Arid West Water Quality Research Project

EVALUATION OF THE EPA RECALCULATION PROCEDURE IN THE ARID WEST

User's Guide

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ACRONYMS

µg/L	microgram(s) per liter
ACR	acute-to-chronic ratio
AW-MDRs	Arid West minimum data requirements
AWQC	ambient water quality criteria
AWWQRP	Arid West Water Quality Research Project
BLM	biotic ligand model
CCC	criterion continuous concentration (chronic criterion)
CEC	Chadwick Ecological Consultants
CMC	criterion maximum concentration (acute criterion)
Cu	copper
DQOs	data quality objectives
EC_{50}	median effect concentration – point estimate for 50% non-mortality effects
ECE	extant criteria evaluation
EDW	effluent-dependent waters
FACR	final acute-to-chronic ratio
FAV	final acute value
FCV	final chronic value
GMAV	genus mean acute value
GMCV	genus mean chronic value
HCS	habitat characterization study
LA_{50}	median lethal accumulation concentration – point estimate for 50% mortality used in biotic ligand model
LC_{50}	median lethal concentration – point estimate for 50% mortality
MAV	mean acute value
MDR	minimum data requirement
mg/L	milligram(s) per liter
NPDES	National Pollutant Discharge Elimination System
NH_3	ammonia, or "unionized" ammonia
NH_4^+	ammonium ion, or "ionized" ammonia
PCWMD	Pima County Wastewater Management Department
SMAV	species mean acute value
SMCV	species mean chronic value
SS-AWQC	site-specific ambient water quality criterion

TA-N	total ammonia as nitrogen
T&E	threatened or endangered
USEPA	U.S. Environmental Protection Agency

1. EXECUTIVE SUMMARY

This User's Guide was developed to provide the general technical background necessary to derive site-specific water quality criteria using the USEPA's Recalculation Procedure. This procedure is one of three primary methods recommended by the USEPA for modifying national Ambient Water Quality Criteria (AWQC) for protection of aquatic life where local conditions indicate these criteria might not be appropriate (i.e., might be over- or underprotective). The Recalculation Procedure makes adjustments to the national toxicity database used in derivation of AWQC so that the database better reflects the composition of aquatic communities at a particular site. If properly conducted, the Recalculation Procedure can provide a straightforward and scientifically-defensible means of modifying default AWQC to more accurately reflect the unique biotic assemblages often encountered in surface waters throughout the U.S.

The Recalculation Procedure may be particularly important for ephemeral and effluent-dependent waters in the arid western U.S. because aquatic assemblages are often unique and contain substantially fewer species compared to perennial waters in other regions (AWWQRP 2002). In such cases, concerns have been raised that the surrogate laboratory species used to develop the national AWQC do not adequately represent those encountered in arid west effluent-dependent waters (EDWs). Furthermore, standard methods for conducting a Recalculation Procedure are less statistically robust when the total numbers and types of aquatic species are low, as is often the case in arid west EDWs.

In response to these concerns, the Arid West Water Quality Research Project (AWWQRP) has conducted a thorough evaluation of the Recalculation Procedure in a companion report to this User's Guide titled *Evaluation of EPA Recalculation Procedure in Arid West Effluent-dependent Waters* (AWWQRP 2005). This report conducts a detailed evaluation of the USEPA Recalculation Procedure using five numeric AWQC (aluminum, ammonia, copper, diazinon, and zinc) applied to six effluent-dependent waters located in arid portions of Arizona, California, and Colorado. These case studies provided the basis upon which to evaluate the scientific rigor of the standard Recalculation Procedure for each of the AWQC, and to recommend alternative methods that better reflect the unique biological conditions encountered in these EDWs.

The purpose of the User's Guide is to assist permit holders in applying the USEPA's Recalculation Procedure given the unique biological conditions often present in EDWs. This will not just be a "how-to" guide for use of the Recalculation Procedure, but rather a description of how to apply the procedure given what we learned from the companion report (AWWQRP 2006), as well as other recent AWWQRP studies (Parametrix et al. 2003, Parametrix and CEC 2005, Parametrix and HydroQual 2005). This User's Guide begins by placing the Recalculation Procedure in the context of the full suite of possibilities for deriving site-specific water quality criteria in EDWs. Our primary goal is to help permit holders decide whether the Recalculation Procedure is appropriate for their specific needs and, if selected, how to proceed with an analysis that would achieve both scientific and regulatory acceptance. A secondary goal is to summarize alternative scientific methods for conducting the Recalculation Procedure as recommended in the companion report (AWWQRP 2006) to this User's Guide, and assist readers as to appropriate selection of these vs. other methods for derivation of SS-AWQC.

1.1 SELECTION OF PROCEDURES FOR DERIVATION OF SITE-SPECIFIC WATER QUALITY CRITERIA

As we discuss in Section 3, National AWQC set maximum threshold concentrations of inorganic and organic contaminants to prevent or minimize the exposure of both freshwater and marine organisms to toxic levels of these contaminants. These criteria are derived from empirical toxicity data and are

designed to protect all but the most sensitive 5% of species in an aquatic community (see Chapter 2 for derivation procedures). AWQC can also be lowered to protect species which are more sensitive than this lower 5th percentile if they are deemed ecologically, economically, or recreationally important.

One major difficulty in applying AWQC to surface waters across the U.S. is that they are derived chiefly from standardized toxicity tests (i.e., uniform types of water and laboratory exposure conditions) using aquatic species that may not be representative of the biota in streams of the arid West. Because the physical and chemical characteristics of surface waters and aquatic community composition varies markedly in different regions of the U.S., AWQC cannot reasonably be expected to provide a consistent level of protection for all species and all surface waters. Recognizing this limitation, USEPA guidance allows for site-specific modification of AWQC using several methods:

- *The Recalculation Procedure*, which provides a means of correcting criteria concentrations if the aquatic species at a particular site are substantially different than those used in the toxicity testing to derive the AWQC.
- *The Water-Effect Ratio (WER)*, which is intended to take into account how water quality characteristics affect the toxicity of contaminants (most typically metals) in laboratory dilution water relative to that in site water.
- *The Resident Species Procedure*, provides a means of taking into account the differential sensitivity of resident species to chemical stressors. In some cases, species resident to a site may be uniquely adapted to naturally elevated contaminant concentrations and, therefore, be more resistant than the species used to derive the National AWQC.

For the most part, selection amongst these three USEPA methods is based on two primary factors (Fig. 3-5). First, one needs to consider whether the physical or chemical conditions encountered in site waters could affect the bioavailability of the criteria chemical. If this is the case, then the WER would be the method most likely to derive SS-AWQC that are protective of aquatic life at the same level (i.e., 95% genera protection) originally intended by the national AWQC. Second, one needs to consider whether aquatic organisms which reside at a particular site are more or less sensitive to the criteria chemical than the organisms contained in the AWQC toxicity database. In this case, it would be appropriate to use the Recalculation Procedure to add or remove organisms from the toxicity database, and recalculate the AWQC so that it better represents the sensitivity of organisms that actually reside at the site. It should be remembered, however, that the Recalculation Procedure only addresses differences in chemical sensitivity owing to the *presence or absence* of a particular species, not whether physiological acclimation or adaptation might influence sensitivity. This latter question is instead addressed by the Resident Species Procedure.

It is also possible that both conditions can exist at a site, whereby both chemical bioavailability and differences in species composition suggest that modifications to the national AWQC would best protect aquatic life. In such a case, two approaches can be followed. First, the most recent USEPA guidance allows for the WER and Recalculation Procedures to be combined to derive a SS-AWQC. The Resident Species procedure can also be used, but this procedure is much more costly, and answers different scientific questions than does the combined WER and Recalculation Procedure. Only the Resident Species procedure addresses differences in chemical sensitivity of the resident vs. laboratory test organisms owing specifically to acclimation and adaptation, not necessarily whether a species in the toxicity database is resident to a particular site. Constructing a new toxicity database from entirely resident species also eliminates uncertainties with extrapolating toxicity data from surrogate species not resident to the site.

1.2 STANDARD USEPA RECALCULATION PROCEDURE

In Section 4, we discuss each step of standard USEPA guidance for conducting the Recalculation Procedure. General guidelines for conducting a site-specific recalculation were originally published as a companion to the USEPA AWQC derivation guidance document (USEPA 1984c). Since then, more specialized documents have been published (USEPA 1994a, 2001). To conduct the Recalculation Procedure, the following basic steps must be followed in this specific order (USEPA 1994a; see also Figure 4-1):

A) *Corrections to the national toxicity database*. The first step of the Recalculation Procedure involves correcting the national toxicity database for a given AWQC to ensure the data are of adequate type and quality for derivation of AWQC according to USEPA guidance.

B) *Updates to the national database*. Updates (or, "additions") to the national database are similar to corrections, except they include data from studies not already included in the database (e.g., were conducted after the AWQC was published).

C) *Deletions of toxicity data for taxa that do not occur at the site*. Perhaps the most critical step in the Recalculation Procedure involves the identification and deletion of organisms which do not occur at the site. First, the updated toxicity database is grouped in decreasing taxonomic order from phylum, class, order, family, genus, and then finally to species. Resident species lists are then used to screen these corrected and updated national databases for each criterion. A detailed process is then used to select which species must be deleted and which species must be retained, and proceeds in a stepwise process in order of increasing taxonomic level from genus to family to order to class, and finally to phylum (Fig. 4-2).

D) *Check minimum data requirements (MDRs).* For the most part, the same MDRs need to be applied to the toxicity database following corrections, additions, and deletions as would any national toxicity database for the original AWQC. However, if a specific MDR can not be satisfied, a taxonomically similar species must be substituted in order to meet the eight MDRs, or new toxicity data would need to be generated. The only exception to this is if the site contains less than a total of eight families, in which case a simplified recalculation procedure may be used.

E) *Recalculate new acute and chronic criteria*. Once all corrections, additions, and deletions are made to the toxicity database, and the MDRs are satisfied, then the acute (CMC) and chronic (CCC) SS-AWQC can be calculated using typical procedures for derivation of national AWQC. And as with national AWQC, the CMC or CCC must be lowered to protect either critical (i.e., ecologically, commercially or recreationally important species) or federally threatened and endangered species if acceptable scientific data suggest the calculated criteria values may not be sufficiently protective of these species

F) *Present results in a report.* Because the Recalculation Procedure critically depends on the comparison and modification of species lists and toxicity databases, the study report must provide detailed documentation to ensure the process is transparent, scientifically defensible, and will achieve regulatory acceptance.

1.3 MODIFIED RECALCULATION PROCEDURE FOR ARID WEST EFFLUENT-DEPENDENT WATERS

Section 5 describes proposed modifications to the standard USEPA Recalculation Procedure that would derive SS-AWQC that should more accurately represent and protect the unique biological conditions typically encountered in arid west effluent-dependent waters. These modifications were based on results from case studies conducted using actual species assemblages in six effluent-dependent waters and AWQC for aluminum, ammonia, copper, diazinon, and zinc. These modifications include the following:

1. *Revise the process of deleting nonresident taxa from the toxicity database*. Two primary changes to the USEPA's deletion process are proposed. First, we recommend that the phrase *occur at the site* be redefined by delineating the organisms that occur at the site into resident and transient species. In the context of conducting the Recalculation Procedure in arid west effluent-dependent waters, *transient* taxa would not be considered to occur at the site, and so would be deleted from the toxicity database. Second, we recommend several changes to the detailed step-wise process used by USEPA with the goal of generating a site-specific toxicity dataset more representative of the species that occur at the site than what would be derived using the standard process.

2. *Revise the 8-family MDRs for FAV calculation*. In many arid west EDWs, the lack of resident salmonid fish or cladoceran zooplankton make it difficult to satisfy these two key MDRs for derivation of the FAV. A possible solution is to create a revised "eight-family rule" that utilizes USEPA methodology and incorporates more typical arid west stream aquatic communities. Redefining the MDRs, or providing suitable surrogate organisms for a particular habitat type would entail replacing current USEPA MDRs that are expected to be non-resident in arid west effluent-dependent streams with organisms of approximately equal sensitivity that would be expected to occur in the river segments. Our proposed revision to the eight-family MDRs are as follows:

   <u>Arid West Stream Eight-Family Rule [AWS-MDRs]</u>

   - a fish in the Family Centrarchidae,
   - a fish in the Family Cyprinidae,
   - a third family in the Phylum Chordata (may be a fish or an amphibian),
   - an aquatic insect,
   - a second aquatic insect in a different order,
   - a benthic crustacean,
   - a family in a phylum other than Arthropoda or Chordata, and
   - a family in any order of insect or any phylum not already represented.

3. *Use SMAVs rather than GMAVs for FAV calculation*. Because arid west EDWs often have small and species-poor aquatic communities, we are proposing that criteria derived during the Recalculation Procedure be calculated from SMAVs rather than GMAVs for a number of reasons. First, the deletion process itself is conducted on a species level rather than a genus level, making it more acceptable to utilize the SMAVs for the FAV calculation. Second, while within-genus toxicity values are relatively consistent (at least more so than higher taxonomic levels), the toxicity of a contaminant to different species within the same genus is not always equivalent. Calculating criteria from the number of species in the database rather than genera can slightly increase the database sample size to help resolve potential sample size effects,

without affecting the protectiveness of the resulting criteria through inclusion of SMAVs for sensitive species (AWWQRP 2006).

It should be noted, however, that these proposed modifications have not yet been submitted to or reviewed by USEPA. Therefore, they should only be considered technical proposals based on the companion study (AWWQRP 2006) and, thus, would have to be approved by regulatory agencies prior to conducting a Recalculation Procedure for a site using any of these modifications.

1.4 COMPARISON OF RECALCULATION PROCEDURE TO OTHER METHODS FOR SITE-SPECIFIC WATER QUALITY STANDARD DERIVATION

While this User's Guide has focused on the Recalculation Procedure, it needs to be placed in the context of other SS-AWQC procedures because no one procedure may be best suited for all sites. Depending on biological or chemical conditions at any given site, the Recalculation Procedure may not always be the best or only choice for maximizing the accuracy of aquatic life protection afforded by water quality criteria. Prior to conducting any SS-AWQC study, therefore, all possible options should be explored and compared to the available data for the site to ensure the approach selected will be both scientifically defensible, and gain regulatory acceptance.

In Section 6, we compare changes in AWQC that might result from procedures other than the Recalculation Procedure (e.g., WER, simplified recalculation, etc.). Case studies from other AWWQRP projects (URS 2002, Parametrix et al. 2003, Parametrix and CEC 2005, Parametrix and HydroQual 2005) provide the basis of this discussion. Specifically, we make these comparisons for acute copper and ammonia criteria given that WER studies were conducted for both criteria chemicals at many of the same study sites. The absolute differences in acute SS-AWQC that would be derived at each case study site are summarized, and we use these as "lessons learned" that may help inform choices among SS-AWQC approaches for effluent-dependent waters in the arid West.

