss	0.347	7.87	12.9	16.81	13.19	Thurston & Russo 1983
Oncorhynchus mykiss	0.474	7.95	12.5	19.75	17.97	Thurston & Russo 1983
Oncorhynchus mykiss	0.440	7.87	13.0	21.15	16.61	Thurston & Russo 1983
Oncorhynchus mykiss	0.392	7.87	12.9	18.99	14.91	Thurston & Russo 1983
Oncorhynchus mykiss	0.426	7.88	13.4	19.43	15.53	Thurston & Russo 1983
Oncorhynchus mykiss	0.400	7.87	13.1	19.08	14.98	Thurston & Russo 1983
Oncorhynchus mykiss	0.497	7.86	13.4	23.71	18.28	Thurston & Russo 1983
Oncorhynchus mykiss	0.421	7.86	13.0	20.70	15.96	Thurston & Russo 1983
Oncorhynchus mykiss	0.758	8.08	12.8	23.05	26.82	Thurston & Russo 1983
Oncorhynchus mykiss	0.572	7.86	12.7	28.77	22.18	Thurston & Russo 1983
Oncorhynchus mykiss	0.570	7.85	12.5	29.77	22.54	Thurston & Russo 1983
Oncorhynchus mykiss	0.673	7.85	13.1	33.59	25.44	Thurston & Russo 1983
Oncorhynchus mykiss	1.09	8.06	13.2	33.64	37.68	Thurston & Russo 1983
Oncorhynchus mykiss	0.641	7.85	12.3	33.99	25.74	Thurston & Russo 1983
Oncorhynchus mykiss	0.696	7.79	12.4	41.97	28.55	Thurston & Russo 1983
Oncorhynchus mykiss	0.772	7.86	14.1	34.95	26.94	Thurston & Russo 1983
Oncorhynchus mykiss	0.683	7.84	13.8	33.09	24.60	Thurston & Russo 1983
Oncorhynchus mykiss	0.812	7.80	12.4	47.87	33.14	Thurston & Russo 1983

Oncorhynchus mykiss	0.632	7.85	13.1	31.55	23.89	Thurston & Russo 1983
Oncorhynchus mykiss	0.618	7.87	12.1	31.80	24.97	Thurston & Russo 1983
Oncorhynchus mykiss	0.410	7.71	11.4	32.02	18.95	Thurston & Russo 1983
Oncorhynchus mykiss	0.390	7.71	11.5	30.22	17.89	Thurston & Russo 1983
Oncorhynchus mykiss	0.752	7.84	13.0	38.69	28.77	Thurston & Russo 1983
Oncorhynchus mykiss	0.662	7.83	13.5	33.55	24.50	Thurston & Russo 1983
Oncorhynchus mykiss	0.763	7.80	13.3	42.02	29.09	Thurston & Russo 1983
Oncorhynchus mykiss	0.250	7.44	12.8	32.49	12.57	Thurston & Russo 1983
Oncorhynchus mykiss	0.449	7.84	12.2	24.54	18.25	Thurston & Russo 1983
Oncorhynchus mykiss	0.392	7.87	12.2	20.02	15.72	Thurston & Russo 1983
Oncorhynchus mykiss	0.464	7.90	11.9	22.65	18.79	Thurston & Russo 1983
Oncorhynchus mykiss	0.243	7.50	14.5	24.20	10.22	Thurston & Russo 1983
Oncorhynchus mykiss	0.635	7.82	13.2	33.67	24.15	Thurston & Russo 1983
Oncorhynchus mykiss	0.510	7.75	12.3	33.94	21.52	Thurston & Russo 1983
Oncorhynchus mykiss	0.623	7.84	12.9	32.30	24.01	Thurston & Russo 1983
Oncorhynchus mykiss	0.833	7.90	13.0	37.41	31.03	Thurston & Russo 1983
Oncorhynchus mykiss	0.432	7.70	13.9	28.54	16.60	Thurston & Russo 1983
Oncorhynchus mykiss	0.796	7.90	13.0	35.75	29.65	Thurston & Russo 1983
Oncorhynchus mykiss	0.714	7.87	13.0	34.32	26.95	Thurston & Russo 1983
Oncorhynchus mykiss	0.326	7.80	9.7	23.65	16.37	Thurston & Russo 1983
Oncorhynchus mykiss	0.404	7.65	14.3	29.02	15.53	Thurston & Russo 1983
Oncorhynchus mykiss	0.389	7.67	14.0	27.30	15.11	Thurston & Russo 1983
Oncorhynchus mykiss	0.375	7.62	14.4	28.62	14.58	Thurston & Russo 1983
Oncorhynchus mykiss	0.364	7.64	13.1	29.28	15.42	Thurston & Russo 1983
Oncorhynchus mykiss	0.382	7.66	13.6	28.27	15.38	Thurston & Russo 1983
Oncorhynchus mykiss	0.367	7.65	13.2	28.64	15.33	Thurston & Russo 1983
Oncorhynchus mykiss	0.392	7.69	13.4	27.51	15.74	Thurston & Russo 1983
Oncorhynchus mykiss	0.281	7.60	12.9	25.14	12.40	Thurston & Russo 1983
Oncorhynchus mykiss	0.456	7.75	11.8	31.53	19.99	Thurston & Russo 1983
Oncorhynchus mykiss	0.432	7.66	12.8	33.97	18.48	Thurston & Russo 1983
Oncorhynchus mykiss	0.268	7.60	13.0	23.80	11.74	Thurston & Russo 1983
Oncorhynchus mykiss	0.307	7.63	12.9	25.65	13.29	Thurston & Russo 1983
Oncorhynchus mykiss	0.351	7.59	12.7	32.62	15.84	Thurston & Russo 1983
Oncorhynchus mykiss	0.448	7.68	13.0	33.15	18.65	Thurston & Russo 1983

Oncorhynchus mykiss	0.552	7.77	13.6	31.81	20.89	Thurston & Russo 1983
Oncorhynchus mykiss	0.580	7.86	10.2	35.31	27.23	Thurston & Russo 1983
Oncorhynchus mykiss	0.484	7.88	10.0	28.60	22.87	Thurston & Russo 1983
Oncorhynchus mykiss	0.297	7.69	10.7	25.62	14.66	Thurston & Russo 1983
Oncorhynchus mykiss	0.327	7.74	10.4	25.76	16.05	Thurston & Russo 1983
Oncorhynchus mykiss	0.289	7.76	10.0	22.44	14.47	Thurston & Russo 1983
Oncorhynchus mykiss	0.262	7.66	9.80	25.95	14.12	Thurston & Russo 1983
Oncorhynchus mykiss	0.312	7.64	10.0	31.85	16.77	Thurston & Russo 1983
Oncorhynchus mykiss	0.201	7.69	10.4	17.75	10.15	Thurston & Russo 1983
Oncorhynchus mykiss	0.234	7.69	10.7	20.18	11.55	Thurston & Russo 1983
Oncorhynchus mykiss	0.249	7.64	9.8	25.82	13.59	Thurston & Russo 1983
Oncorhynchus mykiss	0.192	7.65	9.8	19.46	10.41	Thurston & Russo 1983
Oncorhynchus mykiss	0.163	7.62	7.9	20.53	10.46	Thurston & Russo 1983
Oncorhynchus mykiss	0.677	8.10	13.9	18.14	21.94	Thurston & Russo 1983
Oncorhynchus mykiss	0.662	8.12	13.6	17.34	21.80	Thurston & Russo 1983
Oncorhynchus mykiss	0.636	7.94	12.8	26.49	23.66	Thurston & Russo 1983
Oncorhynchus mykiss	0.694	7.98	12.5	27.02	26.01	Thurston & Russo 1983
Oncorhynchus mykiss	0.764	7.89	12.4	36.73	29.91	Thurston & Russo 1983
Oncorhynchus mykiss	0.921	7.94	12.5	39.25	35.05	Thurston & Russo 1983
Oncorhynchus mykiss	0.856	7.85	16.1	34.17	25.87	Thurston & Russo 1983
Oncorhynchus mykiss	0.801	7.88	16.7	28.60	22.87	Thurston & Russo 1983
Oncorhynchus mykiss	0.897	7.91	19.0	25.36	21.42	Thurston & Russo 1983
Oncorhynchus mykiss	0.942	7.91	19.1	26.44	22.34	Thurston & Russo 1983
Oncorhynchus mykiss	0.931	7.96	19.2	23.21	21.52	Thurston & Russo 1983
Oncorhynchus mykiss	0.158	6.51	14.1	157.35	27.18	Thurston et al. 1981c
Oncorhynchus mykiss	0.184	6.80	14.1	94.05	18.82	Thurston et al. 1981c
Oncorhynchus mykiss	0.454	7.30	14.0	74.20	23.78	Thurston et al. 1981c
Oncorhynchus mykiss	0.799	8.29	14.1	13.85	24.21	Thurston et al. 1981c
Oncorhynchus mykiss	0.684	8.82	13.9	3.95	18.62	Thurston et al. 1981c
Oncorhynchus mykiss	0.648	9.01	14.5	2.51	16.19	Thurston et al. 1981c
Oncorhynchus mykiss	0.683	7.83	12.8	36.49	26.65	Thurston et al. 1981c
Oncorhynchus mykiss	0.704	7.79	12.9	40.88	27.80	Thurston et al. 1981c
Oncorhynchus mykiss	0.564	7.75	12.5	36.97	23.44	Thurston et al. 1981c
Oncorhynchus mykiss	0.610	7.76	12.5	39.08	25.22	Thurston et al. 1981c

Oncorhynchus mykiss	0.497	7.75	12.7	32.09	20.34	Thurston et al. 1981c
Oncorhynchus mykiss	0.643	7.75	13.0	40.58	25.73	Thurston et al. 1981c
Oncorhynchus mykiss	0.56	8.34	5.0	17.32	33.37	Reinbold & Pescitelli 1982b
Oncorhynchus mykiss	0.79	8.28	12.8	15.40	26.39	Reinbold & Pescitelli 1982b
Oncorhynchus mykiss	0.40	8.43	3.0	11.86	27.20	Reinbold & Pescitelli 1982b
Oncorhynchus mykiss	1.02	8.16	14.2	23.39	31.76	Reinbold & Pescitelli 1982b
Oncorhynchus mykiss	0.77	8.60	3.3	15.27	48.41	Reinbold & Pescitelli 1982b
Oncorhynchus mykiss	0.97	8.50	14.9	10.09	26.48	Reinbold & Pescitelli 1982b
Oncorhynchus mykiss	0.26	7.70	3.6	38.52	22.41	West 1985
Oncorhynchus mykiss	0.61	7.70	9.8	55.15	32.09	West 1985
Oncorhynchus mykiss	0.59	7.90	11.3	30.15	25.01	West 1985
Oncorhynchus mykiss	0.43	7.90	16.2	15.23	12.63	West 1985
Oncorhynchus mykiss	1.04	8.30	18.7	12.75	22.72	West 1985
Salmo trutta	0.701	7.86	13.8	32.46	25.02	Thurston & Meyn 1984
Salmo trutta	0.677	7.82	14.2	33.30	23.89	Thurston & Meyn 1984
Salmo trutta	0.597	7.85	13.2	29.58	22.39	Thurston & Meyn 1984
Salvelinus fontinalis	1.05	7.83	13.8	52.03	38.00	Thurston & Meyn 1984
Salvelinus fontinalis	0.962	7.86	13.6	45.21	34.86	Thurston & Meyn 1984
Prosopium williamsoni	0.473	7.84	12.4	25.47	18.94	Thurston & Meyn 1984
Prosopium williamsoni	0.358	7.80	12.3	21.27	14.72	Thurston & Meyn 1984
Prosopium williamsoni	0.143	7.68	12.1	11.33	6.38	Thurston & Meyn 1984
Notemigonus crysoleucas	0.72	7.50	24.5	34.73	14.67	Thurston & Meyn 1984
Notropis lutrensis	2.83	8.30	24.0	24.37	43.43	Hazel et al. 1979
Notropis lutrensis	3.16	9.10	24.0	6.50	47.99	Hazel et al. 1979
Notropis spilopterus	1.20	7.95	26.5	18.52	16.85	Rosage et al. 1979
Notropis spilopterus	1.62	8.15	26.5	16.27	21.67	Rosage et al. 1979
Notropis spilopterus	1.35	7.90	25.7	24.52	20.34	Swigert & Spacie 1983
Notropis whipplei	1.25	7.90	25.7	22.71	18.83	Swigert & Spacie 1983
Campostoma anomalum	1.72	7.80	25.7	38.97	26.97	Swigert & Spacie 1983
Pimephales promelas	1.59	8.05	14.0	47.29	51.97	DeGraeve et al. 1980
Pimephales promelas	1.50	7.91	16.3	51.55	43.55	Thurston et al. 1983
Pimephales promelas	1.10	7.89	13.1	50.16	40.85	Thurston et al. 1983
Pimephales promelas	0.754	7.64	13.6	58.40	30.74	Thurston et al. 1983
Pimephales promelas	0.908	7.68	13.5	64.69	36.40	Thurston et al. 1983