Copper. SS-AWQC for copper were evaluated for several arid west EDWs using the WER, Recalculation Procedure, and the newly proposed Biotic Ligand Model (BLM). Figure 6-1 summarizes SS-AWQC for copper for all of the AWWQRP case study sites. For most sites, a WER would derive the highest (i.e., least restrictive or least conservative) SS-AWQC for copper compared to either the simple hardness equation or the Recalculation Procedure. As discussed in Section 6.1.3, the Recalculation Procedure tends to increase hardness-based AWQC by as much as two-fold if one considers cladocerans (water fleas) to not be true site residents. But further increases in criteria concentrations derived using either the BLM or the WER (particularly for the South Platte and Salt Rivers) suggest that at least for these sites, water quality characteristics can also exert a significant influence on levels of aquatic life protection. Therefore, because both species sensitivity differences *and* water quality characteristics exert strong influences on predicted criteria concentrations, both approaches could be combined to derive a SS-AWQC for these sites.

The BLM results, however, clearly demonstrate the utility of considering the influence of *all* water quality variables when deriving SS-AWQC for waters with elevated hardness. Even though WER-based criteria empirically take into account the influence of all water quality factors on copper toxicity, the WER itself is still applied to a hardness-based copper AWQC which may not be appropriate in some of the hardest waters. This is particularly true for waters with highly elevated hardness levels (e.g., Pinal Creek and Las Vegas Wash) because even though hardness is very high, other water quality characteristics (e.g., very low alkalinity) can still make copper extremely toxic to sensitive organisms such as *C. dubia*. Thus for copper, only the BLM can accurately take into account the influence of all important water quality characteristics on copper toxicity, and so it may be an attractive tool for derivation of fully-protective SS-AWQC. Unfortunately, the application of the Recalculation Procedure to a BLM-derived AWQC is not straightforward owing to the unique method by which the FAV is derived (USEPA 2003). In addition, because many of the toxicity studies did not report all of the water

quality parameters needed to run the BLM, the BLM toxicity database is substantially smaller than that currently used in the hardness-based AWQC (AWWQRP 2006). Application of the Recalculation Procedure to BLM-derived copper criteria represents an area for future study.

Ammonia. The studies described in Section 6.2 suggest that for ammonia, the Recalculation Procedure is not likely to modify acute national AWQC to a significant degree. However, at least for the sites evaluated here, WERs would result in SS-AWQC ranging from a factor of two lower than to a factor of three greater than the national AWQC. For the Recalculation Procedure, site-specific recalculations for ammonia might not be necessary, because the breakdown of warm and cold water habitats proposed in our updated ammonia criteria equations may already account for site-specific differences in arid west streams, making further species-based recalculation efforts unnecessary. Even the 1999 AWQC salmonid-present and salmonid-absent criteria are likely to take into account some of the most significant species-related factors, and so the Recalculation Procedure may be of little utility for ammonia in most cases. The only additional species group to consider would be the unionid clams, which are not adequately addressed in the current national criteria, and so sites with resident populations of unionids might benefit from criteria recalculations to ensure adequate levels of protection.

Even though our studies suggest that WERs may derive SS-AWQC that are up to two-three fold different (both higher and lower) than the national criteria, it is difficult to generalize as to the outcome of a WER study at any other site. This may be because pH and temperature are the most important water quality characteristics modifying ammonia bioavailability and toxicity (but see omission of temperature-based chronic AWQC in the companion report; CEC et al. 2005). Since both factors are already taken into account by the national AWQC equations, few additional factors may warrant selection of the WER (Section 3.3). Unfortunately, our scientific understanding of ammonia bioavailability as a function of hardness, sodium, or other factors is not as well developed as that for metals such as copper (Section 6.1). Therefore, while it is possible that waters with elevated hardness or sodium may alter acute ammonia toxicity enough to support use of a WER, empirical tests would have to be conducted on a site-specific basis to determine whether or to what extent a WER might change site-specific water quality criteria.

2. INTRODUCTION

This User's Guide is intended to provide the general technical background necessary to derive site-specific water quality criteria using the USEPA's Recalculation Procedure. This procedure is one of three primary methods recommended by the USEPA for modifying national Ambient Water Quality Criteria (AWQC) for protection of aquatic life where local conditions indicate these criteria might not be appropriate (i.e., might be over- or underprotective). National AWQC (or criteria) set maximum threshold concentrations of inorganic and organic contaminants to prevent or minimize the exposure of both freshwater and marine organisms to toxic levels of these contaminants. These criteria are derived from empirical toxicity data and are designed to protect all but the most sensitive 5% of species in an aquatic community (and may be lowered to protect sensitive ecologically, recreationally, or economically important species). These AWQC are used for several regulatory purposes, including protection of beneficial uses and derivation of National Pollutant Discharge Elimination System (NPDES) discharge permit levels.

The Recalculation Procedure makes adjustments to the national toxicity database used in derivation of AWQC so that the database better reflects the composition of aquatic communities at a particular site. If properly conducted, the Recalculation Procedure can provide a straightforward and scientifically-defensible means of modifying default AWQC to more accurately reflect the unique biotic assemblages often encountered in surface waters throughout the U.S. This is particularly important for ephemeral and effluent-dependent waters in the arid western U.S. because aquatic assemblages are often unique and contain substantially fewer species compared to perennial waters in other regions (URS 2002). In such cases, concerns have been raised that the surrogate laboratory species used to develop the national AWQC do not adequately represent those encountered in arid west effluent-dependent waters (EDWs). Furthermore, standard methods for conducting a Recalculation Procedure are less statistically robust when the total numbers and types of aquatic species are low, as is often the case in arid west EDWs.

In response to these concerns, the Arid West Water Quality Research Project (AWWQRP) has conducted a thorough evaluation of the Recalculation Procedure in a companion report to this User's Guide titled *Evaluation of EPA Recalculation Procedure in Arid West Effluent-dependent Waters* (AWWQRP 2006). This report conducts a detailed evaluation of the USEPA Recalculation Procedure using five numeric AWQC (aluminum, ammonia, copper, diazinon, and zinc) applied to six effluent-dependent waters located in arid portions of Arizona, California, and Colorado. These case studies provided the basis upon which to evaluate the scientific rigor of the standard Recalculation Procedure for each of the AWQC, and to recommend alternative methods that better reflect the unique biological conditions encountered in these EDWs.

2.1 USER'S GUIDE GOALS AND OBJECTIVES

The purpose of the User's Guide is to assist permit holders applying the USEPA's Recalculation Procedure given the unique biological conditions often present in EDWs. This will not just be a "how-to" guide for use of the Recalculation Procedure, but rather a description of how to apply the procedure given what we learned from the companion report (AWWQRP 2006), as well as other recent AWWQRP studies (Parametrix et al. 2003, Parametrix and CEC 2005, Parametrix and HydroQual 2005). This User's Guide begins by placing the Recalculation Procedure in the context of the full suite of possibilities for deriving site-specific water quality criteria in EDWs. A primary goal of this effort is to help permit holders decide whether the Recalculation Procedure is appropriate for their specific needs and, if selected, how to proceed with an analysis that would achieve both scientific and regulatory acceptance. A secondary goal is to summarize alternative scientific methods for conducting the Recalculation Procedure as recommended in the companion report (AWWQRP

2006) to this User's Guide, and assist readers as to appropriate selection of these vs. standard methods.

2.2 USER'S GUIDE ORGANIZATION

Section 3: Selection of Alternatives for Derivation of Site-specific Water Quality Standards. This section briefly explains USEPA guidance for derivation of AWQC protective of aquatic life, and compares all three standard methods for modifying these AWQC on a site-specific basis: the Recalculation Procedure, the Water-Effect Ratio (WER), and the Resident Species Procedure. Suggestions for selecting the appropriate method will be given.

Section 4: Standard Recalculation Procedure. This section provides a detailed explanation of standard guidance for conduct of the Recalculation Procedure (USEPA 1994a). All six basic steps for conducting a recalculation are reviewed, along with suggestions for their successful implementation and regulatory acceptance. This section does not focus on arid West issues *per se*, but rather on more typical uses of the Recalculation Procedure nationally.

Section 5: Modified Recalculation Procedure for Arid West Effluent-dependent Waters. This section describes modified guidance for conduct of the Recalculation Procedure in arid west EDWs based on results from the companion report to this User's Guide (AWWQRP 2006). This section will also identify primary technical differences and selection criteria differentiating the recommended modifications for arid west EDWs from standard USEPA guidance.

Section 6: Comparison of Recalculation Procedure to Other Methods for Site-specific Water Quality Standard Derivation. While this User's Guide focuses on the Recalculation Procedure, it is essential to place this in the context of other site-specific modification procedures. This is because no one site-specific modification procedure may be best suited for any individual situation. Depending on biological or chemical conditions at any given site, the Recalculation Procedure may not always be the best or only choice for maximizing the accuracy of aquatic life protection afforded by AWQC. Therefore, in this section we compare potential changes in AWQC that might result from procedures other than the Recalculation Procedure (e.g., WER, simplified recalculation, etc.). Case studies from other AWWQRP projects (URS 2002, Parametrix et al. 2003, Parametrix and CEC 2005, Parametrix and HydroQual 2005) provide the basis of this discussion.

Section 7: References.

Appendix A: Full Text of USEPA Recalculation Procedure. The full text of the USEPA's Recalculation Procedure has most recently been published as Appendix B to *Interim Guidance on Determination and Use of Water-Effect Ratios for Metals* (USEPA 1994a). Because it is difficult to obtain an electronic copy of this document, we include here a full-text copy of the Recalculation Procedure guidance for easy reference.

3. SELECTION OF PROCEDURES FOR DERIVATION OF SITE-SPECIFIC WATER QUALITY CRITERIA

As previously discussed, national AWQC set maximum threshold concentrations of inorganic and organic contaminants for both freshwater and marine environments. These criteria are derived from empirical toxicity data and are designed to protect all but the most sensitive 5% of species in an aquatic community. AWQC can also be lowered to protect species which are more sensitive than this lower 5th percentile if they are deemed ecologically, economically, or recreationally important. These AWQC are used for several regulatory purposes, including protection of beneficial uses and derivation of NPDES discharge permit levels.

One major difficulty in applying AWQC to surface waters across the U.S. is that they are derived chiefly from standardized toxicity tests (i.e., uniform types of water and laboratory exposure conditions) using aquatic species that may not be representative of the biota in arid streams of the West. Because the physical and chemical characteristics of the surface waters and the composition of aquatic communities vary markedly in different regions of the U.S., AWQC cannot reasonably be expected to provide a consistent level of protection for all species and all surface waters. Recognizing this limitation, USEPA guidance allows for site-specific modification of AWQC using several methods, including the Recalculation Procedure, the Resident Species Procedure and the Water-Effect Ratio (WER; USEPA 1984c, 1994a). Such methods adjust AWQC magnitudes to reflect differences in species composition and/or water quality characteristics between the site of concern, and those used in laboratory tests to derive the AWQC. The USEPA also expects States and Tribes to adopt AWQC into their own standards either by adopting these criteria, or modifying criteria to reflect site-specific conditions using these or other scientifically defensible methods (USEPA 1999b).

In the sections below, we first briefly summarize general methods and terminology used in derivation of AWQC. Second, we describe the general goals and methods used in each of the three USEPA methods for deriving site-specific AWQC, and close with suggestions for selecting which of these three methods should be used for a particular site or situation.

3.1 AMBIENT WATER QUALITY CRITERIA

To understand how AWQC are developed, the Guidelines and terminology provided by the USEPA (1985c) are briefly summarized below. The first step is to compile acute and chronic toxicity data that meet these USEPA Guidelines. For each species with acceptable acute toxicity data, the species mean acute value (SMAV) is calculated as the geometric mean[1] of available 48 to 96-hr LC/EC50s[2] for each species. The genus mean acute value (GMAV) is then calculated as the geometric mean of available SMAVs for each genus. The 5th percentile of the distribution of available GMAVs is identified as the final acute value (FAV). The FAV is divided by two to determine the criterion maximum concentration (CMC), which is more commonly termed the "acute criterion." This division by two was selected because USEPA recognized that simply using the FAV would represent a chemical concentration that would severely harm 50% of species near the 5th percentile of sensitivity. Dividing

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[1] "Geometric mean" of N numbers is the Nth root of the product of the N numbers. This is used instead of arithmetic means because the distribution of chemical sensitivities of individual species or genera of organisms in toxicity tests are more likely to be lognormal than normal (USEPA 1985c).

[2] These are the two most commonly used endpoints for acute toxicity tests and describe the chemical concentration that impacts 50% of the individuals in a toxicity test population. "LC50" is the median (50%) lethal concentration, and the "EC50" is the median (50%) effects concentration for acute impacts that are not strictly "lethal," such as immobilization."

the FAV by two to calculate the CMC is intended to result in a "LC-low" or concentration that would not adversely affect "too many" of the organisms (USEPA 1985c). The CMC can also be reduced to protect an ecologically, commercially, or recreationally important species if its toxicity test endpoint or SMAV is lower than the FAV (e.g., this was done in the AWQC for cyanide; USEPA 1985b).

Understanding two main aspects of this CMC derivation process are particularly critical to successful implementation of the Recalculation Procedure: (1) minimum toxicity database requirements and (2) the method for calculating the FAV and how it is influenced by the addition or removal of species LC50 values. Sections 3.1.1 and 3.1.2 address these two main aspects of the CMC derivation process.

3.1.1 Minimum Database Requirements

The USEPA uses minimum toxicity database requirements to ensure that the toxicity data collected for a chemical represent a wide taxonomic range of aquatic organisms which, in turn, is assumed to represent the range of species sensitivities in the natural environment (USEPA 1985c). USEPA guidance specifies that, at least for acute criteria development, a minimum dataset must be available for at least eight different families of aquatic organisms, which is more commonly called *the eight-family rule*. In the latest version of USEPA guidance, a minimum of eight was chosen to ensure that the four lowest GMAVs would, by definition, all be in the lowest 50th percentile of available data to limit the amount of extrapolation required to estimate the 5th percentile (FAV) (Stephan 2002). The specific requirements for constructing a toxicity database from at least eight families of freshwater and marine animals are shown in Table 3-1.

Table 3-1. Taxonomic Requirements for Derivation of Freshwater and Marine Final Acute Value

Test Type	Freshwater Families (eight required)	Marine Families (eight required)
Acute	Salmonidae family (Osteichthyes)	Family in phylum Chordata
	Second family in Osteichthyes	Second family in phylum Chordata
	Third family in phylum Chordata	One family in phylum other than Arthropoda or Chordata
	Planktonic crustacean	One member of Mysidae or Penaeidae
	Benthic crustacean	Family not in phylum Chordata
	An aquatic insect	Second family not in Chordata
	Family in phylum other than Chordata	Third family not in Chordata
	Family in any order of insect, or any phylum not already represented	Any other family
Chronic	At least one fish	At least one fish
	At least one invertebrate	At least one invertebrate
	At least one acutely sensitive species (can be marine)	At least one acutely sensitive species (can be freshwater)

3.1.2 Final Acute Value (FAV)

The following summarizes the method used for deriving the FAV, and how it is influenced by additions or deletions of species from the toxicity dataset when conducting the Recalculation Procedure. The FAV is designed to represent the contaminant concentration at which all but the most sensitive 5% of species are protected against acute toxicity (i.e. 50% mortality). Data collected from at least eight families, as designated above (Table 3-1), are reduced down to a ranked set of GMAVs (see Table 3 of most AWQC documents), which are the geometric means of all SMAVs (geometric mean of all acute toxicity data for a given species) for each genus. From these mean acute values (MAVs), a statistical procedure is used to estimate the chemical concentration that corresponds to the 5th percentile level of organism sensitivity from rather than selecting a MAV for a particular species or field situation (Stephan 2002). A curve-fitting procedure is then used to fit a log-triangular distribution model to the GMAV data. If there are fewer than 59 GMAVs available (which is the case for most criteria to date), one only needs to enter the total number of GMAVs, and the toxicity values for the lowest *four* GMAVs in a particular dataset. The equations used by USEPA for calculation of the FAV are as follows:

$$S^2 = \frac{\sum((\ln GMAV)^2) - ((\sum(\ln GMAV))^2 \div 4)}{\sum(P) - ((\sum(\sqrt{P}))^2 \div 4)}$$

$$L = (\sum(\ln GMAV) - S(\sum(\sqrt{P}))) \div 4$$

$$A = S(\sqrt{0.05}) + L$$

$$FAV = e^A$$

Where:

P = cumulative probability for each genus GMAV as $R/(N+1)$

R = rank number of each GMAV in the dataset

N = total number of GMAVs in the dataset

S, L, A = variables for interim calculation steps

This procedure has been shown to provide the most accurate estimate of a FAV that corresponds to a concentration below which all but 5% of the tested species are protected from acute exposure (USEPA 1988). The 1985 Guidelines present a tabular example of a FAV calculation in its Appendix 2 (USEPA 1985c), and is repeated in Table 3-2 below.

Table 3-2. Example Calculation of Final Acute Value from Appendix 2 of USEPA (1985c)

Rank	GMAV	lnGMAV	$(\ln GMAV)^2$	P=R/(N+1)	√P
4	6.4	1.8563	3.4458	0.44444	0.66667
3	6.2	1.8245	3.3290	0.33333	0.57735
2	4.8	1.5686	2.46060	0.22222	0.47140
1	0.4	-0.9163	0.8396	0.11111	0.33333
Sum:		4.3331	10.0750	1.11110	2.04875

N = total number of GMAVs in the dataset = 8

$$S^2 = \frac{10.0750 - (4.3331)^2 \div 4)}{1.11110 - (2.04875)^2 \div 4)} = 87.134$$

$$S = 9.3346$$

$$L = [(4.3331) - (9.3346)(2.04875)] \div 4 = -3.6978$$

$$A = (9.3346)(\sqrt{0.05}) - 3.6978 \ = \ -1.6105$$

$$FAV = e^{-1.6105} \ = 0.1998$$

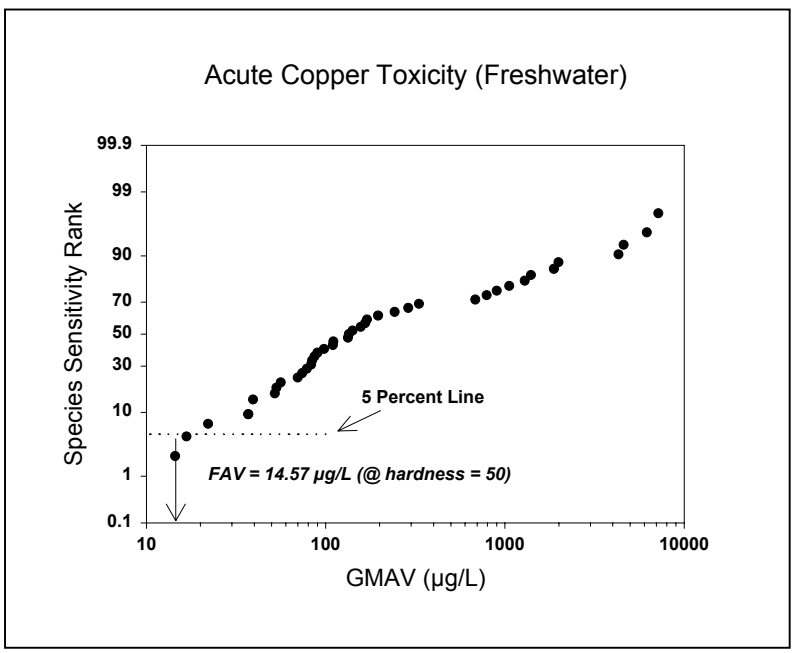


Figure 3-1. Relationship Between FAV and Species Sensitivity Distribution for Copper

A graphical example of the acute species sensitivity distribution for copper—and the FAV concentration estimated from these data—is presented in Fig. 3-1 (USEPA 1996a). Because copper toxicity is hardness-dependent (i.e., acute toxicity values for a given species decrease with increasing hardness), GMAV concentrations and the resulting FAV calculations are normalized to a hardness of 50 mg/L in this example.

One of the most important implications of using this FAV model for the Recalculation Procedure is that the model is inherently more conservative (i.e., generate lower criteria concentrations) when using small toxicity datasets. Numerically, this occurs during calculation of the cumulative probability, *P*, for each MAV which is inversely related to the total number of MAVs, *N*. Therefore, if one were to remove GMAVs from a database, but retain the most sensitive four GMAVs used in the FAV calculation, the resulting FAV would be smaller to be more conservative. While it may be counter-intuitive for a FAV to become smaller after removal of less sensitive species, this is done to be environmentally conservative, and to encourage development of toxicity data (Stephan 2002). As an example, Figure 3-2 illustrates the effect of database size, *N*, on a hypothetical FAV calculation with no changes in the lowest four MAVs. It should be noted that the slope of the line in Figure 3-2 is also highly dependent on the variability amongst the lowest four MAVs, with the effect of database size becoming stronger (i.e., slope of line becoming steeper) when variability is higher.

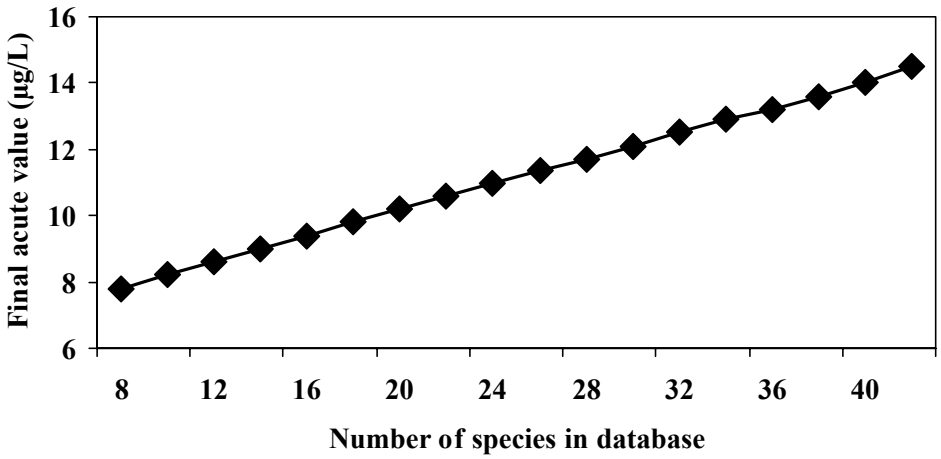


Figure 3-2. Influence of Toxicity Database Size, *N*, on FAV Calculations with No Change to Lowest Four MAVs

3.1.3 Chronic Criterion

The chronic criterion may be derived in a manner similar to the CMC via a FAV-type calculation, but acceptable chronic values must be available for at least eight families (i.e., Table 3-1), which is only rarely achieved. The more common method is to use the Acute-to-Chronic Ratio (ACR), which essentially "corrects" an acute value to provide an estimate of chronic toxicity. In most AWQC, this ratio is used to compare chronic values—of which few typically exist—against acute toxicity values as a means of estimating a Final Chronic Value (FCV). Individual ACRs are derived by dividing each acceptable chronic value (derived from flow-through life-cycle, partial life-cycle, or early life-stage

toxicity test results) into an acute toxicity value for the same species, preferably taken from the same study, or at least the same laboratory using the same and test dilution water conditions (Fig. 3-3). After compiling all acceptable paired data, a species mean ACR is calculated when two or more ACRs exist for a species as the geometric mean of all the individual ACRs. A final ACR (FACR) is then calculated by one of several methods, the most common of which is to take the simple geometric mean of all species mean ACRs. The FCV is then derived by dividing the FACR into the FAV (Fig. 3-3) already derived using the log-triangular curve-fitting procedure described above. Unless other data are available to suggest the FCV is under-protective of the aquatic community (including protection of aquatic plants, and protection from bioaccumulative substances), the criterion continuous concentration (CCC), or chronic criterion, is set equal to the FCV.

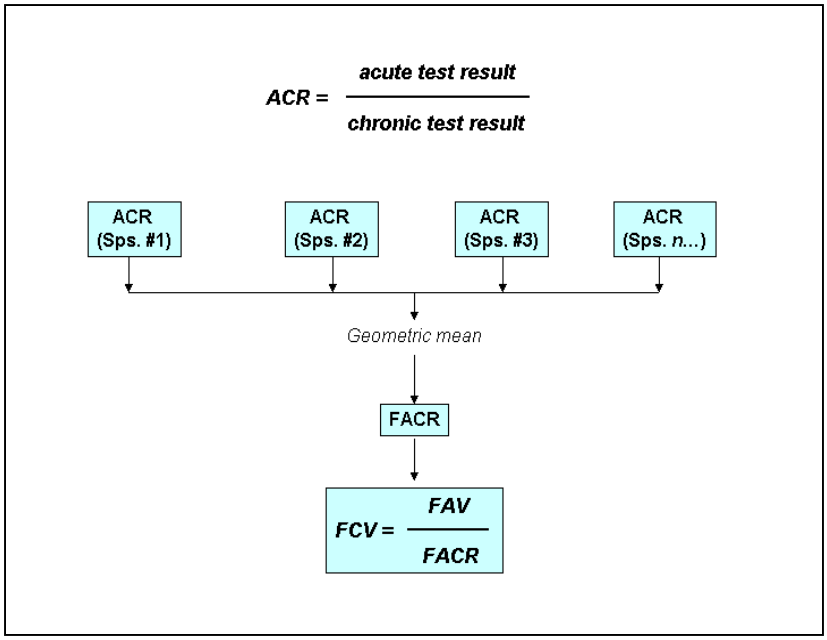


Figure 3-3. Derivation of the Final Chronic Value using the ACR Method

3.2 SITE-SPECIFIC WATER QUALITY CRITERIA

One major difficulty in applying AWQC to surface waters across the U.S. is that they are derived chiefly from standardized toxicity tests (i.e., uniform types of water and laboratory exposure conditions) using aquatic species that may not be representative of the biota in arid streams of the West. Because the physical and chemical characteristics of surface waters and aquatic community composition can vary markedly in different regions of the U.S., AWQC cannot reasonably be expected to provide a consistent level of protection for all species and all surface waters. Toxicity tests have shown that natural waters often can have a substantial effect on the bioavailability[3] and, hence,

[3] "Bioavailability" refers to the degree to which a chemical can be taken up by an organism, subsequently binding to a biologically important site (e.g., cell membrane, gill surface) that initiates a chain of events leading to toxicity.

toxicity of a chemical when compared to typical laboratory waters. Recognizing this limitation, USEPA guidance allows for site-specific modification of AWQC using several methods, including the Recalculation Procedure, the Resident Species Procedure and the Water-Effect Ratio (WER; USEPA 1984c, 1994a). Such methods adjust AWQC magnitudes to reflect differences in species composition and/or water quality characteristics between the site of concern, and those used in laboratory tests to derive the AWQC. General guidance for site-specific criteria modification is available for these three basic sets of procedures, and is outlined in the following sections.

3.2.1 Recalculation Procedure

The Recalculation Procedure provides a means of correcting criteria concentrations if the species at a particular site are substantially different than those used in the toxicity testing to derive the AWQC (USEPA 1984c, 1994a). For example, cold-water fish (e.g., salmonids) often are some of the most sensitive aquatic organisms to metals, but in warm-water streams, an AWQC low enough to protect cold-water fish may be overly conservative, and ecologically irrelevant.

This procedure thus may be used if:

- Some of the toxicity data contained in the AWQC document are for species not resident to a site, *and*;
- No change in the bioavailability or toxicity of the material is anticipated due to water quality characteristics at a particular site.

Under these conditions, species included in the national database, but not resident to the site in question nor representative of species resident to the site, are eliminated and either the FAV or FCV are recalculated as in Section 3.1. Because the Recalculation Procedure is the primary focus of this User's Guide, detailed procedures for its use are presented in Section 4.

3.2.2 Water-Effect Ratio

The Indicator Species, or "Water-Effect Ratio" (WER), procedure is intended to take into account how water quality characteristics affect the toxicity of contaminants (most typically metals) in laboratory dilution water relative to that in site water (USEPA 1984c, 1994a). Briefly, the WER is the quotient of contaminant toxicity (measured as an acute or chronic endpoint) in site water and its toxicity in laboratory water (Fig. 3-4). The default WER is assumed to be equal to one until empirical data can be generated to indicate otherwise. In many cases, the site water toxicity value (e.g., median lethal concentration, LC_{50}), and thus the resulting WER, will be higher than the toxicity value calculated in laboratory waters, as natural waters typically contain materials (e.g., organic carbon) not present in laboratory waters at concentration sufficient to mitigate toxicity. However, in certain situations the toxicity value may be lower in the site water than in laboratory waters. The use of a WER is more likely to provide the intended level of protection (compared to not using a WER) because it takes into account the site-specific modifying factors and potential interactions with other constituents of the site water.