Pimephales promelas	2.73	8.03	22.1	47.60	50.35	Thurston et al. 1983
Pimephales promelas	2.59	8.06	22.0	42.58	47.69	Thurston et al. 1983
Pimephales promelas	0.832	7.67	13.9	58.84	32.55	Thurston et al. 1983
Pimephales promelas	2.33	8.05	13.0	74.65	82.04	Thurston et al. 1983
Pimephales promelas	2.17	8.05	13.6	66.48	73.06	Thurston et al. 1983
Pimephales promelas	1.61	7.94	19.1	42.26	37.75	Thurston et al. 1983
Pimephales promelas	1.27	7.76	19.0	50.28	32.44	Thurston et al. 1983
Pimephales promelas	0.775	7.66	13.4	58.23	31.68	Thurston et al. 1983
Pimephales promelas	1.51	7.87	15.8	58.91	46.25	Thurston et al. 1983
Pimephales promelas	1.85	7.83	22.0	50.58	36.94	Thurston et al. 1983
Pimephales promelas	1.73	7.91	18.9	49.26	41.62	Thurston et al. 1983
Pimephales promelas	1.22	7.77	14.3	66.71	43.80	Thurston et al. 1983
Pimephales promelas	1.31	7.77	14.1	72.71	47.74	Thurston et al. 1983
Pimephales promelas	2.16	8.04	22.2	36.59	39.45	Thurston et al. 1983
Pimephales promelas	2.73	8.08	21.4	44.76	52.10	Thurston et al. 1983
Pimephales promelas	3.44	8.16	21.4	47.39	64.35	Thurston et al. 1983
Pimephales promelas	2.04	7.88	21.7	50.95	40.74	Thurston et al. 1983
Pimephales promelas	1.23	7.68	12.9	91.71	51.60	Thurston et al. 1983
Pimephales promelas	1.10	7.63	13.2	89.85	46.53	Thurston et al. 1983
Pimephales promelas	1.73	7.76	12.9	107.53	69.38	Thurston et al. 1983
Pimephales promelas	2.03	7.84	21.7	55.43	41.22	Thurston et al. 1983
Pimephales promelas	1.09	7.76	13.1	66.73	43.05	Thurston et al. 1983
Pimephales promelas	0.796	7.74	12.8	52.17	32.51	Thurston et al. 1983
Pimephales promelas	1.34	7.91	15.9	47.43	40.07	Thurston et al. 1983
Pimephales promelas	0.240	6.51	13.0	259.96	44.91	Thurston et al. 1981c
Pimephales promelas	0.452	7.01	13.8	145.89	34.27	Thurston et al. 1981c
Pimephales promelas	1.08	7.82	12.0	62.72	45.00	Thurston et al. 1981c
Pimephales promelas	0.793	7.83	11.8	45.71	33.39	Thurston et al. 1981c
Pimephales promelas	1.68	8.51	13.5	18.88	50.50	Thurston et al. 1981c
Pimephales promelas	1.47	9.03	13.2	5.94	39.51	Thurston et al. 1981c
Pimephales promelas	0.73	8.46	4.1	18.54	45.05	Reinbold & Pescitelli 1982b
Pimephales promelas	1.24	8.02	23.9	19.55	20.29	Reinbold & Pescitelli 1982b
Pimephales promelas	0.80	8.26	4.6	30.57	50.41	Reinbold & Pescitelli 1982b
Pimephales promelas	1.65	8.16	25.2	17.65	23.96	Reinbold & Pescitelli 1982b

Pimephales promelas	1.75	7.78	25.9	40.89	27.32	Swigert & Spacie 1983
Pimephales promelas	1.87	7.80	25.6	42.65	29.53	Swigert & Spacie 1983
Pimephales promelas	2.41	7.90	3.4	229.72	190.54	West 1985
Pimephales promelas	1.83	8.10	12.1	56.07	67.81	West 1985
Pimephales promelas	1.97	8.00	17.1	52.22	52.19	West 1985
Pimephales promelas	2.55	8.10	26.1	29.23	35.35	West 1985
Catostomus commersoni	1.40	8.16	15.0	30.28	41.11	Reinbold & Pescitelli 1982c
Catostomus commersoni	1.35	8.14	15.4	29.65	38.73	Reinbold & Pescitelli 1982c
Catostomus commersoni	0.79	7.80	22.5	22.30	15.44	Swigert & Spacie 1983
Catostomus commersoni	0.76	7.80	3.6	89.57	62.00	West 1985
Catostomus commersoni	1.87	8.10	11.3	60.86	73.60	West 1985
Catostomus commersoni	1.73	8.20	12.6	40.85	59.94	West 1985
Catostomus commersoni	2.22	8.20	15.3	43.01	63.10	West 1985
Catostomus platyrhynchus	0.819	7.67	12.0	66.91	37.02	Thurston & Meyn 1984
Catostomus platyrhynchus	0.708	7.73	11.7	51.62	31.62	Thurston & Meyn 1984
Catostomus platyrhynchus	0.668	7.69	13.2	47.59	27.23	Thurston & Meyn 1984
Ictalurus punctatus	2.4	8.70	22.0	10.56	40.26	Colt & Tchobanoglous 1976
Ictalurus punctatus	2.9	8.70	26.0	10.19	38.85	Colt & Tchobanoglous 1976
Ictalurus punctatus	3.8	8.70	30.0	10.88	41.47	Colt & Tchobanoglous 1976
Ictalurus punctatus	1.95	8.40	28.0	10.71	23.19	Colt & Tchobanoglous 1978
Ictalurus punctatus	2.1	8.09	22.0	32.33	38.36	Roseboom & Richey 1977
Ictalurus punctatus	4.2	8.08	28.0	44.44	51.72	Roseboom & Richey 1977
Ictalurus punctatus	1.76	7.98	23.8	30.49	29.35	Reinbold & Pescitelli 1982b
Ictalurus punctatus	1.75	7.94	23.8	33.10	29.57	Reinbold & Pescitelli 1982b
Ictalurus punctatus	1.45	7.80	25.7	32.85	22.74	Swigert & Spacie 1983
Ictalurus punctatus	0.50	8.00	3.5	37.64	37.61	West 1985
Ictalurus punctatus	0.98	8.10	14.6	24.94	30.16	West 1985
Ictalurus punctatus	1.91	8.10	17.0	40.83	49.38	West 1985
Ictalurus punctatus	1.29	7.80	19.6	44.71	30.95	West 1985
Ictalurus punctatus	2.26	8.00	26.0	32.34	32.32	West 1985
Gambusia affinis	2.6	8.00	24.0	42.53	42.51	Wallen et al. 1957
Gambusia affinis	2.4	8.20	19.5	34.54	50.68	Wallen et al. 1957
Gambusia affinis	3.2	7.75	19.0	129.59	82.17	Wallen et al. 1957
Gambusia affinis	2.4	8.50	23.0	14.64	38.41	Wallen et al. 1957

Poecilia reticulata	1.47	7.22	25.0	129.40	37.66	Rubin & Elmaraghy 1976, 1977
Poecilia reticulata	1.59	7.45	25.0	82.95	32.56	Rubin & Elmaraghy 1976, 1977
Poecilia reticulata	1.45	7.45	25.0	75.65	29.69	Rubin & Elmaraghy 1976, 1977
Morone americana	0.15	6.00	16.0	418.44	63.94	Stevenson 1977
Morone americana	0.52	8.00	16.0	14.93	14.92	Stevenson 1977
Lepomis cyanellus	0.61	7.84	12.3	33.09	24.61	Jude 1973
Lepomis cyanellus	1.08	8.28	26.2	8.43	14.45	Reinbold & Pescitelli 1982a
Lepomis cyanellus	0.594	6.61	22.4	254.49	45.86	McCormick et al. 1984
Lepomis cyanellus	1.29	7.20	22.4	142.85	40.64	McCormick et al. 1984
Lepomis cyanellus	1.64	7.72	22.4	55.79	33.59	McCormick et al. 1984
Lepomis cyanellus	2.11	8.69	22.4	9.24	34.60	McCormick et al. 1984
Lepomis gibbosus	0.14	7.77	12.0	9.11	5.98	Jude 1973
Lepomis gibbosus	0.78	7.77	14.5	42.02	27.59	Thurston 1981
Lepomis gibbosus	0.86	7.77	14.0	48.09	31.58	Thurston 1981
Lepomis gibbosus	0.61	7.71	15.7	34.43	20.38	Thurston 1981
Lepomis macrochirus	0.89	8.11	18.5	16.73	20.62	Emery & Welch 1969
Lepomis macrochirus	2.97	8.24	18.5	42.01	66.62	Emery & Welch 1969
Lepomis macrochirus	2.57	8.75	18.5	12.70	52.95	Emery & Welch 1969
Lepomis macrochirus	0.55	8.07	22.0	8.85	10.10	Roseboom & Richey 1977
Lepomis macrochirus	0.68	8.00	22.0	12.75	12.74	Roseboom & Richey 1977
Lepomis macrochirus	1.1	7.93	22.0	24.08	21.11	Roseboom & Richey 1977
Lepomis macrochirus	1.8	8.20	28.0	14.81	21.72	Roseboom & Richey 1977
Lepomis macrochirus	0.50	8.40	4.0	14.64	31.68	Reinbold & Pescitelli 1982b
Lepomis macrochirus	1.98	8.12	25.0	23.37	29.37	Reinbold & Pescitelli 1982b
Lepomis macrochirus	0.26	8.16	4.5	12.55	17.04	Reinbold & Pescitelli 1982b
Lepomis macrochirus	1.35	8.09	24.8	17.22	20.43	Reinbold & Pescitelli 1982b
Lepomis macrochirus	0.94	7.60	21.7	44.03	21.72	Smith et al. 1983
Lepomis macrochirus	1.35	7.80	24.2	33.88	23.45	Swigert & Spacie 1983
Lepomis macrochirus	1.75	7.60	26.5	58.69	28.95	Swigert & Spacie 1983
Lepomis macrochirus	1.76	7.80	26.6	37.52	25.97	Swigert & Spacie 1983
Micropterus dolomieu	0.694	6.53	22.3	359.93	62.67	Broderius et al. 1985
Micropterus dolomieu	1.01	7.16	22.3	123.43	33.60	Broderius et al. 1985

Micropterus dolomieu	1.20	7.74	22.3	39.30	24.49	Broderius et al. 1985
Micropterus dolomieu	1.78	8.71	22.3	7.56	29.33	Broderius et al. 1985
Micropterus salmoides	1.0	7.96	22.0	20.48	18.99	Roseboom & Richey 1977
Micropterus salmoides	1.7	8.04	28.0	19.59	21.12	Roseboom & Richey 1977
Etheostoma spectabile	0.90	8.40	21.0	7.65	16.55	Hazel et al. 1979
Etheostoma spectabile	1.07	8.10	22.0	16.12	19.49	Hazel et al. 1979
Stizostedion vitreum	0.85	8.08	18.2	17.43	20.29	Reinbold & Pescitelli 1982a
Stizostedion vitreum	0.52	7.90	3.7	48.37	40.12	West 1985
Stizostedion vitreum	1.10	7.70	11.1	89.93	52.33	West 1985
Stizostedion vitreum	0.51	8.30	19.0	6.12	10.91	West 1985
Cottus bairdi	1.39	8.02	12.4	49.83	51.73	Thurston & Russo 1981

[a] The species and tests are in the same order as in Table 1 in the 1984/1985 ammonia criteria document. The scientific names of various salmonids have been updated. Two values for the rainbow trout by Calamari et al. (1977,1981) were deleted because they were "greater than" values; this had no effect on the FAV because the SMAV for rainbow trout was lowered to protect large rainbow trout (see Table 1 in this 1998 Update). A few values for pH and temperature were corrected and ranges were replaced with point estimates to facilitate conversion of acute values from un-ionized ammonia at the test pH to total ammonia nitrogen at pH=8.