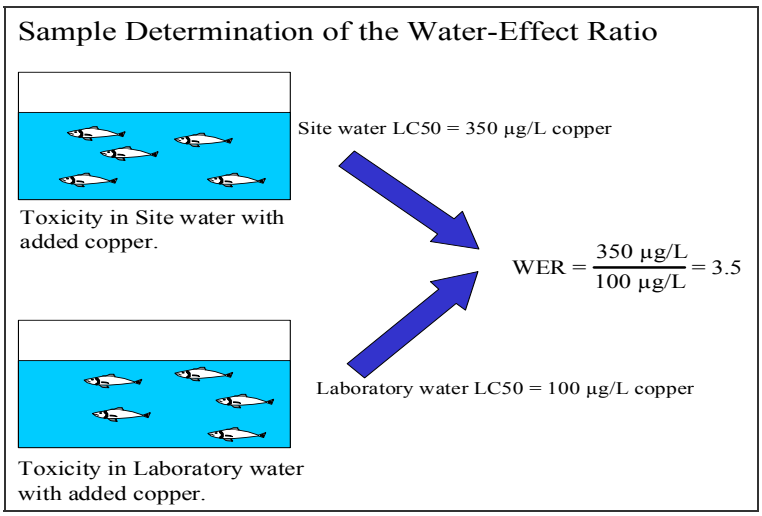


Figure 3-4. Example Calculation of a Water-Effect Ratio

At a minimum, USEPA guidance (USEPA 1994a) requires three rounds of testing when determining a WER, two rounds when flows are 1-2 times design flows (flow used for steady-state wasteload allocation modeling, e.g., the $7Q_{10}$) and one round of testing when flows are between 2-10 times design flows. Standard WER guidance requires that a "primary" test species (i.e., species used for the majority of testing) must be used during these three rounds of testing. It also requires that a "secondary" test species be tested during at least one round of testing to confirm results obtained using the primary species. A "streamlined" WER method is also available specifically for copper in which only one species and fewer testing rounds are required (USEPA 2001). WERs can be applied to total recoverable or dissolved criteria, however, a dissolved WER must be applied to a dissolved criterion and a total recoverable WER must be applied to a total recoverable criterion. The final site-specific AWQC (SS-AWQC) is derived using the WER as follows:

$$SS - AWQC_{TR} = AWQC_{TR} \times WER_{TR}$$

$$SS - AWQC_{D} = AWQC_{D} \times WER_{D}$$

Where:

D = dissolved

TR = total recoverable

3.2.3 Resident Species Procedure

In some situations, it may be desirable to derive criteria based only upon resident species to eliminate uncertainties inherent in extrapolating the chemical sensitivity of non-resident surrogate species to those present at a site. Furthermore, species resident to a particular site may be uniquely adapted to naturally elevated contaminant concentrations and, therefore, be more resistant than the species used to derive the National AWQC. The Resident Species Procedure provides a means of eliminating surrogate species extrapolations, and may also take into account the differential sensitivity of resident species to chemical stressors.

This procedure may be used if:

- The toxicity data contained in the AWQC are for species not resident to a site, *and;*
- Data suggest that significant differences may also exist in the bioavailability or toxicity of the material due to water quality characteristics at a particular site.

The required database for generating a national AWQC is regenerated using resident species exposed to the material in question in site water. However, recent USEPA guidance (USEPA 1994a) on the development and implementation of WERs have reduced the utility of this procedure, because the guidance allows for combining the methods of the Recalculation Procedure and Indicator Species Procedures when generating a site-specific criterion.

3.3 SELECTION OF APPROPRIATE METHOD

If properly conducted, any of three USEPA methods for derivation of SS-AWQC should provide the same levels of aquatic life protection originally intended by the national AWQC. However, as we reviewed in Section 3.2, each method addresses fundamentally different characteristics of the receiving water environment that might indicate whether national AWQC are providing appropriate levels of protection at a given site. Selecting which method, or combination of the available USEPA methods, is most appropriate should thus be based on a thorough scientific understanding of the physical, chemical, and biological conditions at the site of interest.

For the most part, selection amongst these three USEPA methods is based on two primary factors (Fig. 3-5). First, one needs to consider whether the physical or chemical conditions encountered in site waters could affect the bioavailability of the criteria chemical. Because most toxicity tests used to derive national AWQC are conducted in very clean laboratory waters essentially free of total suspended solids, dissolved organic carbon, etc., the bioavailability of test chemicals tends to be relatively high. However, natural waters contain many common chemical constituents that can diminish the bioavailability of a toxic chemical for a variety of reasons. Most of these constituents (e.g., dissolved organic carbon, alkalinity, or total dissolved solids) will tend to reduce the bioavailability by chemically binding to many toxic chemicals before they can interact with an organism, thereby reducing toxicity. If either of these conditions are known to exist for a particular criteria chemical (Fig. 3-5), then the WER would be the method most likely to derive SS-AWQC that are protective of aquatic life at the same level (i.e., 95% genera protection) originally intended by the national AWQC (USEPA 1994a).

Second, one needs to consider whether aquatic organisms which reside at a particular site are more or less sensitive to the criteria chemical than the surrogate organisms contained in the AWQC toxicity database. This is often a difficult question to answer because only a limited number of test species have been successfully used in laboratory toxicity testing, yet their range of response to toxic chemicals is expected to represent the sensitivity of the much wider range of aquatic organisms encountered in natural environments (USEPA 1985c, Stephan 2002). However, some clear differences can be observed between the aquatic community at a particular site and the species represented in the toxicity database for a criteria chemical. For example, entire taxonomic groups of organisms (e.g., genera) might not reside at a site, yet species representative of a non-resident genus may not only be present in the criteria database, but be critical to criteria derivation itself (i.e., be amongst the lowest four GMAVs used to calculate the FAV). In this case, it would be appropriate to use the Recalculation Procedure (Fig. 3-5) to remove this genus from the toxicity database, and recalculate the FAV so that it better represents the sensitivity of organisms that actually reside at the site. It should be remembered, however, that the Recalculation Procedure only addresses differences in chemical sensitivity owing to the *presence or absence* of a particular species, not whether physiological acclimation or adaptation might influence sensitivity. This latter question is instead addressed by the Resident Species Procedure (Section 3.2.3).

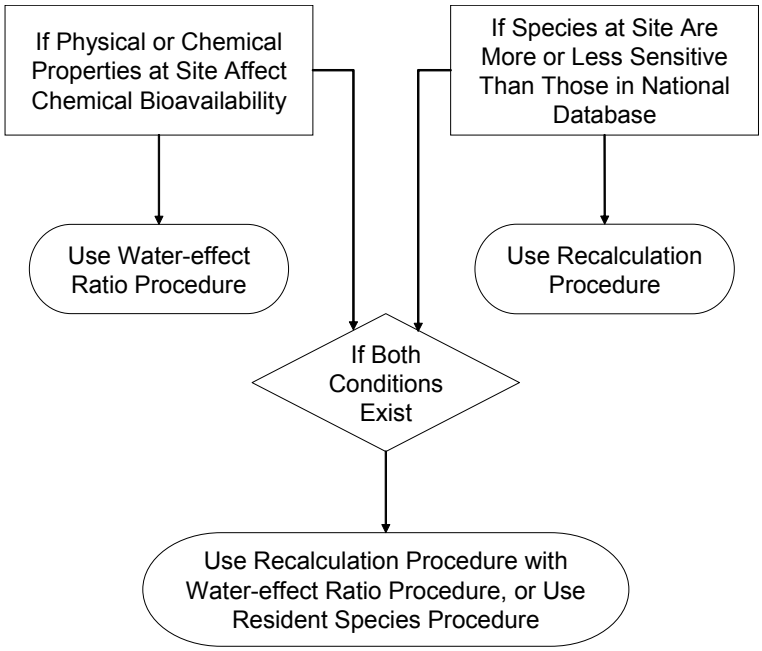


Figure 3-5. Decision Criteria for Selection of Appropriate Method for SS-AWQC Derivation

It is also possible that chemical bioavailability and differences in species composition at a site both will suggest that modifications to the national AWQC would best protect aquatic life. In such a case, two approaches can be followed. First, the most recent USEPA guidance allows for the WER and Recalculation Procedures to be combined (Fig. 3-5) to derive a SS-AWQC (USEPA 1994a). This process entails application of the WER to the recalculated AWQC after appropriate additions, corrections, or deletions of resident species are applied to the toxicity database (Section 4). The combined WER and Recalculation Procedure has the advantage of relative simplicity and low cost, yet still relies on a toxicity database that may not adequately represent all resident species (i.e., some non-resident species sometimes must be retained as surrogates of resident species).

The Resident Species procedure can also be used when both conditions exist at a site (Fig. 3-2), but this procedure is much more costly, and answers different scientific questions than does the combined WER and Recalculation Procedure. Only the Resident Species procedure addresses differences in chemical sensitivity of the resident vs. laboratory test organisms owing specifically to acclimation and adaptation, not necessarily whether a species in the toxicity database is resident to a particular site. Constructing a new toxicity database from entirely resident species also eliminates uncertainties with extrapolating toxicity data from surrogate species not resident to the site. Because of the logistic difficulty in collecting, acclimating, and testing with resident species, this procedure has rarely been used (for an example using dissolved oxygen, see: Camp Dresser & McKee 1994). Yet this procedure holds promise for generating site- or even regional-specific criteria for ephemeral and effluent-dependent streams in the arid West (Parametrix et al. 2003). Species which are resident (or endemic) to these waters are likely to be acclimated or adapted to water quality characteristics that would influence their sensitivity to chemical contaminants (e.g., elevated hardness for metals toxicity). For example, it is well known that the sensitivity of aquatic organisms to metals can depend on both acclimation and adaptation to local conditions (Mulvey and Diamond 1991, Erickson et al. 1997, Welsh et al. 2000a, Barata et al. 2002, Naddy et al. 2003). However, the specific impacts of acclimation or adaptation on the accuracy of criteria concentrations have as yet received little study.

4. STANDARD RECALCULATION PROCEDURE

In this section, we present a detailed guide for conducting the Recalculation Procedure according to the most recent USEPA guidance. This guide will explain each of the steps necessary for successfully changing the toxicity database for a criteria chemical, and explain the underlying biological conditions and constraints inherent in the USEPA's procedure. For reference, we also include the exact text of the Recalculation Procedure as reprinted from Appendix B of the *Interim guidance on determination and use of water-effect ratios for metals* (USEPA 1994a). We conclude this section with a discussion of State-specific considerations that may be needed for successful implementation of the Recalculation Procedure in the arid western U.S.

4.1 OVERVIEW OF THE USEPA RECALCULATION PROCEDURE

National AWQC are to be derived from the most up-to-date toxicity databases for species resident to North America. Established methods for data selection and national criteria derivation are published in *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (USEPA 1985c), as well as Appendix B: The Recalculation Procedure in *Interim Guidance on Determination and Use of Water-Effect Ratios for Metals* (USEPA 1994a; and reprinted as Appendix A of this Users Guide).

General guidelines for conducting a site-specific recalculation were originally published as a companion to the USEPA AWQC derivation guidance document (USEPA 1984c). Since then, more specialized documents have been published (USEPA 1994a, 2001) to ensure the method is properly used. To conduct the Recalculation Procedure, the following basic steps must be followed in this specific order (USEPA 1994a; see also Figure 4-1):

A) Corrections to the national toxicity database,

B) Updates to the national database,

C) Deletions of toxicity data for taxa that do not occur at the site,

D) If new database does not meet minimum data requirements (MDRs), generate the toxicity data necessary to meet MDRs,

E) Recalculate new acute and chronic criteria based on the revised and updated toxicity databases, and

F) Present results in a report.

An explanation of the tasks required for each of these steps is given in Sections 4.1.1 – 4.1.6 below.

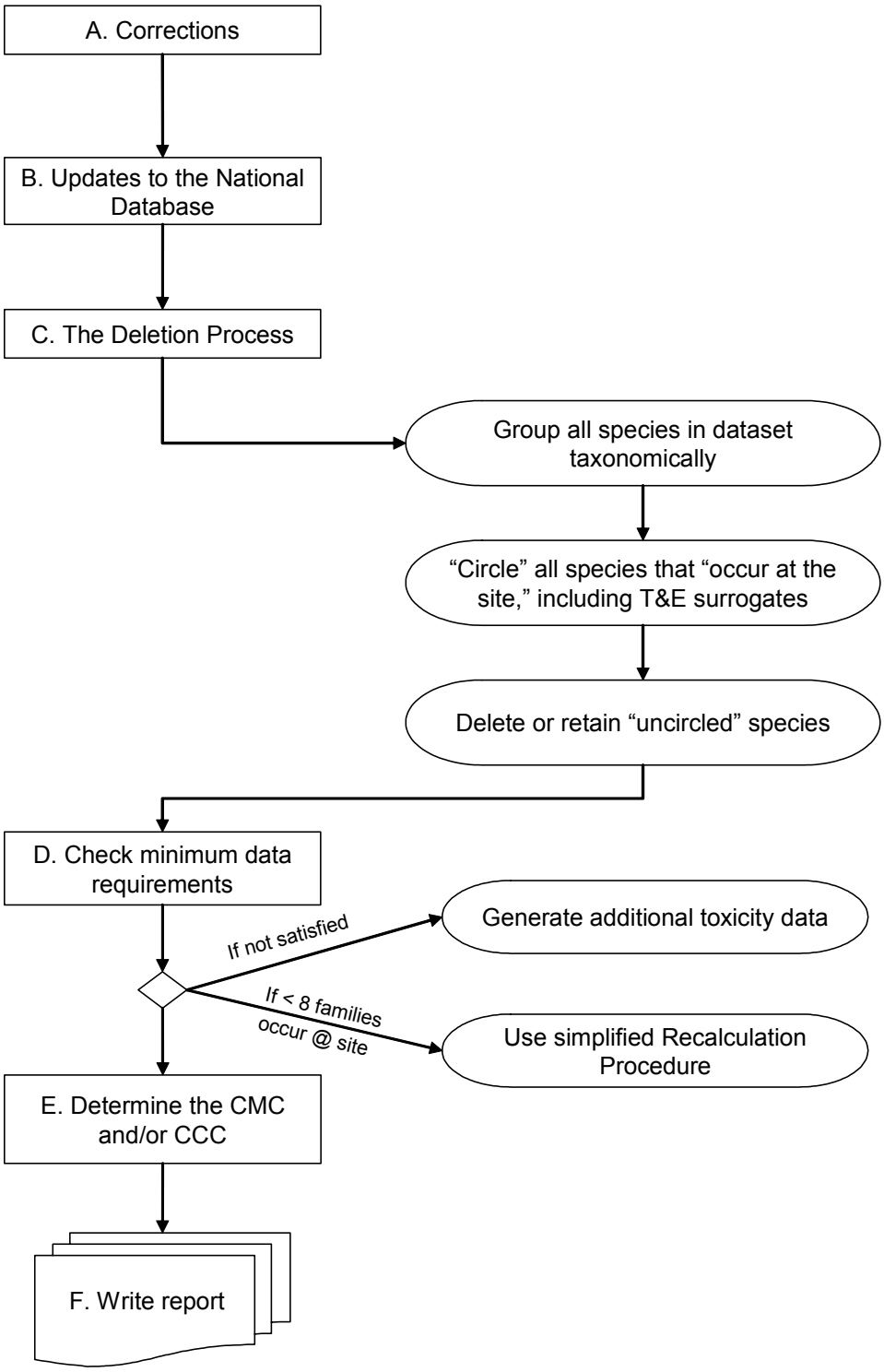


Figure 4-1. Outline of the USEPA Recalculation Procedure

4.1.1 Corrections to the National Database

The first step of the Recalculation Procedure involves correcting the national toxicity database for a given AWQC to ensure the data are of adequate type and quality for derivation of AWQC according to USEPA guidance (USEPA 1985c). These corrections can be of two types:

1. Corrections that are known to, and have been approved by, the USEPA. The best example of such corrections include the "1995 updates" which compiled corrected and updated toxicity data (including new criteria calculations in some cases) for several AWQC (USEPA 1996a).

2. Corrections that have been submitted to the USEPA for approval.

It should be noted that selective corrections are typically not allowed by the USEPA unless pre-approved as in #2 above. Furthermore, the concept of "correction" as defined by the USEPA focuses on data that should have been included in the national database in the first place. As further explained in USEPA guidance:

The concept of "correction" does not include removal of a datum from the national dataset just because the quality of the datum is claimed to be suspect. If additional data are available for the same species, the USEPA will decide which data should be used, based on the available guidance (USEPA 1985c); also, data based on measured concentrations are usually preferable to those based on nominal concentrations.

Therefore, to ensure regulatory acceptance of any criteria calculation, USEPA guidance states it is essential that USEPA approval of any proposed corrections is obtained prior to advancing further in the process. However in practice, corrections to national databases have been accepted by regional USEPA offices and state agencies, without updating the national database, in the derivation of site-specific standards.

4.1.2 Updating the National Database

Updates (or, "additions") to the national database are similar to corrections, except they include data from studies not already included in the database (e.g., were conducted after the AWQC was published). Again, selective additions to the database are typically not allowed, and must be pre-approved by the USEPA. Also similar to corrections, additions consist of two different kinds:

1. Additions that are known to, and have been approved by, the USEPA.

2. Additions that are submitted to the USEPA for approval.

Updating the national toxicity database insures the deletion process is initiated with the most robust database possible and is of particular importance for older criteria (e.g., aluminum and zinc) and criteria with limited national databases.

4.1.3 Deletions of Nonresident Taxa

Perhaps the most critical step in the Recalculation Procedure involves the identification and deletion of organisms which do not occur at the site. According to USEPA guidance, this step can *only* proceed *after* corrections and additions have been made. Furthermore, selective deletions are not allowed, and the specific process outlined below must be followed to ensure regulatory acceptance.

A key component of the recalculation procedure, specifically with regard to deletion of non-resident taxa from the database, is the definition of the phrase "occur at the site." This is a key factor in the potential deletion of *non-resident taxa* from the national toxicity database. The USEPA (USEPA 1994a) defines "occur at site" as the species, genera, families, orders, classes and phyla that:

- are usually present at the site,
- are present at the site only seasonally due to migration,
- are present intermittently because they periodically return to or extend their ranges into the site,
- were present at the site in the past, are not currently present at the site due to degraded conditions, and are expected to return to the site when conditions improve, and
- are present in nearby bodies of water, are not currently present at the site due to degraded conditions, and are expected to be present at the site when conditions improve.

Given the incomplete nature of biological data at some sites, making these determinations can sometimes be difficult, and require significant expert opinion. The ability to do so will directly relate to the quality of the available data, and new data may have to be generated *before* undertaking the deletion process as indicated in USEPA guidance (USEPA 1994a):

a. *Acceptable pertinent toxicological data* ***must*** *be available for at least one species in each class of aquatic plants, invertebrates, amphibians, and fish that contains a species that is a critical species at the site.*

b. *For each aquatic plant, invertebrate, amphibian, and fish species that occurs at the site and is listed as threatened or endangered under section 4 of the Endangered Species Act, data* ***must*** *be available or generated for an acceptable surrogate species. Data for each surrogate species* ***must*** *be used as if they are data for species that occur at the site.*

If these additional data are generated, studies conducted must use acute toxicity test procedures known to be acceptable for derivation of AWQC (USEPA 1985c). Typically, chronic toxicity tests do not need to be conducted because the final ACR for a given criterion (Section 3.1.3, Fig. 3-3) can be used to derive the site-specific FCV.

After a list of resident species for a site is compiled, the formal USEPA (USEPA 1994a, Stephan and Hansen 1997) deletion process can be exercised. First, the updated toxicity database is grouped in decreasing taxonomic order from phylum, class, order, family, genus, and then finally to species. Resident species lists are then used to screen these corrected and updated national databases for each criterion. The deletion process specifies which species must be deleted and which species must be retained, and proceeds in a stepwise process in order of increasing taxonomic level from genus to family to order to class, and finally to phylum (Fig. 4-2).

The first step in the USEPA process is to "circle" all species that are found at the site that are also in the toxicity database; these species must not be deleted (USEPA 1994a). It is important to note the significance of this first step. The USEPA places greater significance on the circled species since they *occur at the site*, and assumes these species better represent a family, order, or class than species that do not occur at the site, but would be retained by the subsequent step-wise process. Such emphasis on "circled species" is very important since the circled species can override the retention of other taxa, while the lack of a circled species can lead to the retention of multiple taxa that are only distantly related.

The remaining species in the toxicity database are then subject to further screening (Fig. 4-2) that is designed to ensure that:

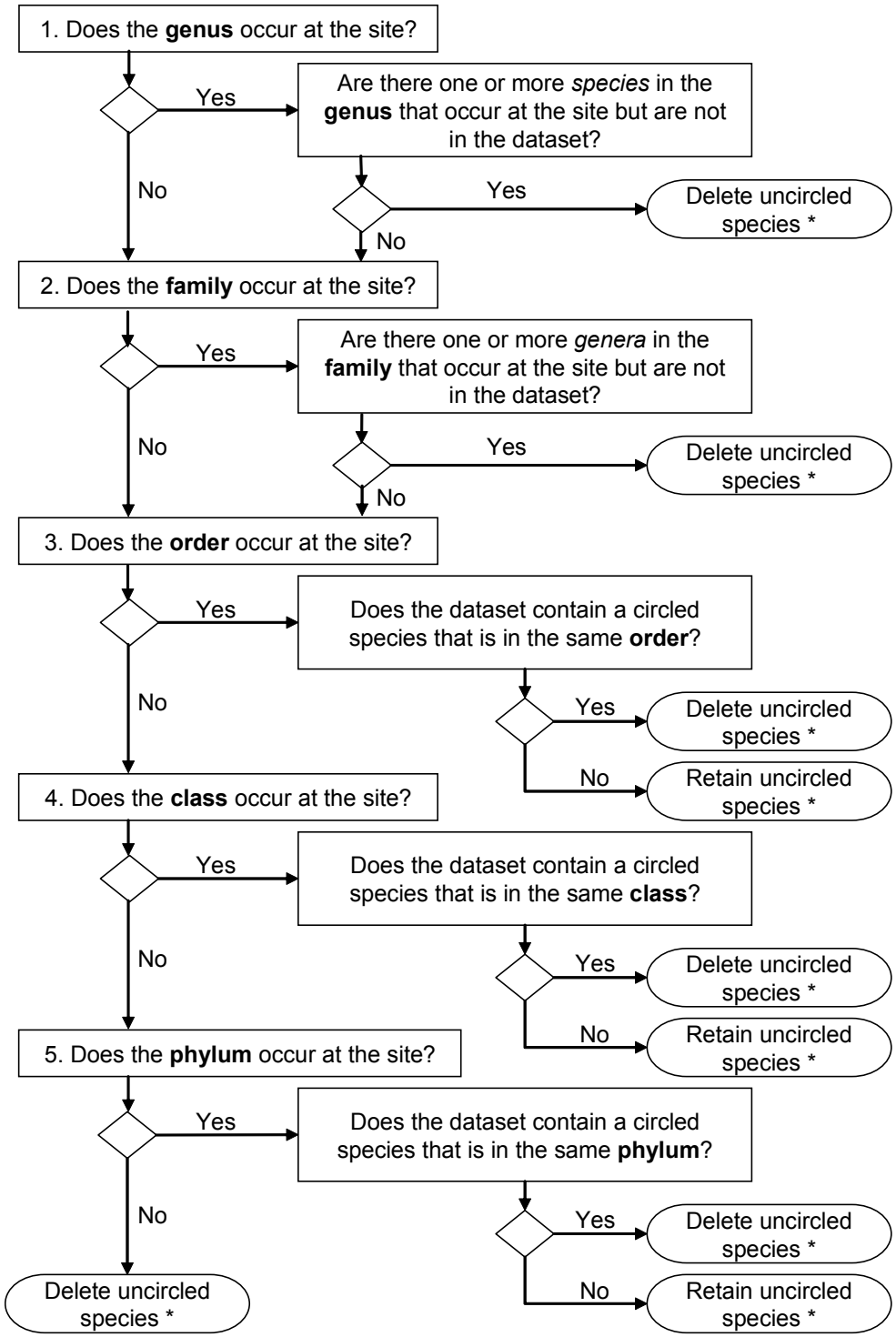
1. Each species that occurs both in the national database and at the site also occurs in the site-specific database,

2. Each species that occurs at the site but does not occur in the national database is represented in the site-specific database by all species in the national data set that are in the same genus,

3. Each genus that occurs at the site but does not occur in the national database is represented in the site-specific database by all genera in the national database that are in the same family, and

4. Each order, class, and phylum that occurs both in the national database and at the site is represented in the site-specific database by one or more species in the national database that are most closely related to a species that occurs at the site (emphasis added).

In its detailed guidance for conducting the Recalculation Procedure (USEPA 1994a), the USEPA provided an example of the deletion process using three different phyla of aquatic organisms. We expanded this example below to represent a more diverse aquatic community and a more realistic scenario, first with a tabulation of the species that occur at the site (Table 4-1).



* Continue deletion process by starting at step 1 for another uncircled species unless all uncircled species in the dataset have been considered.

Figure 4-2. Schematic Representation of the Step-wise Deletion Process

Table 4-1. Species That Occur at the Site

Phylum	Class	Order	Family	Species
Annelida	Hirudinea	Rhynchobdellida	Glossiphoniidae	*Glossiphonia complanata*
Bryozoa	(No species in this phylum occur at the site.)			
Mollusca	Gastropoda	Basomatophora	Physidae	*Physa interga*
Mollusca	Gastropoda	Neritopsina	Neritidae	*Nerita plicata*
Chordata	Osteichthyes	Cypriniformes	Cyprinidae	*Carassius auratus*
Chordata	Osteichthyes	Cypriniformes	Cyprinidae	*Notropis anogenus*
Chordata	Osteichthyes	Cypriniformes	Cyprinidae	*Phoxinus eos*
Chordata	Osteichthyes	Cypriniformes	Catostomidae	*Carpiodes carpio*
Chordata	Osteichthyes	Salmoniformes	Osmeridae	*Osmerus mordax*
Chordata	Osteichthyes	Perciformes	Centrarcidae	*Lepomis cyanellus*
Chordata	Osteichthyes	Perciformes	Centrarcidae	*Lepomis humilis*
Chordata	Amphibia	Caudata	Ambystomidae	*Ambystoma gracile*

Next, the USEPA provided a tabulation of species in these same three phyla that occur in the national database for this hypothetical example (Table 4-2). Note that for each species in the national database, a letter code is assigned that designates which species was deleted or retained (according the process depicted in Figure 4-2), and why each decision was made. These codes are as follows:

S = retained because this species occurs at the site. *This is equivalent to being a "circled" species.*

G = retained because there is a species in this genus that occurs at the site but not in the national database.

F = retained because there is a genus in this family that occurs at the site but not in the national database.

O = retained because this order occurs at the site and is not represented by a lower taxon.

C = retained because this class occurs at the site and is not represented by a lower taxon.

P = retained because this phylum occurs at the site and is not represented by a lower taxon.

D = deleted because this species does not satisfy any of the requirements for retaining species.

Table 4-2. Species That Are in the National Database

Phylum	Class	Order	Family	Species	Code
Annelida	Oligochaeta	Plesiopora.	Tubificidae	*Tubifex tubifex*	P
Bryozoa	Phylactolaemata	Plumatellina	Lophopodidae	*Lophopodella carteri*	D
Mollusca	Gastropoda	Architaenioglossa	Viviparidae	*Campeloma decisum*	C
Mollusca	Gastropoda	Basomatophora	Planorbidae	*Gyraulus circumstriatus*	C
Mollusca	Gastropoda	Basomatophora	Physidae	*Physella gyrina*	C
Mollusca	Gastropoda	Basomatophora	Physidae	*Physa Columbiana*	G
Mollusca	Gastropoda	Basomatophora	Physidae	*Physa heterostropha*	G
Chordata	Cephalaspidomorph	Petromyzontiformes	Petromyzontidae	*Petromyzon marinus*	D

Table 4-2. Species That Are in the National Database

Phylum	Class	Order	Family	Species	Code
Chordata	Osteichthyes	Cypriniformes	Cyprinidae	*Carassius auratus*	S
Chordata	Osteichthyes	Cypriniformes	Cyprinidae	*Notropis hudsonius*	G
Chordata	Osteichthyes	Cypriniformes	Cyprinidae	*Notropis stramineus*	G
Chordata	Osteichthyes	Cypriniformes	Cyprinidae	*Phoxinus eos*	S
Chordata	Osteichthyes	Cypriniformes	Cyprinidae	*Phoxinus oreas*	D
Chordata	Osteichthyes	Cypriniformes	Cyprinidae	*Tinca tinca*	D
Chordata	Osteichthyes	Cypriniformes	Catostomidae	*Ictiobus bubalus*	F
Chordata	Osteichthyes	Salmoniformes	Salmonidae	*Oncorhynchus mykiss*	O
Chordata	Osteichthyes	Perciformes	Centrarcidae	*Lepomis cyanellus*	S
Chordata	Osteichthyes	Perciformes	Centrarcidae	*Lepomis macrochirus*	G
Chordata	Osteichthyes	Perciformes	Percidae	*Perca flavescens*	D
Chordata	Amphibia	Anura	Pipidae	*Xenopus laevis*	C

From this example, five out of a total of 15 species in the national database would be deleted (designated as "D"), and the rest retained because the species itself occurs at the site (designated as "S" or a "circled species"), or because related species occur at the site as defined by USEPA's step-wise process (Figure 4-2). However, this process can generate a few results that appear to deviate from the goal of deriving a database more representative of the site. For example, strictly following USEPA guidance, all Gastropods would be retained at the class level to represent *Nerita plicata,* even though two species were retained at the genus level. If a direct match or a "circled species" existed (*P. integra* was in the database rather than *P. heterostropha*), none of the other species would need to be retained.

Presuming a circled species to be more representative of a distantly related species (e.g., at the class or phylum level) than a species that was retained, but not circled, at a lower level of identification is not necessarily realistic. This is because toxicity tends to most similar among closely-related organisms (e.g., within the same genus) rather than among distantly-related organisms (e.g., within the same class). The resulting database contains additional data that may, or may not be representative of the species at the site. As a result, we have recommended modest changes to USEPA's stepwise deletion process that will result in a toxicity database more representative of a site. These recommendations are included in Section 5 as part of our overall recommendations for changes to the Recalculation Procedure for arid west EDWs.