Appendix 5. Histopathological Effects

Fewer results of the effects of chronic exposure of aquatic life to ammonia are available than results of the effects of acute exposures. The available data indicate that ammonia can have adverse effects on aquatic life at relatively low concentrations, approaching 0.001 to 0.006 mg NH_3-N/L. These reported adverse effects include quantitative data showing that decreased survival, growth, and reproduction are correlated to increasing concentrations of ammonia. These more conventional measures of chronic toxicity are generally regarded as a suitable basis for projecting the potential chronic toxic effects of pollutants, including ammonia, to aquatic life populations and communities.

In addition to the reported chronic toxic effects of ammonia to aquatic life based on these more conventional measures, the literature contains some information concerning the effects that chronic exposure to low levels of ammonia can have on the structure and function of select tissues and organs. These include reduced swimming stamina and performance, increased respiratory distress, hormonal dysfunction, and damage to gill, kidney, brain, and liver tissues. Some investigators have reported other pathological changes in the test animals' physiology, histochemistry, and biochemistry. None of these reported abnormalities in test organisms have been quantitatively correlated with the ammonia exposure or with effects on the survival, growth, or reproduction of the test organisms; potential adverse effects on populations and communities are unavailable.

Salmonid species subjected to un-ionized ammonia concentrations ranging from 0.002 mg NH_3-N/L at pH=6.4 to 0.06 mg NH_3-N/L at pH=7.7 on a chronic exposure basis have demonstrated significant effects on growth. Rice and Bailey (1980) observed growth effects on pink salmon embryos and fry when un-ionized ammonia exceeded 0.002 to 0.003 mg NH_3-N/L at pH=6.4. Burkhalter and Kaya (1977) observed that un-ionized ammonia concentrations somewhat less than 0.05 mg NH_3-N/L at pH=7.5 inhibited growth rates of rainbow trout embryos and fry. Samylin (1969), in tests with Atlantic salmon embryos and fry, reported effects on growth rates when un-ionized ammonia exceeded 0.06 mg NH_3-N/L at pH=7.1. The calculated "no apparent effect" concentrations for these tests are 0.002 mg NH_3-N/L at pH=6.4 for pink salmon, 0.008 mg NH_3-N/L at pH=7.1 for the Atlantic salmon, and less than 0.05 mg NH_3-N/L at pH=7.5 for the rainbow trout. Non-salmonid fish species have exhibited similar effects, with the calculated "no apparent growth effect" concentrations ranging from 0.03 mg NH_3-N/L at pH=6.6 to 0.05 mg NH_3-N/L at pH=8.68. Reported growth

effect concentrations were 0.11 mg NH_3-N/L at pH=7.78 for the bluegill (Smith et al. 1984), 0.32 mg NH_3-N/L at pH=7.95 for the channel catfish (Reinbold and Pescitelli 1982a), and 0.40 mg NH_3-N/L at pH=7.9 for the green sunfish (McCormick et al. 1984). Broderius et al. (1985), in tests with smallmouth bass, observed that the growth effects of un-ionized ammonia were not constant with pH. The growth effect concentrations ranged from 0.05 mg NH_3-N/L at pH=6.6 to 0.71 mg NH_3-N/L at pH=8.68. Thurston et al. (1986) reported the results of life-cycle tests with the fathead minnow. The tested un-ionized ammonia concentrations ranged from 0.07 to 0.96 mg NH_3-N/L at pH=8.0. No effects on growth or survival of parental fish were reported at 0.44 mg NH_3-N/L, or on embryo viability or production up to 0.37 mg NH_3-N/L; adverse effects were reported for all of these endpoints at 0.91 mg NH_3-N/L. First filial generation animals did not demonstrate any adverse effects on growth or survival at 0.36 mg NH_3-N/L, the highest tested concentration. Embryo hatching success was adversely affected at 0.37 mg NH_3-N/L but not at 0.19 mg NH_3-N/L. Parental fish and first filial generation fish exhibited a high incidence of brain lesions at an un-ionized ammonia concentration of 0.21 mg NH_3-N/L, but not at 0.11 mg NH_3-N/L.

Histopathological effects of chronic exposure of rainbow trout to un-ionized ammonia are evident within the range of un-ionized concentrations producing effects on growth. Calamari et al. (1977,1981) observed alterations of the epidermis of newly hatched rainbow trout fry exposed to un-ionized ammonia concentrations of 0.02 mg NH_3-N/L and greater at pH=7.4 for 21 to 24 days. Concentrations of 0.06 mg NH_3-N/L and greater at pH=7.4 produced pathological alterations of kidney tissues of newly hatched rainbow trout fry. Increases in the severity of these pathological states corresponded to increasing un-ionized ammonia concentrations; fifty percent mortality was reported with animals exposed to concentrations of 0.06 mg NH_3-N/L and greater at pH=7.4 for 72 days (Calamari et al. 1977,1981).

Thurston et al. (1984b) exposed rainbow trout to five concentrations of un-ionized ammonia ranging from 0.008 to 0.06 mg NH_3-N/L at pH=7.7. The parental (P) fish were exposed for eleven months, the first filial generation (F_1) for 48 months, and the second filial generation (F_2) for five months. Animals from the parental, first filial, and second filial generations were examined for chronic effects of un-ionized ammonia. Data collected during the tests included mortality, reproductive success, and growth. Histological examinations were performed on select tissues from fish of all three generations.

No statistically significant difference in survival, growth, or reproduction was observed at any of the tested concentrations.

Blood from the parental fish exposed to concentrations of 0.05 mg NH_3-N/L and greater showed reduced hematocrits and, to a lesser extent, reduced hemoglobin content. The first filial generation (F_1) did not show any significant alteration in hematocrits or hemoglobin, although there was a strong correlation between blood ammonia values and ambient ammonia concentrations.

Histological examinations of spleen, heart, gill, liver, and kidney tissues were performed on animals from all three generations and correlated to test concentrations. Histological alterations of gill and kidney tissues were remarkable and showed a positive correlation with un-ionized ammonia concentrations; histopathological alterations increased in severity with increasing ammonia concentrations. Gill lamellae obtained from parental fish exposed to un-ionized ammonia concentrations ranging from 0.02 mg NH_3-N/L to 0.05 mg NH_3-N/L for four months, and 0.05 mg NH_3-N/L and 0.06 mg NH_3-N/L for seven and eleven months, showed mild to moderate fusion, aneurysms, and separation of the epithelia from the underlying basement membrane. Test animals that had been exposed for seven months at un-ionized ammonia concentrations of 0.05 mg NH_3-N/L and subsequently allowed to 'recover' in an ammonia-free environment for the remaining four months, did not show any evidence of gill tissue damage, suggesting that the animals might have recovered.

The gill tissues of fish from the first filial generation exposed to concentrations of 0.03 mg NH_3-N/L and greater evidenced mild to severe tissue injury. The degree of injury exhibited a positive correlation with the un-ionized ammonia concentrations. Symptoms included hypertrophy of the gill lamellae, with accompanying basal hyperplasia, separation of epithelia from the underlying basement membranes, necrosis, aneurysms, and mild to moderate fusion of gill lamellae. This suite of symptoms is analogous to obstructive bronchopulmonary disease, e.g., emphysema, in humans and has been reported to affect swimming performance and stamina in trout (Smith and Piper 1985). Pathologic conditions were most apparent in both the parental and F_1 fish when un-ionized ammonia reached and exceeded 0.03 mg NH_3-N/L at pH=7.7. No effects were reported on survival, growth, or reproduction at the highest tested concentration of 0.06 mg NH_3-N/L.

Second filial generation rainbow trout exposed to un-ionized ammonia concentrations of 0.02 mg NH_3-N/L and greater exhibited histological alterations similar to those of the first filial generation. In addition to the histopathological alterations, the second filial generation also became infected with a protozoan. It is not known whether the protozoan infection was related to an increased susceptibility associated with the

ammonia exposure. These alterations are generally viewed as pathological and strongly indicative of organ dysfunction. Survival and growth of the second filial generation were unaffected at the highest tested ammonia concentration of 0.06 mg NH_3-N/L.

In addition to the recovery noted by Thurston et al. (1984b), other investigators have reported recovery and compensation. Smith and Piper (1975) reported recovery of rainbow trout when in water to which ammonia was not added. Burrows (1964) observed recovery of chinook salmon in uncontaminated water at 14°C, but not at 6°C. Schulze-Wiehenbrauck (1976) found that growth of rainbow trout juveniles was reduced during two-week exposures, but the decrease was completely compensated for during the next three or four weeks. Burkhalter and Kaya (1977) reported compensation for reduced growth at the lowest tested concentration.

Endpoint indices of abnormalities such as reduced growth, impaired reproduction, reduced survival, and gross anatomical deformities are clinical expressions of altered structure and function that originate at the cellular level. Any lesion observed in the test organism is cause for concern and such lesions often provide useful insight into the potential adverse clinical and subclinical effects of such toxicants as ammonia. For purposes of protecting human health or welfare these subclinical manifestations often serve useful in establishing 'safe' exposure conditions for certain sensitive individuals within a population.

With fish and other aquatic organisms the significance of the adverse effect can be used in the derivation of criteria only after demonstration of adverse effects at the population level, such as reduced survival, growth, or reproduction. Many of the data indicate that the concentrations of ammonia that have adverse effects on cells and tissues do not correspondingly cause adverse effects on survival, growth, or reproduction. No data are available that quantitatively and systematically link the effects that ammonia is reported to have on fish tissues with effects at the population level. This is not to say that the investigators who reported both tissue effects and population effects within the same research did not correlate the observed tissue lesions and cellular changes with effects on survival, growth, or reproduction, and ammonia concentrations. Many did, but they did not attempt to relate their observations to ammonia concentrations that would be safe for populations of fish under field conditions nor did they attempt to quantify (e.g., increase in respiratory diffusion distance associated with gill hyperplasia) the tissue damage and cellular changes (Lloyd 1980;

Malins 1982). Additionally, for the purpose of deriving ambient water quality criteria, ammonia-induced lesions and cellular changes must be quantified and positively correlated with increasing exposures to ammonia.

In summary, the following have been reported:

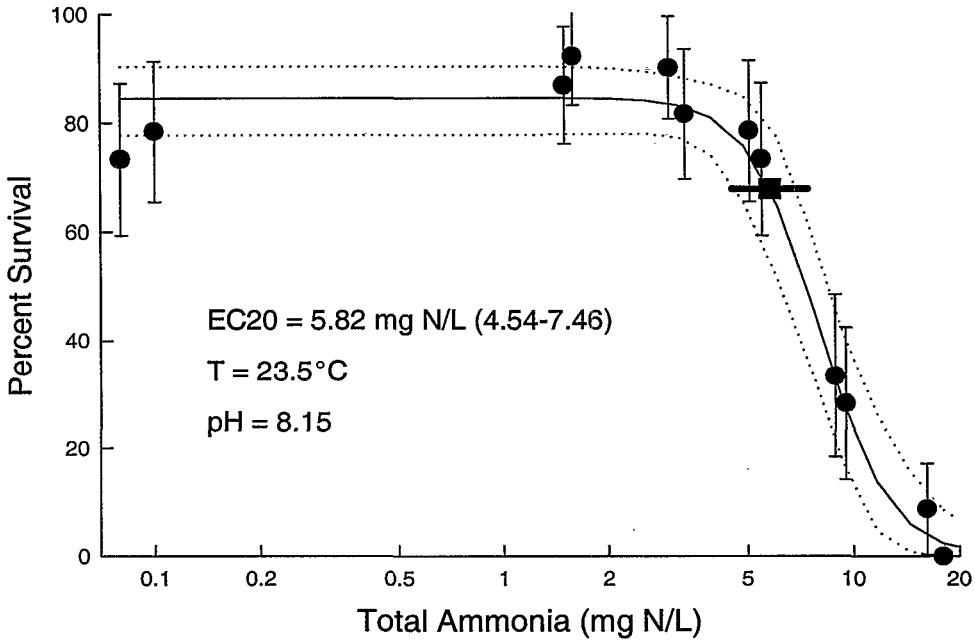
1. Fish recover from some histopathological effects when placed in water that does not contain added ammonia.
2. Some histopathological effects are temporary during continuous exposure of fish to ammonia.
3. Some histopathological effects have occurred at concentrations of ammonia that did not adversely affect survival, growth, or reproduction during the same exposures.

Because of the lack of a clear connection between histopathological effects and effects on populations, histopathological endpoints are not used in the derivation of the new criterion, but the possibility of a connection should be the subject of further research.

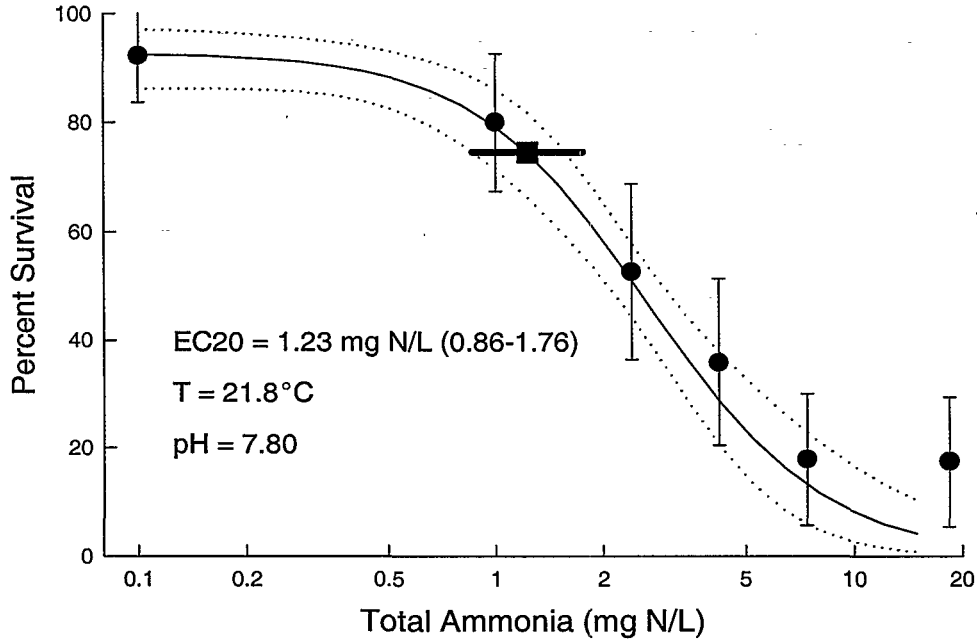
Appendix 6. Results of Regression Analyses of Chronic Data

The following pages contain figures and other information related to the regression analyses that were performed to calculate chronic EC20s and LC20s. Circles denote measured responses and confidence limits (if available), solid lines denote estimated regression lines, and dotted lines denote 95% confidence limits on the regression lines. Squares with solid thick lines denote estimated EC20s and 95% confidence limits.

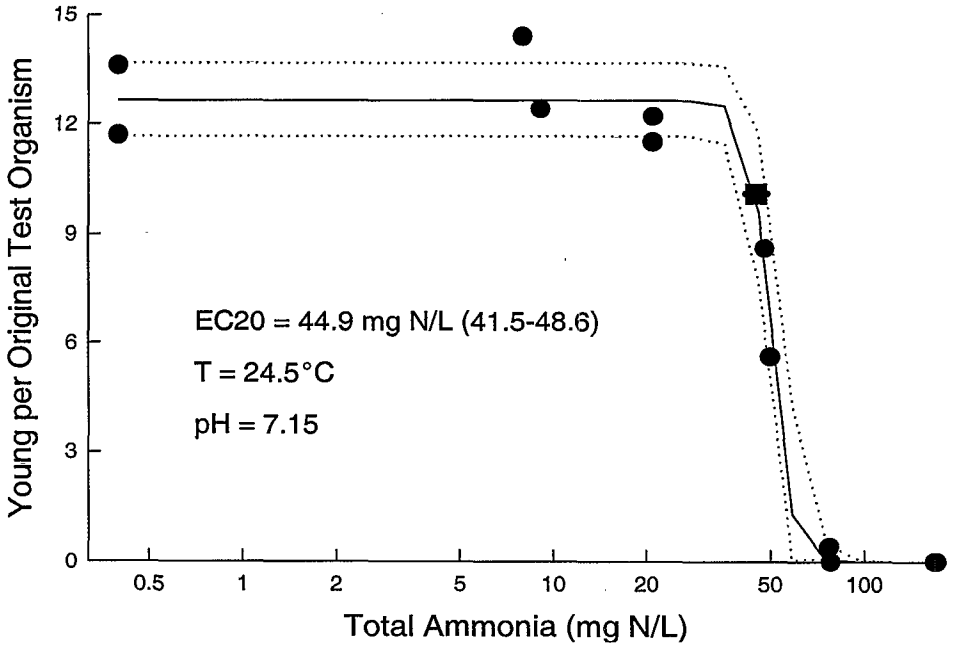
FINGERNAIL CLAM, 42-DAY JUV, ANDERSON ET AL. 1978



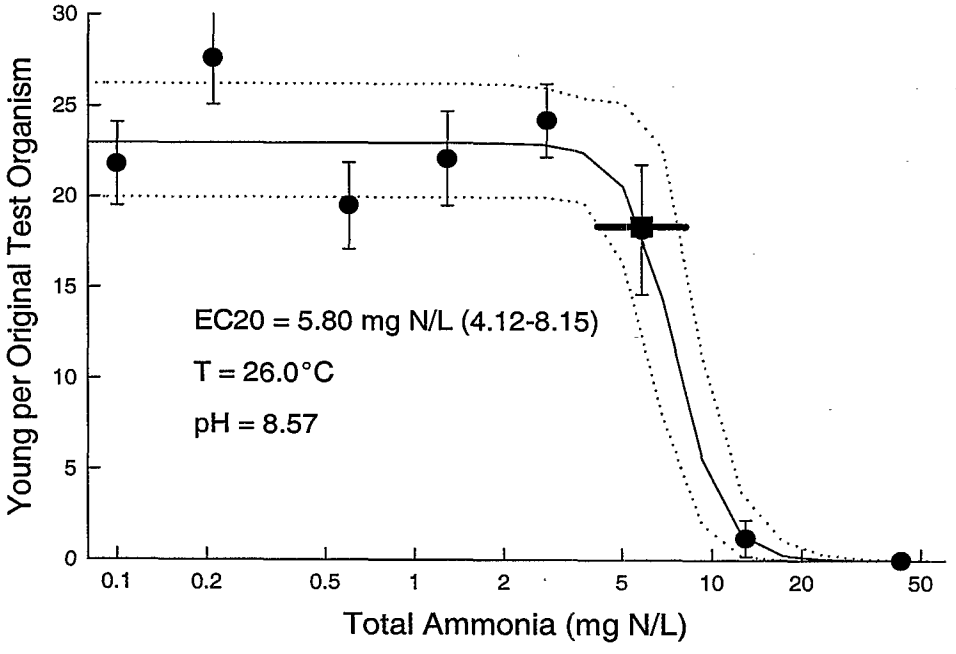
FINGERNAIL CLAM, 42-DAY JUV, SPARKS AND SANDUSKY 1981



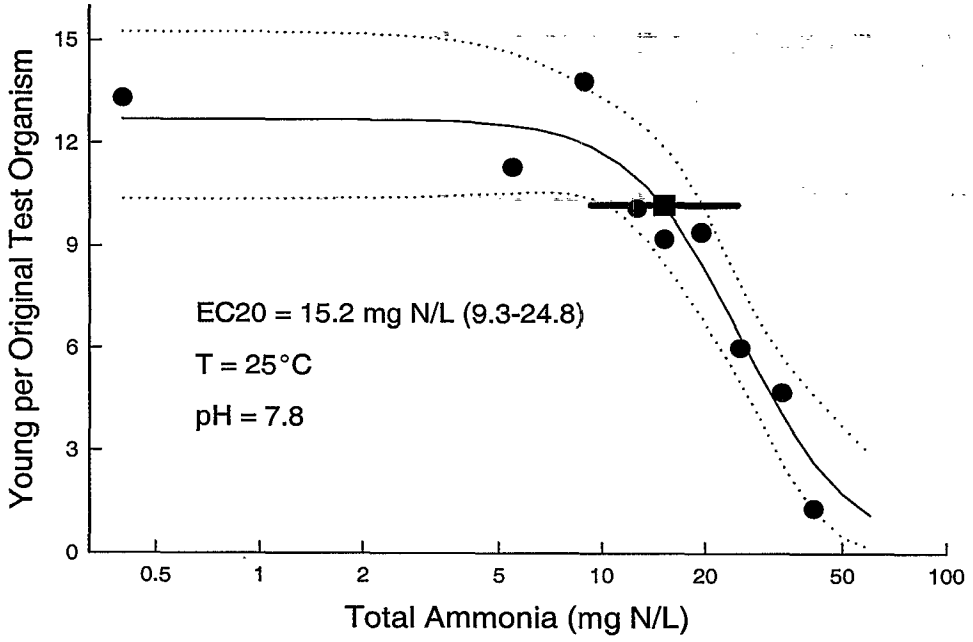
CERIODAPHNIA ACANTHINA, LIFE CYCLE, MOUNT 1982



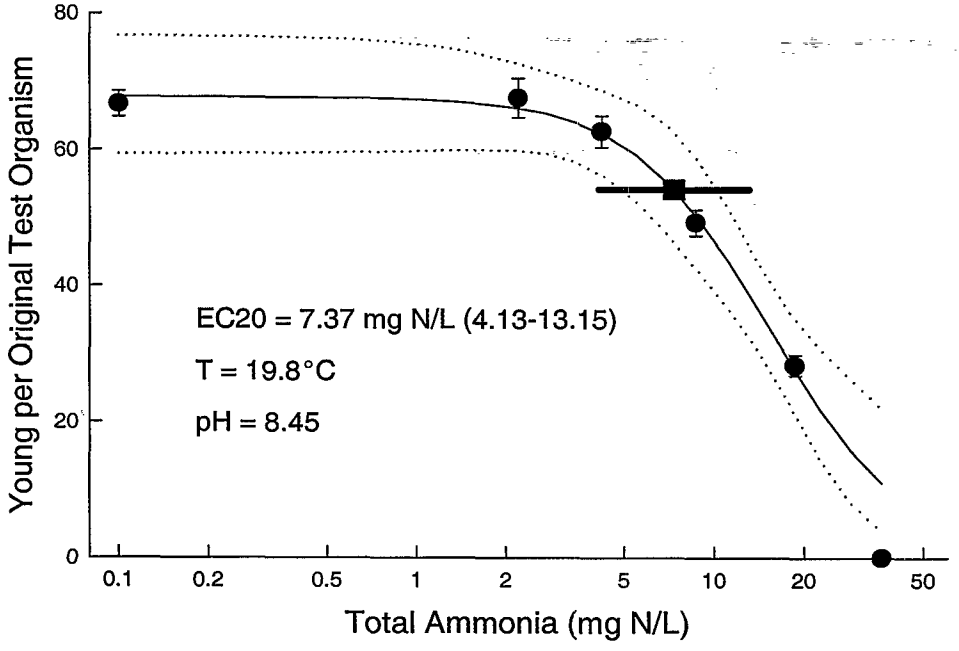
CERIODAPHNIA DUBIA, LIFE CYCLE, WILLINGHAM 1987