4.1.4 Checking Minimum Database Requirements

For the most part, the same MDRs need to be applied to the toxicity database following corrections, additions, and deletions as would any national toxicity database for the original AWQC (the *eight-family rule*; Section 3.1.1). However, if a specific MDR can not be satisfied, a taxonomically similar species must, according to USEPA guidance (USEPA 1994a), be substituted in order to meet the eight MDRs:

- *If no species of the kind required occurs at the site, but a species in the same* order *does, the MDR can only be satisfied by data for a species that occurs at the site and is in that* order.
- *If no species in the same order occurs at the site, but a species in the* class *does, the MDR can only be satisfied by data for a species that occurs at the site and is in that* class.
- *If no species in the same class occurs at the site, but a species in the* phylum *does, the MDR can only be satisfied by data for a species that occurs at the site and is in the* phylum.

- *If no species in the same phylum occurs at the site, any species that occurs at the site and is not used to satisfy a different MDR can be used to satisfy the MDR.*

If, after this process, the MDRs can still not be satisfied, additional toxicity data should be collected for the appropriate species to ensure all eight MDRs (even as modified above) can be met. Any additional data would need to be conducted according to procedures which are acceptable for use in AWQC derivation (USEPA 1985c), and then the Recalculation Procedure must be started again at step B (see Figure 4-1) with the addition of these new data. If for some reason the MDRs can still not be met, the AWQC can not be modified according to the standard Recalculation Procedure. However, USEPA does provide a "simplified" procedure that can be used under specific circumstances. This is summarized in Section 4.2 below.

4.1.5 Recalculate CMC and/or CCC

Once all corrections, additions, and deletions are made to the toxicity database, and the MDRs are satisfied, then the acute (CMC) and chronic (CCC) SS-AWQC can be calculated using typical procedures for derivation of national AWQC (Section 3.1). Briefly, the site-specific FAV is first calculated from the lowest four site-specific GMAVs and the total number of GMAVs, then this number is divided by two to derive the site-specific CMC. If a site-specific chronic criterion is needed, then the site-specific FAV is divided by the national FACR, or a site-specific FACR is derived (see Appendix A for details). And as with national AWQC, the CMC or CCC must be lowered to protect either critical (i.e., ecologically, commercially or recreationally important species) or federally threatened and endangered species if acceptable scientific data suggest the calculated criteria values may not be sufficiently protective of these species.

4.1.6 Writing the Report

Because the Recalculation Procedure critically depends on the comparison and modification of species lists and toxicity databases, the study report must provide detailed documentation to ensure the process is transparent, scientifically defensible, and will achieve regulatory acceptance. According to USEPA guidance, the following elements must be included in any report providing the outcome of a Recalculation Procedure study:

1. *A list of all species of aquatic invertebrates, amphibians, and fishes that are known to "occur at the site," along with the source of the information.*
2. *A list of all aquatic plant, invertebrate, amphibian, and fish species that are critical species at the site, including all species that occur at the site and are listed as threatened or endangered under section 4 of the Endangered Species Act.*
3. *A site-specific version of Table 1 from a criteria document produced by the USEPA after 1984.*
4. *A site-specific version of Table 3 from a criteria document produced by the USEPA after 1984.*
5. *A list of all species that were deleted.*
6. *The new calculated FAV, CMC, and/or CCC.*
7. *The lowered FAV, CMC, and/or CCC, if one or more were lowered to protect a specific species.*

4.2 SIMPLIFIED RECALCULATION PROCEDURE

In some cases, a site can either be so small or possess such a limited aquatic community that less than a total of eight families actually occur at the site. Therefore, no matter what national AWQC toxicity data might be available, it would be impossible to satisfy MDRs for the Recalculation Procedure as outlined in Section 4.1.4. USEPA provides "simplified" Recalculation guidance for such a circumstance (USEPA

1994a; see also Appendix A). In this simplified procedure, if less than eight families occur at a site, then the FAV can be set to the lowest available species mean acute value (SMAV). The acute and chronic criteria (CMC and CCC) are calculated from this FAV using typical approaches (Section 4.1.5). According to USEPA guidance, however, this simplified method can *only* be used if:

- Less than a total of eight families occur at the site, *and*
- Data are available for at least one species in each of the families that occur at the site.

In earlier AWWQRP studies (Parametrix et al. 2003), it seemed plausible that this simplified Recalculation Procedure would be particularly useful in ephemeral or effluent-dependent waters given that aquatic assemblages are often quite limited in these waters (URS 2002). In fact, the state of Arizona currently uses a similar approach for deriving site-specific AWQC for metals in ephemeral waters[4] (AZDEQ 1996, 2002). However, at least for the detailed case studies we conducted for six different EDWs in the companion report to this User's Guide (AWWQRP 2006), none of the aquatic assemblages were so limited that an eight-family MDR could not be satisfied. The simplified Recalculation Procedure was thus not required, at least for these effluent-dependent waters.

4.3 APPLICATION BY STATES IN THE WESTERN U.S.

While the Recalculation Procedure is provided as National guidance for derivation of SS-AWQC, its ultimate application to individual sites will be subject to State water quality and NPDES regulations. To evaluate how widely the Recalculation Procedure can be applied from a regulatory point of view, we reviewed water quality regulations in 12 western States with significant arid lands (Table 4-3). While most (nine) of the 12 western States allow for the development of SS-AWQC in their water quality regulations, only Arizona, Colorado, and Idaho specifically mention that the Recalculation Procedure is acceptable for use. Five others, California, New Mexico, Washington, Wyoming, and Montana, can be assumed to allow the Recalculation Procedure based on appropriate references to *The Water Quality Standards Handbook* (USEPA 1994b). For Nevada, SS-AWQC are not mentioned in their water quality regulations, and because it is still a National Toxics Rule (NTR) State, USEPA guidance dictates that the Recalculation Procedure can *not* be used (USEPA 1994a). Therefore, it appears that no more than eight western States specifically allow for use of the Recalculation Procedure.

[4] Arizona is the only state to have an "ephemeral waters" aquatic life use designation, and metals criteria are calculated on the basis of a simplified, generic aquatic assemblage (AZDEQ 1996, 2002).

Table 4-3. Summary of Recalculation Procedure Application in Western States

State	Allow Development of Site-Specific WQS	Regulations Specifically State Recalculation Procedure is Acceptable for Site-Specific WQS Development	Can be Assumed Recalculation Procedure is Acceptable for Site-Specific WQS Development Based on Reference to USEPA (1994b) or Other Guidance	Do Not Mention Development of Site-Specific WQS in Their Regulations
Arizona	X	X [1]		
California	X		X	
Colorado	X	X		
Idaho	X	X		
Montana	X		X	
Nevada				X
New Mexico			X	
Oregon	X [2]			
Texas	X			
Utah	X			
Washington	X		X	
Wyoming	X		X	
Total # of States	10	3	5	1

[1] Recalculation procedure is being explicitly identified as an acceptable site-specific approach under the current triennial review.

[2] Regulations state the department may establish alternative site-specific criteria, but it is unclear if this is related to chemicals.

5. MODIFIED RECALCULATION PROCEDURE FOR ARID WEST EFFLUENT-DEPENDENT WATERS

In the companion report to this User's Guide (AWWQRP 2006), we propose several modifications to the standard USEPA Recalculation Procedure (Section 4) that would derive SS-AWQC that should more accurately represent and protect the unique biological conditions typically encountered in arid west effluent-dependent waters. These modifications were based on results from case studies conducted using actual species assemblages in six effluent-dependent waters and AWQC for aluminum, ammonia, copper, diazinon, and zinc. These modifications include the following:

1. Revise the process of deleting nonresident taxa from the toxicity database (Section 4.1.3)
2. Revise the eight-family MDRs for FAV calculation (Section 3.1.1)
3. Use SMAVs rather than GMAVs for FAV calculation (Section 3.1.2)

Each of these proposed modifications are outlined in sections 5.2 – 5.4 below. It should be noted, however, that these proposed modifications have not yet been submitted to or reviewed by USEPA. Therefore, they should only be considered technical proposals based on the companion study (AWWQRP 2006) and, thus, would have to be approved by regulatory agencies prior to conducting a Recalculation Procedure for a site using any of these modifications.

5.1 SUMMARY OF CASE STUDIES AND RESIDENT SPECIES LISTS

Fish and invertebrate taxa lists were compiled from a literature review to determine what taxa currently occur or could potentially occur at the effluent-dependent streams in this analysis. All stream segments were located downstream of wastewater treatment plants (WWTP) that discharge treated effluent into streams that would otherwise have low or no flow during most of the year (i.e., effluent-dependent stream segments). These sites included:

- Santa Ana River, California
- Salt/Gila Rivers Arizona,
- Santa Cruz River, Arizona (two sites),
- Fountain Creek, Colorado
- South Platte River, Colorado

The effluent-dependent stream sites chosen for this study produced a composite fish species list containing a total of 75 taxa. The number of taxa collected at each stream segment varied from only three non-native fish taxa collected from sites on the Santa Cruz River near Tucson to 40 fish taxa collected from sites on the Salt/Gila Rivers. The taxonomic composition of native fish species found at each stream were most similar to those from nearby geographic locations; this was expected due to the presence of historic/biogeographical barriers (URS 2002).

The effluent-dependent streams chosen for this study produced a composite invertebrate species list containing a total of 561 taxa. The total number of taxa collected over the period of record used in this analysis for each stream varied from 41 taxa collected from the Santa Cruz River near Tucson to 282 taxa collected from the Santa Ana River. As with the fish cluster analysis using all fish taxa, the grouping of the invertebrate communities in these streams seems to be highly influenced by the number of studies, the number of years studied, and methods used in those studies. These species lists were used

as the basis of criteria recalculations for each of the five model AWQC (aluminum, ammonia, copper, diazinon, and zinc), and for proposing modifications to the eight-family MDRs described below (Section 5.3).

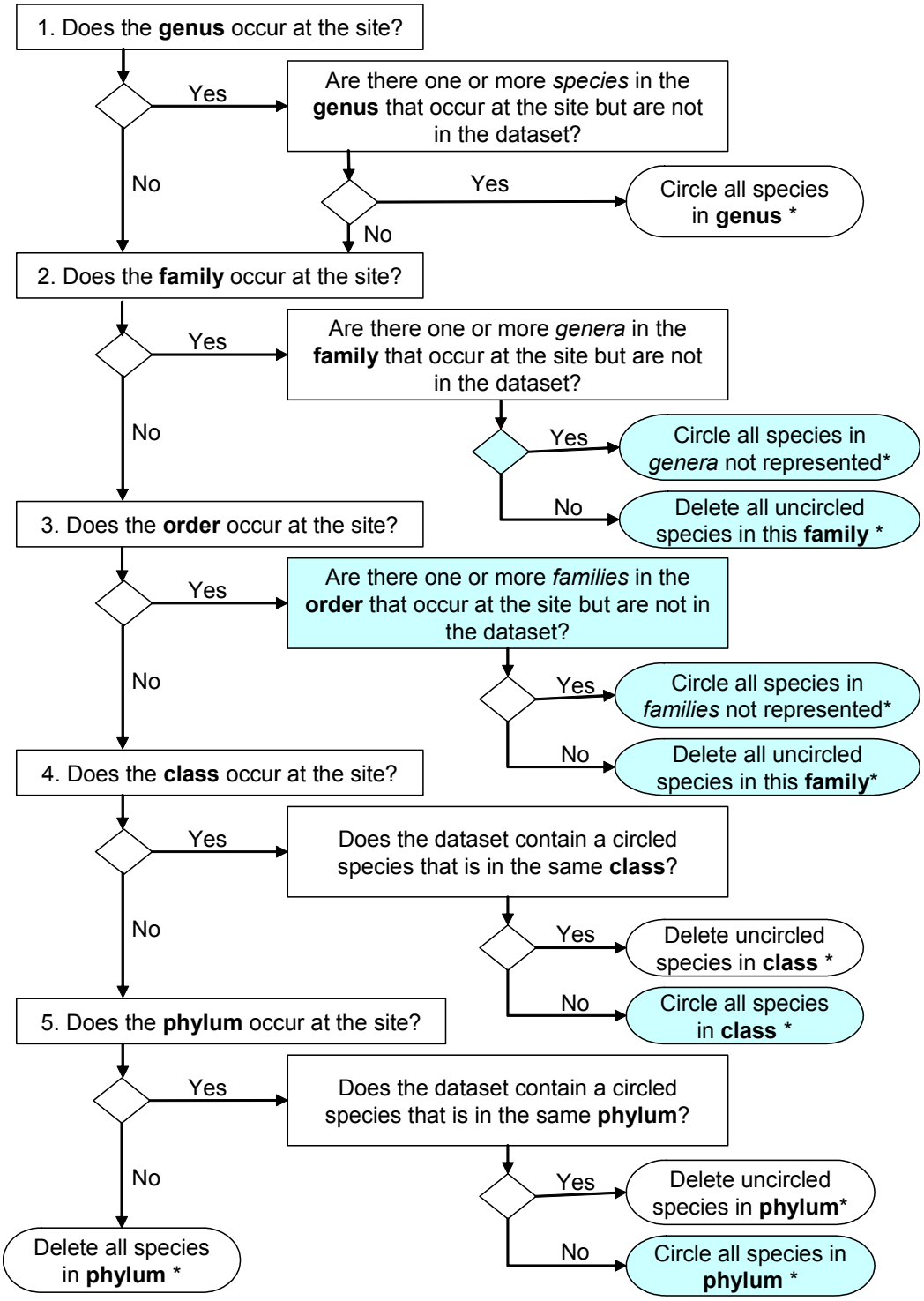
5.2 REVISED DELETION PROCESS

Based on the results of these case studies, two primary changes to the USEPA's deletion process are proposed. First, we recommend that the phrase *occur at the site* be redefined by delineating the organisms that occur at the site into resident and transient species. According to the USEPA, the phrase "occur at the site" includes fish or invertebrates that are usually present at the site, either as year-round residents or as seasonal or intermittent residents, or if not currently present, they are expected to reside within the streams when conditions improve (Section 4.1.3). For our analysis, "occur at this site" is further separated on the basis of whether these organisms are *resident* or *transient* taxa. A resident species is defined as an organism using (or could be expected to use) the habitat located at the site for reproduction, foraging, and/or refuge. A transient species, on the other hand, is defined here as a species that may *occur at the site*, but does not utilize the habitat for these functions, is only passively moving through the site, and is not an important food source for resident species. So in the context of conducting the Recalculation Procedure in arid west effluent-dependent waters, *transient* taxa would not be considered to occur at the site, and so would be deleted from the toxicity database.

Second, we recommend several changes to the detailed step-wise process used by USEPA (Section 4.1.3). After reviewing the standard deletion process, we identified a possible conflict between 1) the step-wise process described by USEPA (Figure 4-2), 2) their accompanying tables that show an example of the deletion process using three phyla (see Tables 4-1 and 4-2), and 3) the previously stated goal of deriving a site-specific database that contains *the most closely related taxa* to taxa found at the site.