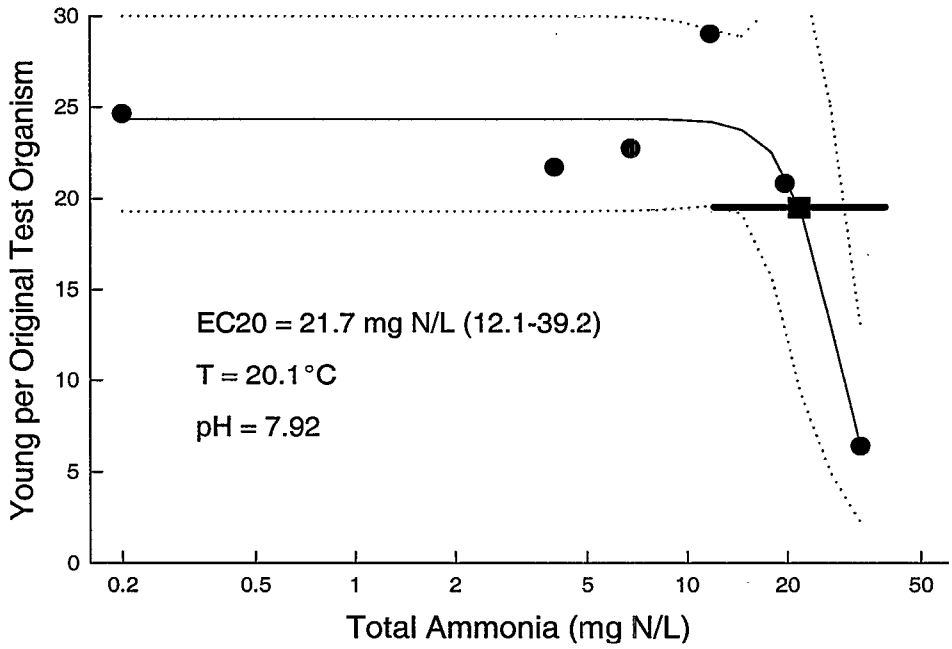
CERIODAPHNIA DUBIA, LIFE CYCLE, NIMMO ET AL 1989



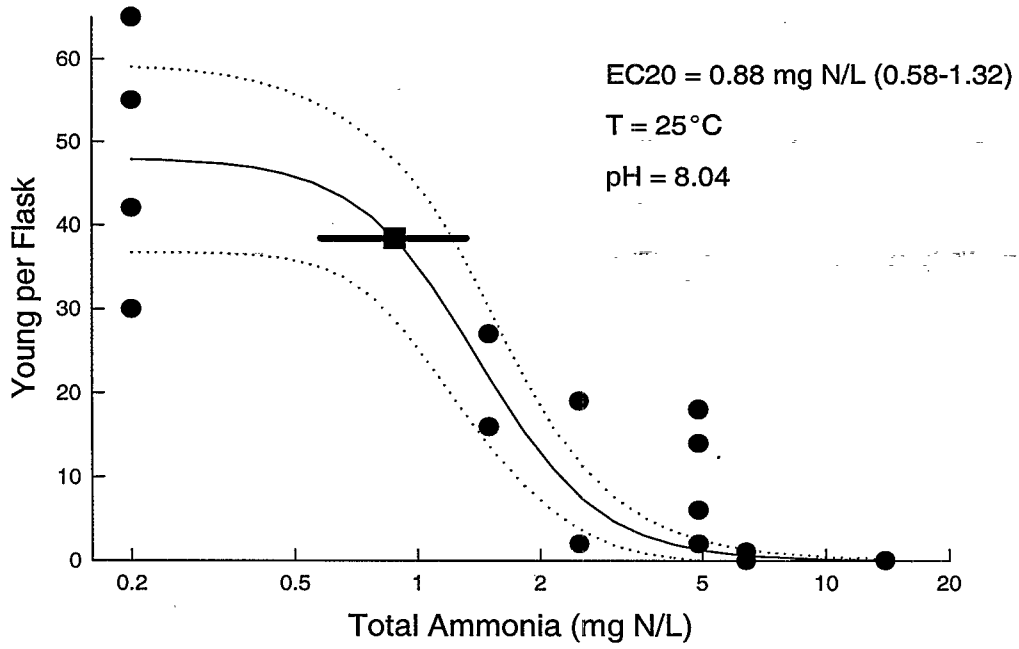
DAPHNIA MAGNA, LIFE CYCLE, GERSICH ET AL. 1985



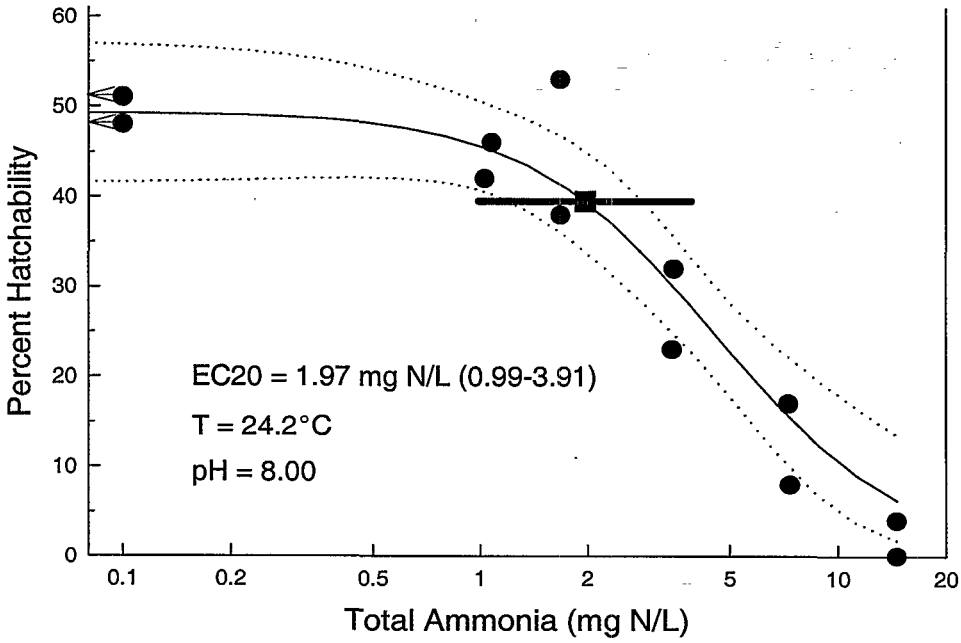
DAPHNIA MAGNA, LIFE CYCLE, REINBOLD AND PESCITELLI 1982a



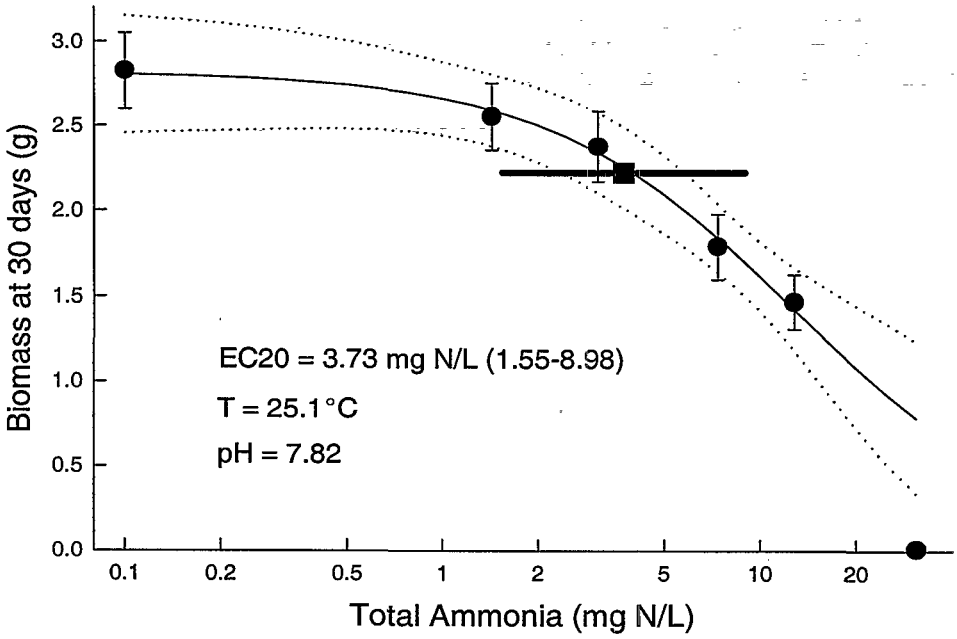
HYALELLA AZTECA, LIFE CYCLE, BORGMANN 1994



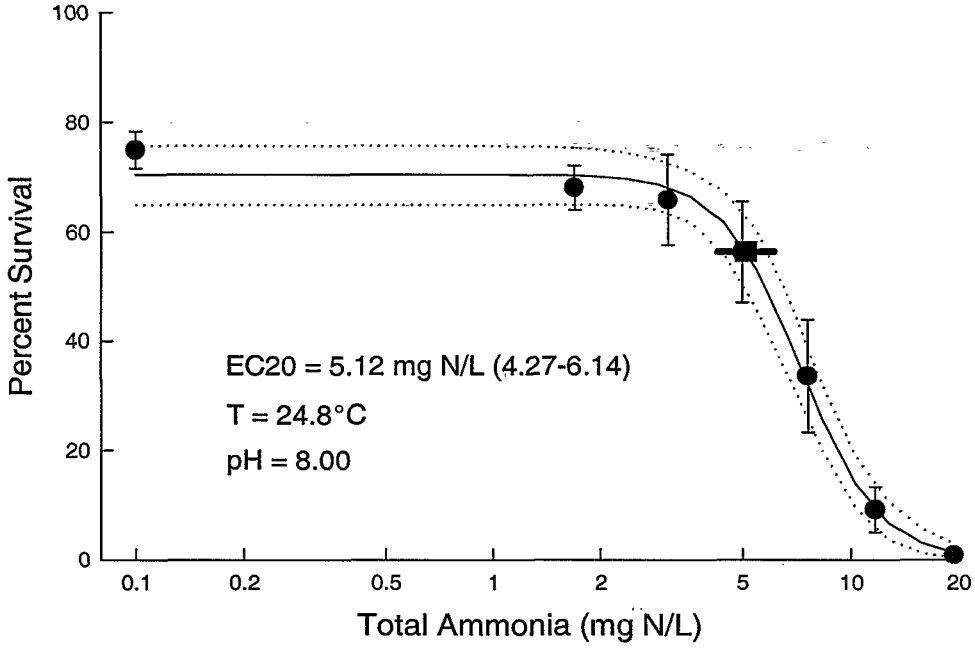
FATHEAD MINNOW, LIFE CYCLE, THURSTON ET AL. 1986