The discrepancy occurs during the retention of a species based on an order-level commonality or higher. According to the USEPA step-wise procedure (Figure 4-2), a species is retained only at the order level when the national database does not contain a circled species in the same order of the species being screened. Conversely, the explanation of the *order* code given in the example provided by the USEPA states that the species being screened will be retained if the order occurs at the site and is not represented by a lower taxon, which may or may not be a circled species. This last phrase, *not represented by a lower taxon*, is not consistent with the step-wise procedure when a species is retained, but not circled, at other lower levels of identification (e.g., family). Furthermore, retaining some taxa on a high level of identification (e.g., class and phylum), when a representative in a lower taxon is already retained, but not circled, generally results in a "muddied" database, which is counterintuitive to the primary goal in the recalculation procedure of revising the national database to retain taxa that are most closely related to the species that occur at the site.

To resolve these conflicts, we suggest refining the USEPA step-wise process with the goal of generating a site-specific toxicity dataset more representative of the species that occur at the site than what would be derived using the standard process (Fig 5-1).



* Continue deletion process by starting at step 1 for another uncircled species unless all uncircled species in the dataset have been considered.

Figure 5-1. Schematic Representation of the Modified Stepwise Deletion Process (shaded cells denote changes relative to USEPA process)

The first step would remain the same, which is "circling" all species that satisfy the definition of "occur at the site." Note that circled taxa may be at a higher level of identification than species if no lower level of identification is available for taxa at the site. Some studies used to develop the resident species lists only identified invertebrates to order, family, or genus. When this occurred in the companion case studies, all species in the lowest level of identification are initially circled. For example, we only have "Trichoptera" sampled at the Santa Cruz River site near Nogales. In this situation, all species in the Order Trichoptera were initially circled.

Following the initial circling process, a refined step-wise *circling*[5] *process*, described in Fig. 5-1, is used to determine which of the remaining species in the toxicity database must be deleted and which must be retained. This results in a database that best reflects the taxonomic profile of each site for each criterion. Upon completion of each site-specific database, each database must still satisfy the MDRs in order to proceed with site-specific AWQC derivation for that site. Otherwise, additional toxicity data would have to be generated to create a site-specific database that satisfies MDRs. This was not considered necessary for any of the sites we evaluated in the companion study (AWWQRP 2006).

5.3 REVISED EIGHT-FAMILY MINIMUM DATA REQUIREMENTS

As previously stated, the MDRs for direct calculation of a criterion (Table 3-1) require that the toxicity database contains data for eight diverse families (USEPA 1985c). However, we believe along with the clarification of the deletion procedure outlined in Section 5.2 above, slight modifications of the MDRs may also be warranted given the habitats and organisms expected to occur in arid west effluent-dependent waters. For example, all sites under consideration for recalculation are classified as warm-water segments; therefore, we would not expect to find cold-water taxa such as trout or salmon at, or downstream of, these sites. This can be verified by the review of the resident species lists of the arid west study streams (AWWQRP 2006). Only one of the five sites under consideration for recalculation, Fountain Creek, contains a salmonid (although those fish could arguably be classified as *transients* based on their sampling location and underlying size structure). Eliminating all non-resident trout and salmon for all other sites violates the generalized USEPA MDRs, since a member of the Family Salmonidae is required for a direct criteria calculation.

Furthermore, we would not expect many arid west effluent-dependent stream sites to have resident zooplankton communities. However, the exclusion of zooplankton, including planktonic crustaceans, would be another violation of the "eight-family rule." Of course, zooplankton are not equally represented in all aquatic ecosystems with respect to abundance and ecological significance. In lake ecosystems, these small invertebrates are an important primary consumer, with high biomass and rapid population growth (Wetzel 2001). Zooplankton are essential to lake ecosystem function and an integral component to many food webs. In stream ecosystems, however, the presence of zooplankton are greatly reduced and frequently absent due to habitat limitations, since by definition, zooplankton are unable to withstand stream current. If zooplankton are sampled from high velocity streams/rivers, it is likely that these organisms were washed out of an upstream off-channel lake, pond, or reservoir and have no means of sustaining a population within the stream system without continual contributions from the source populations (Hynes 2001). Zooplankton washed into stream channels are generally thought to be transient species, since densities rapidly decline with downstream distance from the source population (Chandler 1937, Ward 1975, Novotny and Hoyt 1982, Thorp et al. 1994, Phillips 1995, Hynes 2001, Walks and Cyr 2004). In the case of effluent-dominated streams, the source population of zooplankton

[5] Note that in this context, we are changing the terminology from "retained" to "circled" for all conditions in which species should be retained, even those that do not occur at the site. In contrast, USEPA guidance considers "circled" species to only be those that occur at the site.

sampled just downstream of a WWTP discharge could likely be the WWTP tanks and/or ponds themselves (CEC, unpublished sampling data).

A possible solution is to create a revised "eight-family rule" that utilizes USEPA methodology and incorporates more typical arid west stream aquatic communities. Redefining the MDRs, or providing suitable surrogate organisms for a particular habitat type would entail replacing current USEPA MDRs that are expected to be non-resident in arid west effluent-dependent streams with organisms of approximately equal sensitivity that would be expected to occur in the river segments. For example, requiring a salmonid in the database serves two purposes. First, these fish are the dominant top predators in cold-water aquatic ecosystems. Second, salmonids tend to be relatively sensitive to contaminants. However, if obligate cold-water fish are not a resident species, an appropriate surrogate fish Family for the salmonid requirement would be an organism within the Family Cyprinidae or Centrarchidae. Cyprinids represent 22-42% of fish taxa for each of the streams under consideration for recalculation, excluding one site with a limited fish population (AWWQRP 2006). The second most abundant Family represented is Centrarchidae, which can be the top predator in many warm water stream systems. Furthermore, Cyprinids are the most sensitive warm-water fish for three (zinc, ammonia, and diazinon) of the five contaminants considered in the companion case studies. Thus, we suggest that the first two rules of the eight-family rule should be changed to include an organism in the Family Centrarchidae and one in Cyprinidae.

Including zooplankton as a resident species of the arid west streams will likely need to be evaluated on a site-specific basis. If zooplankton are determined to be non-resident, once again the site will be in violation of the MDRs and a surrogate family needs to be established. A potential surrogate for a planktonic crustacean maybe an additional aquatic insect in a family not already represented in the database. The percentage of invertebrate taxa in the arid west streams that are aquatic insects ranges from 59% to 86% (AWWQRP 2006). The toxicity database would better represent invertebrate communities of arid west streams if toxicity databases included information on at least two aquatic insect Orders. Furthermore, all databases under consideration for recalculation contain toxicity data for two aquatic insect families, making this substitution feasible without additional toxicity testing.

Considering the non-resident taxa in the EPA MDRs and the relative importance of other taxa not included in the EPA MDRs, a revised eight-family rule specific for arid west streams is proposed below. *Note that this revised eight-family rule is for the protection of warm water aquatic communities residing in arid west effluent-dependent stream habitats, not in lakes and/or ponds.*

<u>Arid West Stream Eight-Family Rule [AWS-MDRs]</u>

1. a fish in the Family Centrarchidae,
2. a fish in the Family Cyprinidae,
3. a third family in the Phylum Chordata (may be a fish or an amphibian),
4. an aquatic insect,
5. a second aquatic insect in a different order,
6. a benthic crustacean,
7. a family in a phylum other than Arthropoda or Chordata, and
8. a family in any order of insect or any phylum not already represented.

Although the AWS-MDRs better represent potential aquatic communities residing in arid west streams than national MDRs, further exceptions to MDRs may be necessary if one of the above eight families does not reside at a particular site. For example, as noted in the companion study (AWWQRP 2006), San Timoteo Wash (a tributary to the Santa Ana River) does not contain fish due to naturally intermittent flows. Three of the eight families in the AWS-MDRs are for fish (or vertebrates), making it impossible

to meet all eight of the AWS-MDRs (or USEPA MDRs, as well). Additionally, the Santa Cruz River and San Timoteo Wash do not have resident benthic crustaceans. In these situations, requiring at least an eight-family database may be acceptable, or perhaps species that *could* potentially occur at the site may be retained, or the criteria could default to a generalized regional arid west stream criterion. The exact procedure used in these situations will need to be determined on site-specific basis.

5.4 USE OF SPECIES MEAN ACUTE VALUE FOR FAV CALCULATION

AWQC are presently derived from ranked genus mean acute values (GMAV) calculated as the geometric mean of species mean acute values (SMAV; Section 3.1). Furthermore, the number of genera represented in the database rather than the number of species determines database size, or *N*, used in the FAV model (Section 3.1.2). The decision to rank the toxicity databases at the generic level of identification was made for the latest version of the USEPA guidelines (USEPA 1985c), whereas species and even family levels were used in previous versions (Stephan 2002). In the 1985 (latest) version of the guidelines, the genus level was chosen because:

> *On the average, species within a genus are toxicologically much more similar than species in different genera, and so the use of the Genus Mean Acute Values will prevent data sets from being biased by an overabundance of species in one or a few genera.*

For the analysis presented herein, we are instead proposing that criteria derived during the recalculation process be calculated from SMAVs rather than GMAVs for a number of reasons. First, the deletion process itself is conducted on a species level rather than a genus level, making it more acceptable to utilize the SMAVs for the FAV calculation (Great Lakes Environmental Center 2005). Second, while within-genus toxicity values are relatively consistent (at least more so than higher taxonomic levels), the toxicity of a contaminant to different species within the same genus is not always equivalent. Even though the difference in toxicity between species may be small (< a factor of 10; e.g., *Physa* sp. for zinc), using a GMAV still effectively dilutes the numeric impact of the more sensitive species. Other genera contain species with highly divergent (> a factor of 10-100) toxicity values (e.g., *Catostomus*, *Oncorhynchus*, *Daphnia*, *Morone*, *Gammarus*). In these situations, only the SMAV for the most sensitive species is used in the GMAV calculation and valid data for other species in the genus are lost. Third, little overlap of arid west resident species lists and species within the various toxicity databases can lower the criterion if derived at the GMAV level. This is because the FAV derivation procedure calculates a more conservative criterion when database size is small (Section 3.1.2; Fig. 3-2). A lower criterion due to a reduction in database sample size, rather than the presence of more sensitive species, may thus be artificially over-protective of the arid west stream community. Calculating criteria from the number of species in the database rather than genera can slightly increase the database sample size to help resolve potential sample size effects, without affecting the protectiveness of the resulting criteria through inclusion of SMAVs for sensitive species (AWWQRP 2006).

6. COMPARISON OF RECALCULATION PROCEDURE TO OTHER METHODS FOR SITE-SPECIFIC WATER QUALITY STANDARD DERIVATION

While this User's Guide has focused on the Recalculation Procedure, it needs to be placed in the context of other SS-AWQC procedures because no one procedure may be best suited for all situations. Depending on biological or chemical conditions at any given site, the Recalculation Procedure may not always be the best or only choice for maximizing the accuracy of aquatic life protection afforded by water quality criteria. Prior to conducting any SS-AWQC study, therefore, all possible options should be explored and compared to the available data for the site to ensure the approach selected will be both scientifically defensible, and gain regulatory acceptance.

In this section we will compare changes in AWQC that might result from procedures other than the Recalculation Procedure (e.g., WER, simplified recalculation, etc.). Case studies from other AWWQRP projects (URS 2002, Parametrix et al. 2003, Parametrix and CEC 2005, Parametrix and HydroQual 2005) will provide the basis of this discussion. Specifically, we will make these comparisons for acute copper and ammonia criteria given that WER studies were conducted for both criteria chemicals at many of the same study sites. The absolute differences in acute SS-AWQC that would be derived at each case study site will be summarized, and we will use these as "lessons learned" that may help inform choices among SS-AWQC approaches for effluent-dependent waters in the arid West.

6.1 COPPER

The most recent AWQC document for copper (Cu) was published in 1984 (USEPA 1984a), with updates to the acute and chronic toxicity database and criteria calculations being published in the "1995 Updates" (USEPA 1996a). Like many metals, the freshwater copper criteria are hardness-dependent, with toxicity decreasing linearly with increasing hardness. While it is widely known that "hardness" is only a surrogate indicator of the actual mechanistic role of numerous other chemical factors (Section 6.1.4), until now it has been the chemical factor of choice for modifying metals AWQC to represent site-specific chemical conditions. For such metals, AWQC magnitudes (i.e., concentrations) are presented as hardness-dependent mathematical equations, rather than as single values:

$$CMC = e^{(0.9422[\ln(hardness)]-1.700}\,,$$

and:

$$CCC = e^{(0.8545[\ln(hardness)]-1.702)}\,.$$

At hardness values of 50, 100, and 200 mg/L, this is equivalent to a CMC of 7.3, 14, and 27 µg/L, respectively, and a CCC of 5.2, 9.3, and 17 µg/L, respectively. No changes were made to these criteria in the most recent compilation of national AWQC (USEPA 2004), except that these criteria can also be expressed as dissolved metal concentrations after multiplying by a default correction factor of 0.960.

6.1.1 Hardness-dependent Criteria in Very Hard Waters

Since the inception of the hardness-based criteria for copper, the upper limit for regulatory application of these equations has been set at 400 mg/L as $CaCO_3$ (USEPA 1984b, 2002). However, copper toxicity in very hard surface waters, such as effluent-dependent streams of the arid western U.S., may not be accurately represented by this equation because hardness can far exceed this value in many cases (URS 2002, Parametrix et al. 2003, Parametrix and HydroQual 2005). At the present time, there are only two formal recommendations from the USEPA for calculating a site-specific copper criterion in waters with hardness greater than 400 mg/L as $CaCO_3$ (USEPA 2002):

1. calculate the criterion using a default WER of 1.0 and using a hardness of 400 mg/L in the hardness equation; or
2. calculate the criterion using a WER and the actual ambient hardness of the surface water in the equation.

The first alternative simply suggests that all wastewater discharges into streams with hardness greater than 400 mg/L as $CaCO_3$ should be permitted at 400 mg/L regardless of actual site water hardness. The second uses WER studies to empirically verify the extent to which all chemical characteristics at a site might influence toxicity, and is applied to an AWQC at the ambient hardness.

To evaluate the impact of these approaches on SS-AWQC, we compiled hardness-dependent copper criteria for each of seven effluent-dependent waters with hardness values ranging from 65 mg/L to over 1000 mg/L (Table 6-1; Parametrix and HydroQual 2005). In this table, we list the study sites, their hardness, several methods for deriving SS-AWQC for copper, and the acute toxicity (expressed as a median lethal concentration or LC50) of copper in each of the site waters to the most sensitive test organism used in the study, the water flea *Ceriodaphnia dubia*. The purpose of including these toxicity data is that if a SS-AWQC is adequately protective of aquatic life, this criterion concentration should be lower than the LC50 of the most acutely sensitive species when exposed to copper in a given site water. Conversely, if the SS-AWQC is greater than the LC50 of the most sensitive species in a given site water, then the SS-AWQC may not be adequately protective of aquatic life.