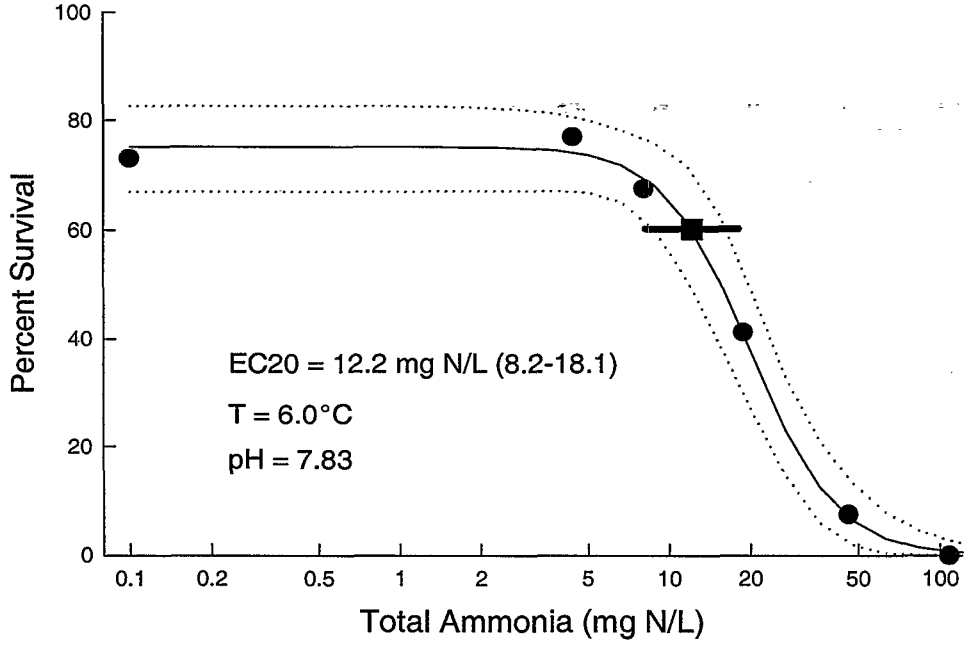
FATHEAD MINNOW, 30-DAY ELS, SWIGERT AND SPACIE 1983



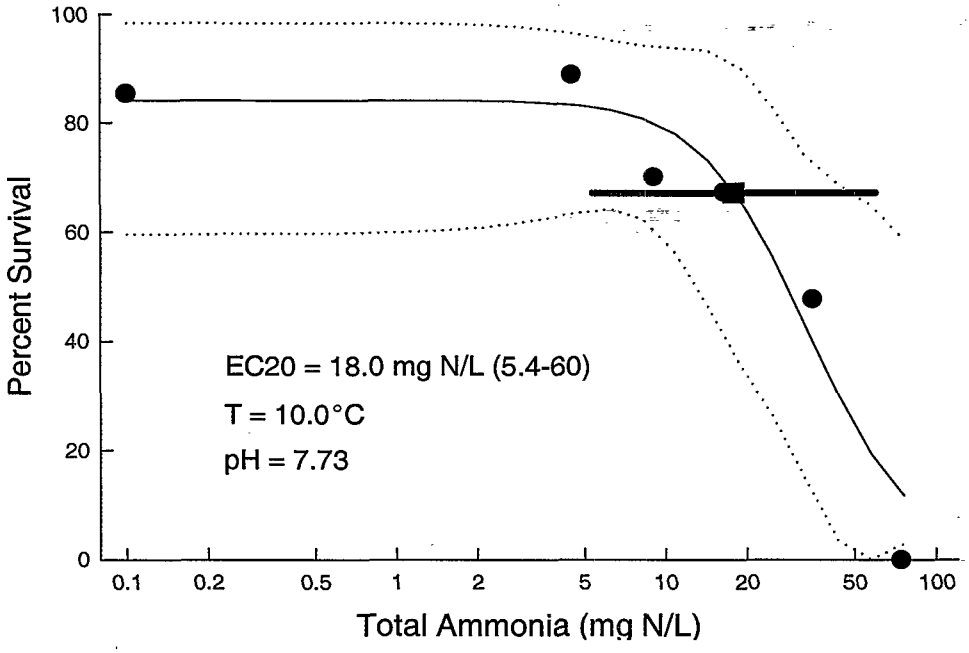
FATHEAD MINNOW, 28-DAY ELS, MAYES ET AL. 1986



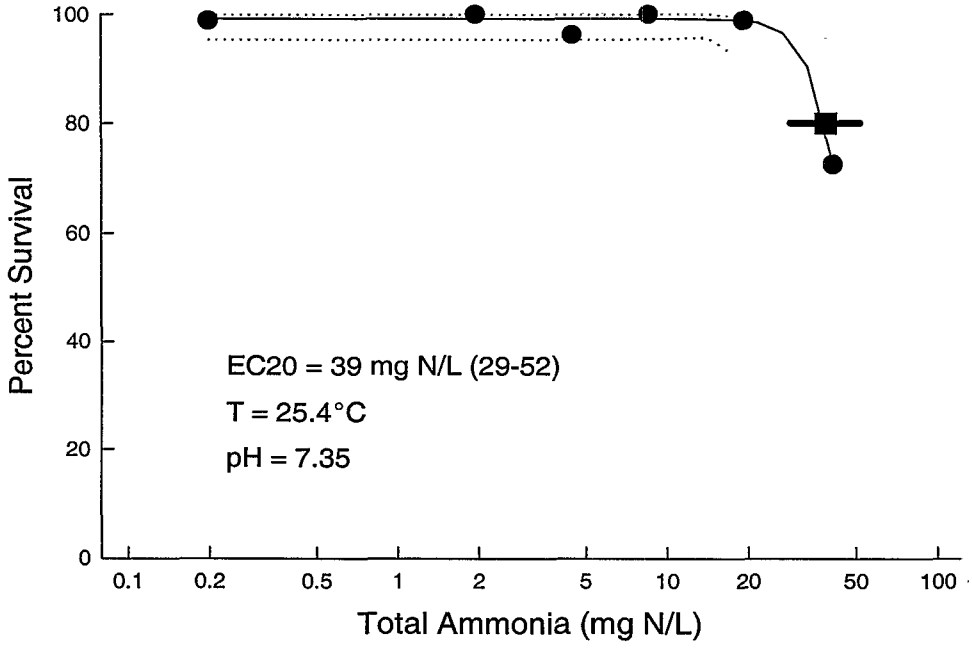
FATHEAD MINNOW, 30-DAY JUVENILE, DEGRAEVE ET AL. 1987



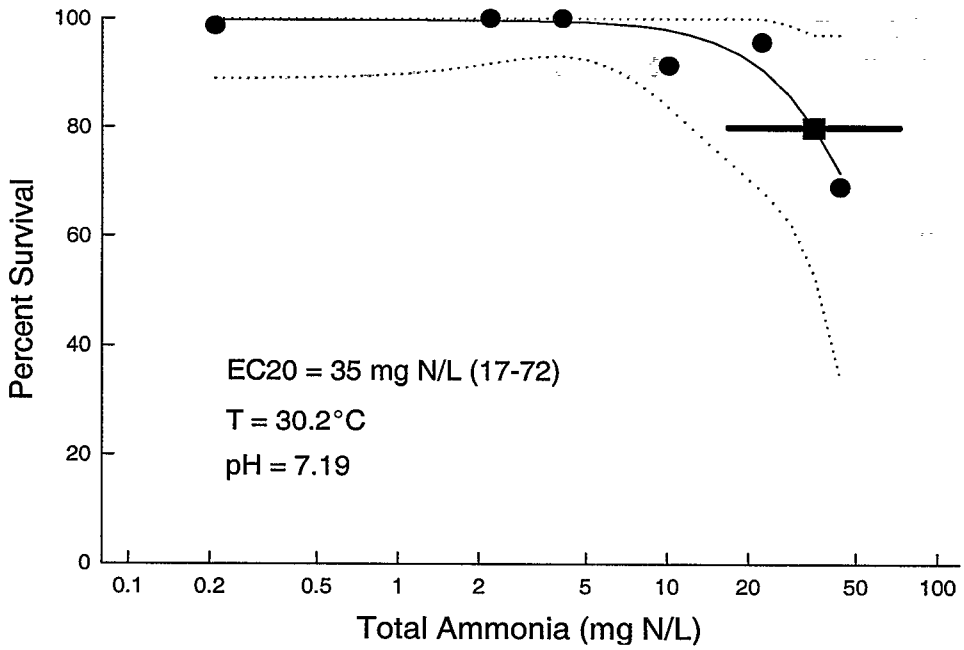
FATHEAD MINNOW, 30-DAY JUVENILE, DEGRAEVE ET AL. 1987



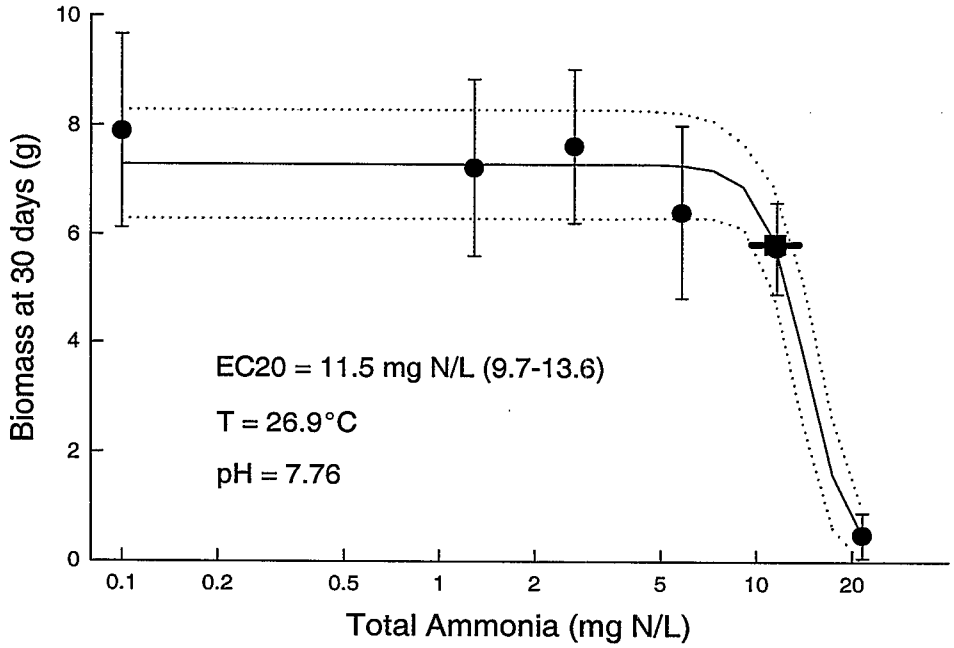
FATHEAD MINNOW, 30-DAY JUVENILE, DEGRAEVE ET AL. 1987



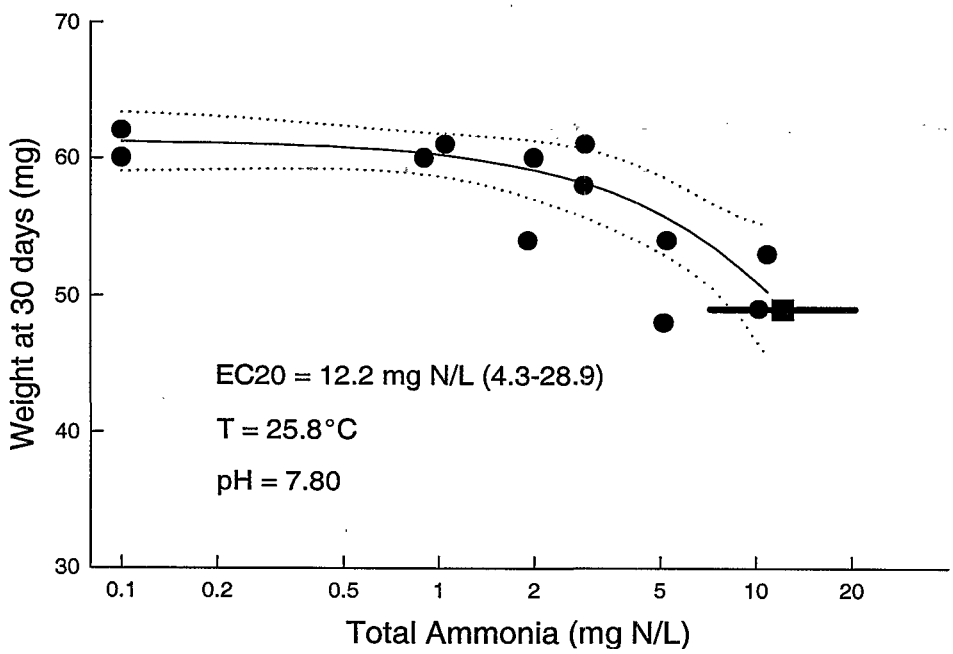
FATHEAD MINNOW, 30-DAY JUVENILE, DEGRAEVE ET AL. 1987



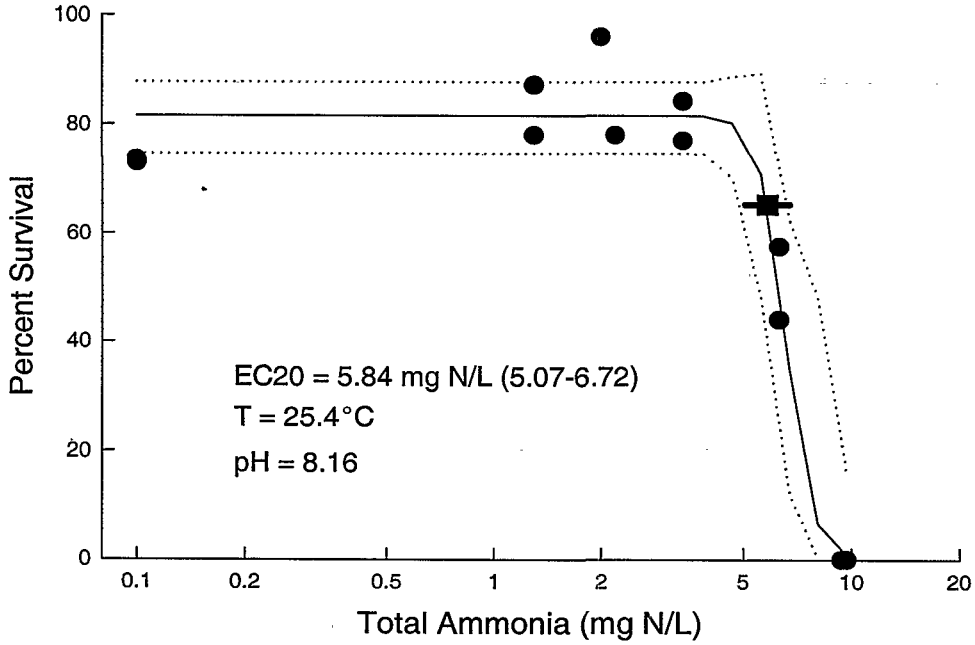
CHANNEL CATFISH, 30-DAY ELS, SWIGERT AND SPACIE 1983



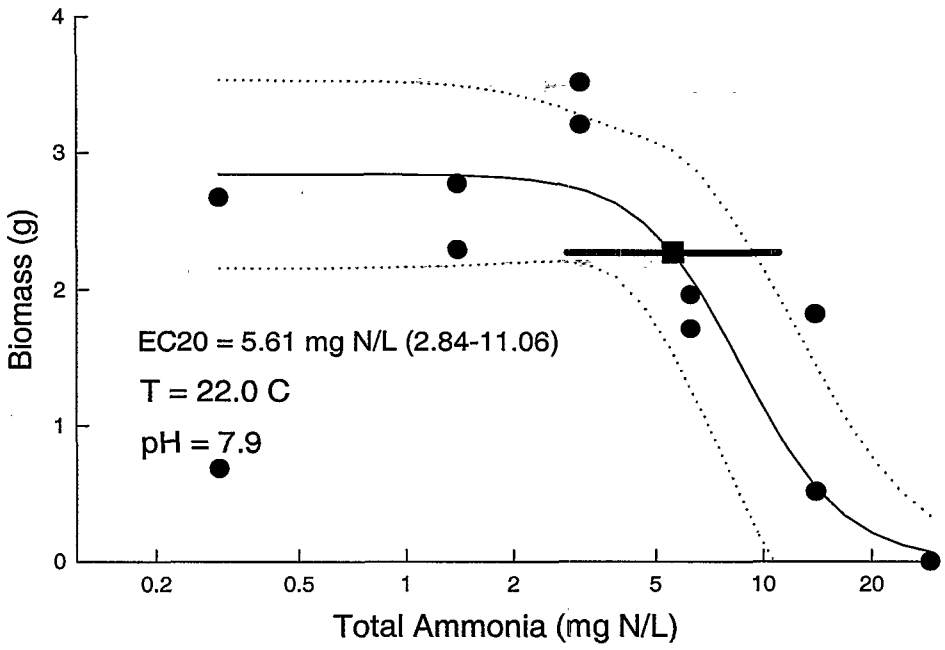
CHANNEL CATFISH, 30-DAY ELS, REINBOLD AND PESCITELLI 1982a



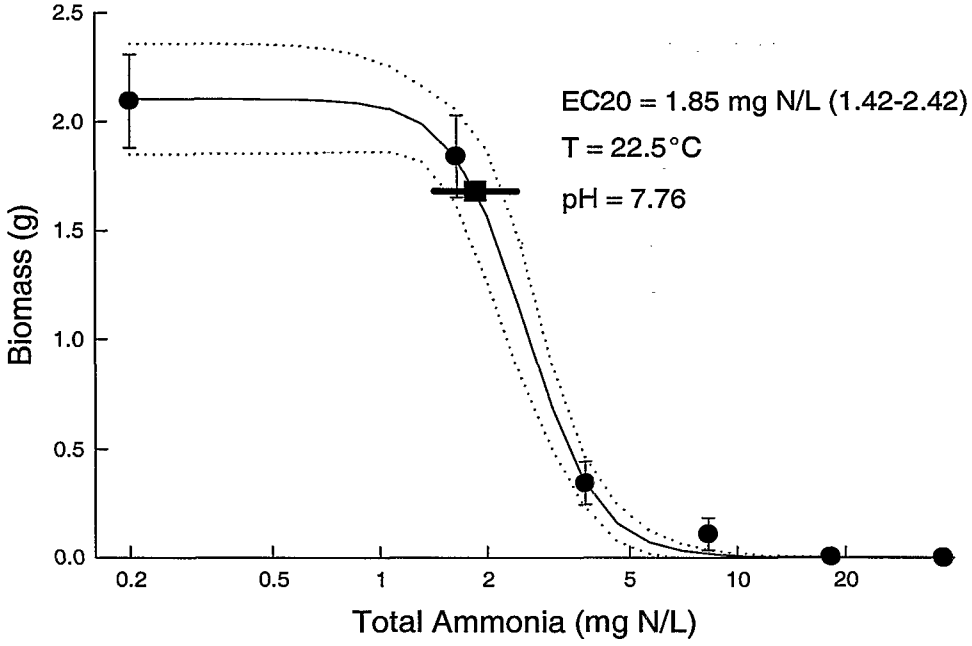
GREEN SUNFISH, 30-DAY ELS, REINBOLD AND PESCITELLI 1982a



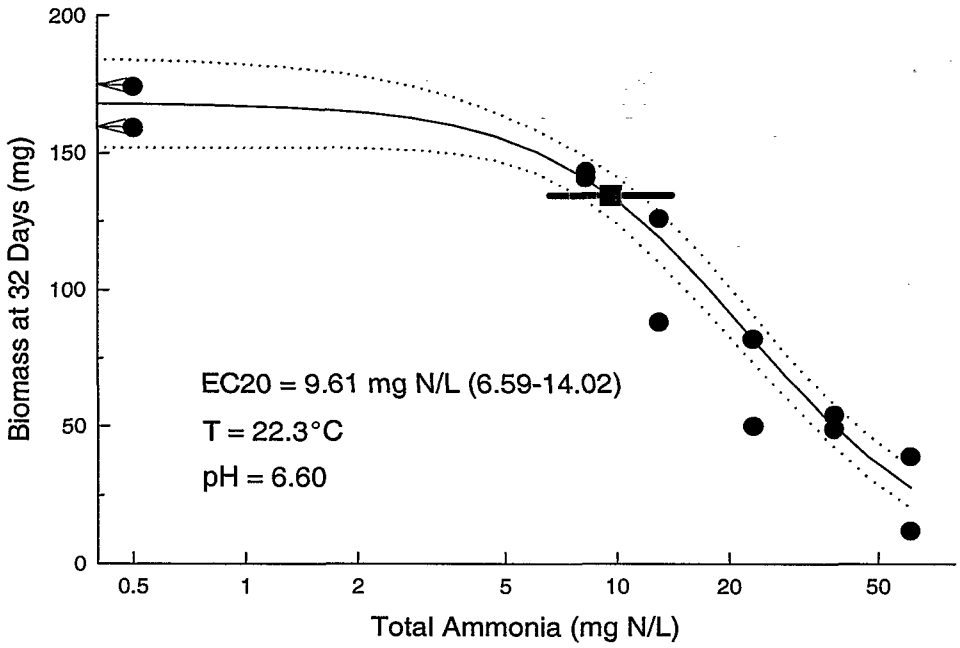
GREEN SUNFISH, 30-DAY ELS, MCCORMICK ET AL. 1984



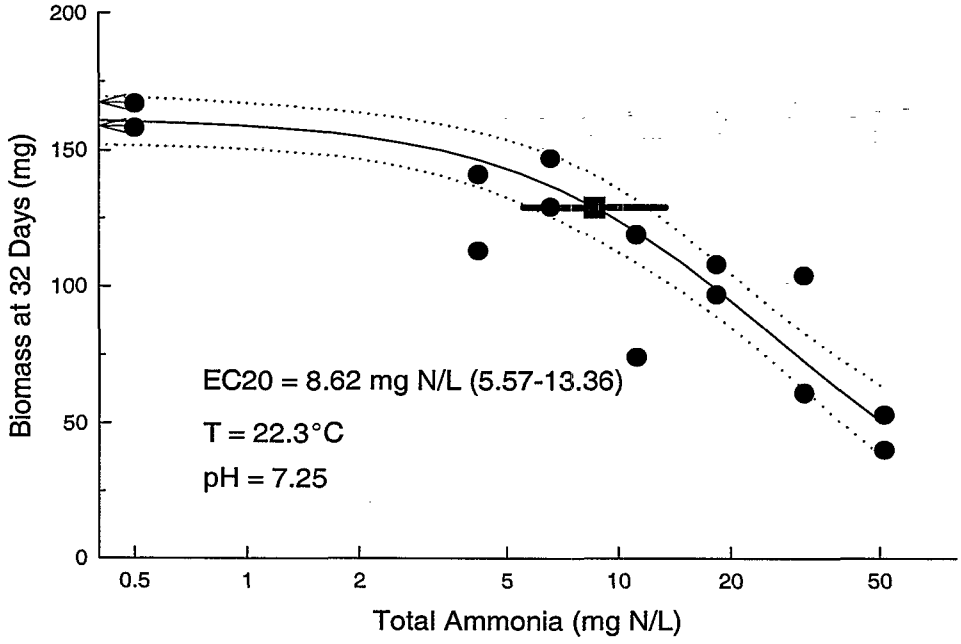
BLUEGILL, 30-DAY ELS, SMITH ET AL. 1984



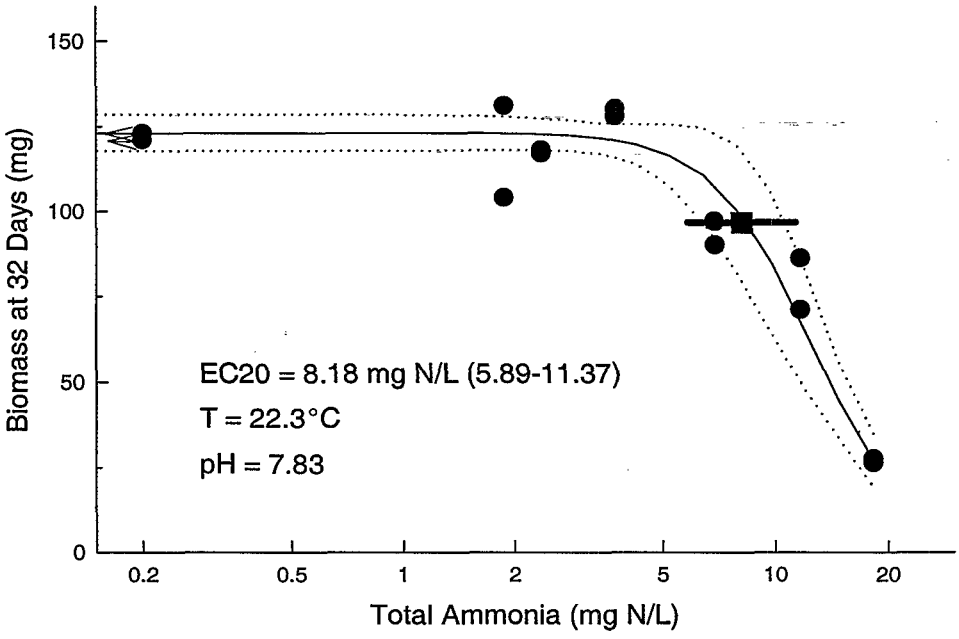
SMALLMOUTH BASS, 32-DAY ELS, BRODERIUS ET AL. 1985



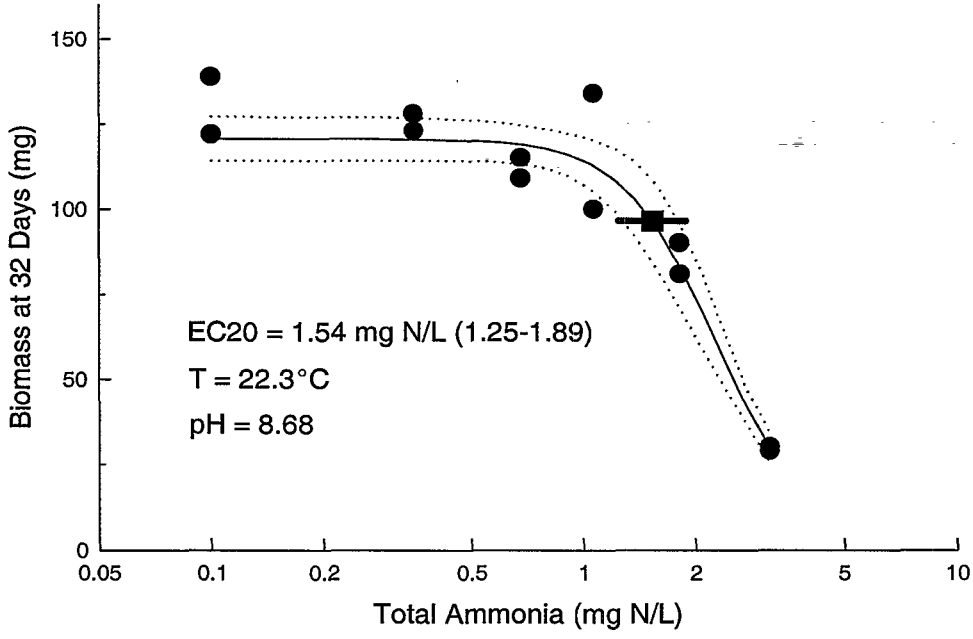
SMALLMOUTH BASS, 32-DAY ELS, BRODERIUS ET AL. 1985



SMALLMOUTH BASS, 32-DAY ELS, BRODERIUS ET AL. 1985



SMALLMOUTH BASS, 32-DAY ELS, BRODERIUS ET AL.



Appendix 7. Acute-Chronic Ratios

Although the CCC was calculated directly from Chronic Values using the fifth percentile procedure (U.S. EPA 1985b), it is of interest to consider how this compares with the use of Acute-Chronic Ratios (ACRs). Therefore, ACRs were determined for all of the EC20s in Table 2 that are used in the derivation of a GMCV and for which comparable acute values were found. (Sufficient ACRs are available for freshwater species that ACRs determined with saltwater species were not considered.) Because the acute toxicity of total ammonia is related to pH differently from its chronic toxicity, all relevant acute and chronic values were adjusted to pH=8 and are expressed in terms of mg N/L, where N is total ammonia nitrogen. The resulting ACRs are given in Table 5, along with the resulting Genus Mean Acute-Chronic Ratios (GMACRs).

When ACRs are used, it is hoped that if the acute and chronic tests are conducted with the same test species in the same water, any biological or chemical factor that affects the result of one of the tests will have a proportional effect on the result of the other test so that the ACR is more constant than the result of either individual test. In addition, it is hoped that the ACRs within a genus agree well. The ACRs within the genera *Ceriodaphnia* and *Daphnia* agree well (Table 5).

The available ACRs at pH=8 for the fathead minnow range from 6.5 to 20.7, but the range can probably be explained because of the different kinds of chronic tests on which they are based. The ACR of 20.7 was based on the life-cycle test of Thurston et al. (1986) whereas the early life-stage tests of Swigert and Spacie (1983) and Mayes et al. (1986) gave ACRs of 6.5 and 9.7. The range of ACRs for the early life-stage tests is small, and it is not surprising that a life-cycle test gave a higher ACR than the early life-stage test. The range of the nine 96-hr LC50s from three laboratories was only 27.2 to 51.5 mg N/L when adjusted to pH=8.

Table 6 gives the GMACRs beside the ranked GMAVs to demonstrate whether there is a trend, because ACRs for some chemicals are higher for resistant species than for sensitive species (U.S. EPA 1985b). No trend is obvious and the range of the GMACRs is 1.9 to 10.9.

A major problem with use of the ACR procedure for calculating a CCC for ammonia is that ACRs are not available for *M. transversum* and *H. azteca*, which are very sensitive in chronic tests; the

data in the 1984/1985 ammonia criteria document indicate that *M. transversum* is not very sensitive in acute tests, which implies a large ACR. In these circumstances, direct calculation of the CCC using the fifth percentile calculation procedure is certainly much more appropriate than calculation using the ACR procedure. In addition, the CCC obtained using the fifth percentile procedure agrees well with the available chronic data.

Table 5. Genus Mean Acute-Chronic Ratios

Species	Chronic Results				Acute Results[a]			Adjusted to pH=8			
	Ref[b]	Temp	pH	EC20[c]	Temp	pH	LC50[c]	EC20	LC50	ACR[d]	GMACR
M. transversum	1	23.5	8.15	5.82	----	----	-----	7.30	----	----	----
	2	21.8	7.80	1.23	----	----	-----	0.94	----	----	----
C. acanthina	3	24.5	7.15	44.9	24.0	7.06	105.	19.8	24.4	1.2	1.9
C. dubia	4	26.0	8.57	5.80	26.0	8.61	14.8	14.1	48.6	3.4	
	5	25.0	7.8	15.2	25.0	7.8	41.3	11.6	31.5	2.7	
D. magna	6	19.8	8.45	7.37	20.0	8.50	26.4	15.1	70.2	4.6	5.3
	7	20.1	7.92	21.7	19.7	8.34	61.3	19.4	119.	6.1	
H. azteca	8	25.0	7.94	<1.58	----	----	-----	<1.45	----	----	----
P. promelas	9	24.2	8.0	1.97	22.1	8.03	48.6[e]	1.97	51.5	20.7	10.9
					22.0	8.06	42.6[e]		47.8		
					19.1	7.94	42.3[e]		37.7		
					19.0	7.76	50.4[e]		32.2		
					22.0	7.83	50.6[e]		36.7		
					18.9	7.91	49.3[e]		41.5		
	10	25.1	7.82	3.73	25.9	7.78	41.0	2.92	27.2	9.7	
					25.6	7.8	42.8		29.4		
	11	24.8	8.0	5.12	22.0	8.14	25.2	5.12	33.1	6.5	
C. commersoni	7	18.6	8.32	>2.9	15.0	8.16	30.3[f]	>4.79	41.4	<8.4	<8.4
					15.4	8.14	29.7[f]		39.0		
I. punctatus	10	26.9	7.76	11.5	25.7	7.8	32.8	8.35	22.6	2.7	2.7
L. cyanellus	7	25.4	8.16	5.84	26.2	8.28	8.6	7.44	14.8	2.0	7.6
	12	22.0	7.9	5.61	22.4	7.7	57.	4.88	32.8	6.7	

L. macrochirus	13	22.5	7.76	1.85	21.7	7.6	44.2	1.35	21.4	15.9	
M. dolomieu	14	22.3	6.60	9.61	22.3	6.53	371.	3.57	59.3	16.6	7.4
		22.3	7.25	8.62	22.3	7.16	117.	4.01	30.4	7.6	
		22.3	7.83	8.18	22.3	7.74	39.5	6.50	24.4	3.8	
		22.3	8.68	1.54	22.3	8.71	7.43	4.65	29.3	6.3	

[a] If acute values were available at more than one pH, the acute value(s) at a pH close to the pH of the chronic value were used. Dashes indicate that a comparable acute test was not found. When an acute test listed above was in Table 1 of the 1984/1985 ammonia criteria document (U.S. EPA 1985a), the values given in Table 1 for pH and temperature were used unless inspection of the reference indicated that an incorrect value was in Table 1. If given in the reference, an LC50 based on total ammonia was used, after conversion to total ammonia nitrogen if necessary. If a total ammonia LC50 was not given in the reference, an LC50 based on un-ionized ammonia was used, after conversion to un-ionized ammonia nitrogen if necessary. Each LC50 based on un-ionized ammonia nitrogen was converted to total ammonia nitrogen in the table above, using the speciation relationship derived by Emerson et al. (1978).

[b] (1) Anderson et al. 1978; (2) Sparks and Sandusky 1981; (3) Mount 1982; (4) Willingham 1987; (5) Nimmo et al. 1989; (6) Gersich et al. 1985; (7) Reinbold and Pescitelli 1982a; (8) Borgmann 1994; (9) Thurston et al. 1986; (10) Swigert and Spacie 1983; (11) Mayes et al. 1986; (12) McCormick et al. 1984; (13) Smith et al. 1984; (14) Broderius et al. 1985.

[c] Expressed as total ammonia nitrogen (mg N/L). Three digits are retained in intermediate calculations to reduce roundoff error in subsequent calculations.

[d] One ACR was calculated for each EC20 for which a comparable acute value was available; if more than one comparable acute value was available, the geometric mean of the acute values was used.

[e] These are the results of the six acute tests given by Thurston et al. (1983) in their appendix that were conducted with fish that were 0.1 to 1.0 g and whose test temperature was closest to the temperature of the chronic test.

[f] Reinbold and Pescitelli 1982b.

Table 6. Ordered Genus Mean Acute-Chronic Ratios

RANK	GENUS	GMAV ADJUSTED TO pH=8	GMACR
34	Philarctus	388.8	
33	Orconectes	246.0	
32	Asellus	210.6	
31	Ephemerella	189.2	
30	Callibaetis	115.5	
29	Stenelmis	113.2	
28	Crangonyx	108.3	
27	Tubifex	97.82	
26	Helisoma	93.52	
25	Arcynopteryx	77.10	
24	Physa	73.69	
23	Cottus	51.73	
22	Gambusia	51.06	
21	Pimephales	43.55	10.9
20	Catostomus	38.11	<8.4
19	Daphnia	36.82	5.3
18	Salvelinus	36.39	
17	Musculium	35.65	
16	Ictalurus	34.44	2.7
15	Simocephalus	33.99	
14	Poecilia	33.14	
13	Dendrocoelum	32.82	
12	Morone	30.89	
11	Campostoma	26.97	
10	Micropterus	26.50	7.4
9	Stizostedion	26.11	
8	Ceriodaphnia	25.78	1.9
7	Notropis	25.60	
6	Salmo	23.74	
5	Lepomis	23.61	7.6
4	Oncorhynchus	21.95	
3	Etheostoma	17.96	
2	Notemigonus	14.67	
1	Prosopium	12.11	