While the first approach (use no greater than 400 mg/L in the hardness equation) would be protective for the Las Vegas Wash (i.e., *C. dubia* LC50 value was greater than the hardness-based criterion noted as "HB" in Table 6-1), a criterion for Pinal Creek would be extremely under-protective of acutely sensitive species (i.e., *C. dubia* LC50 value was ten times lower than the recommended hardness-based criterion; Table 6-1). Results from the Albany Drainage Swale (hardness = 294 mg/L as $CaCO_3$) draw a similar conclusion of under-protection where the hardness equation produced a criterion equal to the LC50 for *C. dubia*. Deriving a criterion using the hardness equation capped at 400 mg/L thus can generate SS-AWQC that are equal to or greater than observed LC50 values for *C. dubia* from the actual site water.

6.1.2 Water-Effect Ratio (WER)

In AWWQRP's Biotic Ligand Model (BLM) validation study (Parametrix and HydroQual 2005), we conducted WER studies in seven effluent-dependent waters using the water fleas *C. dubia* and *Daphnia magna*, as well as the fathead minnow, *Pimephales promelas*. For *C. dubia*, WERs under base-flow conditions ranged from near 1 (Pinal Creek, AZ, and drainage swale, Albany, OR) to over 12 in Sandia Canyon. Thus, for all but two sites, water quality characteristics were such that copper toxicity was reduced 2-12 fold, meaning that SS-AWQC could be 2-12 times higher (i.e., less restrictive) than the national criteria, yet provide the same levels of protection to aquatic life.

To convert this WER into a SS-AWQC, the WER was multiplied by the ambient hardness-based AWQC (criteria labeled as "SS" in Table 6-1). Whereas the first USEPA option for addressing very hard waters (cap the hardness equation at 400 mg/L with no WER; Section 6.1.1) is thought to result in a more protective aquatic life criterion, the second hardness-based option (multiply actual site hardness by the WER) is expected to result in the level of protection that is intended from the original guidelines (USEPA 1984c, 1985c). However, this scenario can still result in SS-AWQC that are under-protective of the most acutely sensitive species. For example, SS-AWQC generated from the hardness equation (based on ambient hardness) multiplied by observed WER values for *C. dubia* in water from Las Vegas Wash and the Salt River were approximately two times greater than their corresponding LC50 values in each of the site waters (Table 6-1). Even the Sandia Canyon site (relatively low hardness) would result in a site-specific criterion equal to the observed LC50 for *C. dubia*. Thus, even though WER-based criteria tended to be less restrictive than hardness-based criteria, both of USEPA's currently available options

concerning the influence of water quality on site-specific copper criteria may not be adequately protective of the most acutely sensitive aquatic life in all waters with elevated hardness.

Table 6-1. Comparison of Different Methods for Deriving SS-AWQC for Copper (Parametrix and HydroQual 2005)

Location		Hardness	*C. dubia* 48-h	Dissolved Cu	Copper Acute Water Quality Criterion (µg/L)				
(City, State)	Drainage	(mg/L as $CaCO_3$)	LC50 (µg/L)	WER	HB[b]	SS[c]	BLM (1)[d]	BLM (2)[e]	BLM (3)[f]
Los Alamos, NM	Sandia Canyon	65.8	130.7	12.53	9.4	118.2	51.3	51.2	53.3
Las Vegas, NV	Las Vegas Wash	794.4	206.9	5.24	51.7	516.5	85.1	79.7	91.9
Globe, AZ	Pinal Creek	1213.8	5.4	1.07	51.7	157.8	3.5	3.4	3.7
Albany, OR	Drainage Swale	293.8	36.3	0.98	38.6	38.0	17.1	14.0	17.1
Riverside, CA	Santa Ana R.	218.1	58.7	2.35	29.2	68.6	34.4	31.5	35.8
Denver, CO	South Platte R.	230.9	151.2	8.35	30.8	257.1	84.7	83.4	91.6
Phoenix, AZ	Salt R.	283.6	178.5	9.03	37.4	337.7	88.2	86.5	94.1

[a]Hardness values, dissolved Cu median-lethal concentrations (LC50), Water-Effect Ratio (WER) values, and BLM predictions for sites with multiple sampling events (i.e., Santa Ana River, South Platte River, and Salt River) are presented as the geometric mean for all events.

[b]Hardness-based (HB) criterion = exp(0.9422*ln(Hardness)-1.700); hardness greater than 400 mg/L as CaCO3 calculated as 400 mg/L (USEPA 1984a).

[c]Site-specific (SS) criterion = Hardness-based criterion (ambient hardness) * dissolved Cu WER.

[d]BLM (1) predictions based on measured quality data (i.e., unmodified input data).

[e]BLM (2) predictions based on adjustments to alkalinity, calcium, and magnesium concentrations following considerations of carbonate complexation/precipitation.

[f]BLM (3) predictions based on adjustments to alkalinity, calcium, and magnesium concentrations following consideration of carbonate complexation/precipitation and incorporation of magnesium-gill interaction (i.e., Mg-gill included in model; affinity characterized by long K = 3.6).

6.1.3 Recalculation Procedure

In the companion Recalculation Procedure study, SS-AWQC for copper (and ammonia, see Section 6.2) were derived using the modified procedure (Section 5) for six case study sites in five effluent-dependent waters (Table 6-2). For comparisons of recalculated SS-AWQC to national criteria, the equations or CMC values for each contaminant and each site were solved for the mean hardness (copper) and pH (ammonia) of each site, as appropriate. Historical ambient water quality data for the study streams were derived using water quality data presented in the Arid West Habitat Characterization Study (HCS) (AWWQRP 2006) and from the BLM validation study (Parametrix and HydroQual 2005).

Table 6-2: SS-AWQC Derived Using the Modified Recalculation Procedure (AWWQRP 2006)

	Site-Specific CMC					
	Santa Ana River	Santa Cruz River		Salt/ Gila River	Fountain Creek	South Platte River
		Near Nogales	Near Tucson			
Hardness (mg/L)	188	170	150	388	218	280
pH	7.2	7.5	7.2	7.4	7.4	7.4
Ammonia (mg TA-N/L)	28.35 (27.52)	18.53 (18.53)	28.47 (27.52)	21.16 (21.40)	22.05 (21.40)	21.62 (21.40)
Copper (µg dissolved Cu/L)	29.93 (16.96)	27.84 (15.36)	21.32 (13.59)	63.36 (34.49)	35.18 (19.57)	45.68 (25.05)

NOTES: NA = Data were not available to derive criteria for that site – see Chapter 9 for discussion. Values in () = updated national acute criterion, given site hardness or pH, for comparison.

The modified recalculation procedure for copper provided substantial site-specific differences in criteria concentrations in arid west study streams compared to updated national criteria (Table 6-2). Unlike ammonia (Section 6.2), we found substantial increases in all site-specific criteria (i.e., were less restrictive) compared to national or updated national AWQC. This was primarily a result of deleting non-resident cladocerans.

6.1.4 Biotic Ligand Model

Recent AWWQRP research has shown that hardness is a poor predictor of copper toxicity in very hard surface waters (Gensemer et al. 2002, Parametrix et al. 2003). Therefore, hardness-based SS-AWQC are not likely to provide accurate measures of aquatic life protection, and more complex approaches are required. Despite extensive research related to the chemical interactions between copper toxicity and individual water quality parameters (e.g., pH, dissolved organic matter and major ions) and their effects on aquatic biota (Lauren and McDonald 1986, Welsh et al. 1993, Erickson et al. 1996, Welsh et al. 2000a, Welsh et al. 2000b, De Schemphelaere and Janssen 2002), EDWs present unique combinations of water quality parameters that are not adequately represented by hardness-based equations. For example, hardness and alkalinity do not necessarily co-vary in surface waters of the arid western U.S. as they do in most natural systems (Gensemer et al. 2002, Parametrix et al. 2003). As a result, hardness equations may not accurately represent the more realistic and complex factors which control copper toxicity in very hard waters.

USEPA's latest draft AWQC for copper (USEPA 2003) uses the BLM, which is a new scientific approach for deriving SS-AWQC based on several water quality parameters, and their collective effect on the bioavailability of copper to aquatic organisms. Unlike the hardness equation, this type of model explicitly accounts for the mechanistic influences of individual water quality variables on copper toxicity. The basic presumption is that any changes in water quality that decrease the concentrations of copper (primarily Cu^{2+} and $CuOH^{-}$ to a lesser degree) which can chemically bind to biological surfaces (i.e., the "biotic ligand") are associated with decreasing copper toxicity (Di Toro et al. 2001, USEPA 2003). For example, increases in pH, alkalinity, or natural organic matter would all tend to decrease copper bioavailability and, hence, increase median-lethal concentrations (LC50) for copper (Erickson et al. 1996). Copper bioavailability may also be affected by competitive chemical interactions at the biotic ligand (e.g., fish gill) with calcium and sodium, thereby increasing copper LC50 values (Erickson et al. 1996).

Predictions of copper toxicity are made by assuming that the dissolved copper LC50, which varies with water chemistry, is always associated with a fixed critical level of copper accumulation at the biotic ligand. This critical level is termed the median lethal accumulation level, or "LA50." To derive the FAV in the 2003 draft copper AWQC, copper sensitivity was ranked relative to species-specific LA50 values and the acute criterion was established that simulated the physiology of a hypothetical organism that was more sensitive than 95% of all freshwater fauna. As a tool for deriving site-specific criteria, the BLM predicts the acutely toxic concentration of copper for this hypothetical organism, based on the actual water quality conditions in the waterbody of concern. This approach represents a significant departure from the current hardness-based copper criteria, and so long as the BLM is adequately validated for a wide range of water quality conditions, it should provide more scientifically-defensible site-specific water quality criteria.

In the AWWQRP BLM validation study (Parametrix and HydroQual 2005), results suggested that the BLM offers an improved alternative to both of these current site-specific methods for modifying copper criteria, particularly for situations where the hardness equation and WER approach would continue to under-protect sensitive aquatic life. To illustrate this, the BLM was used to predict LC50 values and calculate SS-AWQC for copper at each of the sites used in this study (Table 6-1). In contrast to either of the standard USEPA methods discussed in Sections 6.1.2 and 6.1.3, the BLM-derived acute criterion for copper was protective of *C. dubia* for all seven study sites (i.e., was lower than LC50 values), regardless of the manipulations that were made to the input data (Table 6-1). This was true whether no modifications were made to the model or input data (results labeled BLM(1) in Table 6-1), or whether modifications were made to improve the accuracy of model predictions (results labeled BLM(2) and BLM(3) in Table 6-1).

6.1.5 Summary

The examples presented here for copper illustrate the range of potential criteria concentrations that can be calculated using existing (Recalculation Procedure and WER) and proposed (BLM) approaches for deriving SS-AWQC. While selecting any one of these approaches should be based primarily upon the chemical vs. biological conditions at the site of interest (Section 3.3), final selections can sometimes be guided by the anticipated change in the SS-AWQC vs. the default national criteria. It is always advisable to conduct a preliminary feasibility study that evaluates the strengths and weaknesses of each SS-AWQC approach before embarking on a definitive study.

Figure 6-1. Comparisons of Acute SS-AWQC for Copper

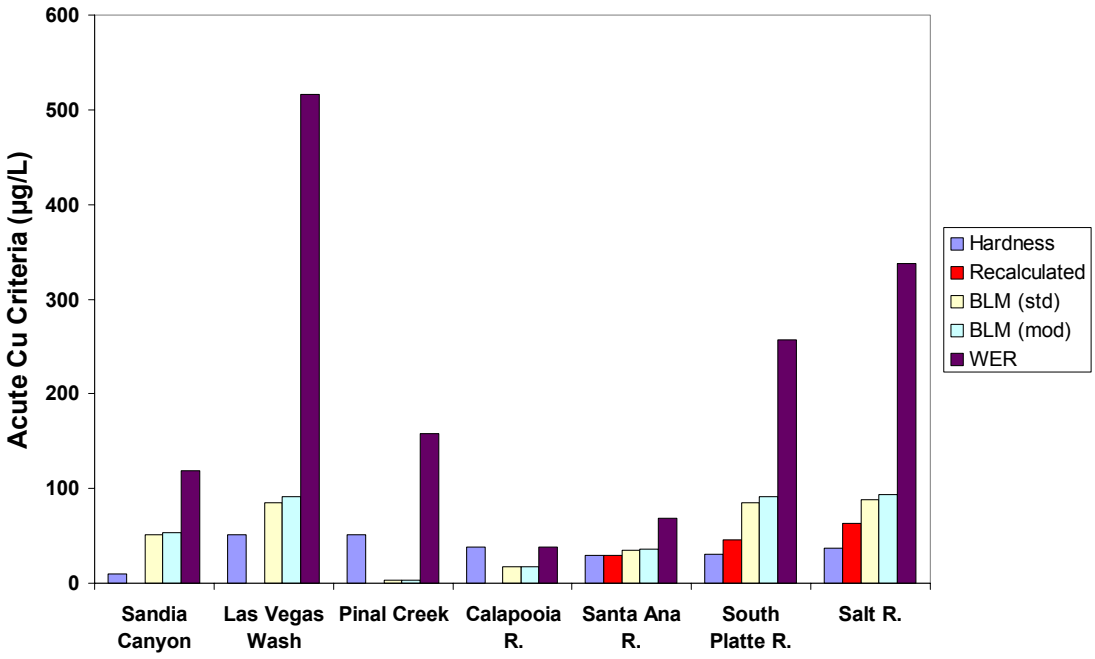


Figure 6-1 summarizes SS-AWQC for copper for all of the AWWQRP case study sites. For most sites, a WER would derive the highest (i.e., least restrictive or conservative) SS-AWQC for copper compared to either the simple hardness equation or the Recalculation Procedure. As we pointed out in Section 6.1.3, the Recalculation Procedure tends to increase hardness-based AWQC by as much as two-fold if one considers cladocerans (water fleas) to not be true site residents. But further increases in criteria concentrations derived using either the BLM or the WER (particularly for the South Platte and Salt Rivers; Fig. 6-1) suggest that at least for these sites, water quality characteristics can also exert a significant influence on levels of aquatic life protection. Therefore, because both species sensitivity differences *and* water quality characteristics exert strong influences on predicted criteria concentrations, USEPA guidance suggests that both approaches should be combined to derive a SS-AWQC for these sites (Section 3.3).

The BLM results, however, clearly demonstrate the utility of considering the influence of *all* water quality variables when deriving SS-AWQC for waters with elevated hardness. Even though WER-based criteria empirically take into account the influence of all water quality factors on copper toxicity, the WER itself is still applied to a hardness-based copper AWQC which may not be appropriate in some of the hardest waters. This is particularly true for waters with strongly elevated hardness levels (e.g., Pinal Creek and Las Vegas Wash) because even though hardness is very high, other water quality characteristics (e.g., very low alkalinity) can still make copper extremely toxic to sensitive organisms such as *C. dubia*. Thus for copper, only the BLM can accurately take into account the influence of all important water quality characteristics on copper toxicity, and so it may be an attractive tool for derivation of fully-protective SS-AWQC. Unfortunately, the application of the Recalculation Procedure to a BLM-derived AWQC is not straightforward owing to the unique method by which the FAV is derived (USEPA 2003). In addition, because many of the toxicity studies did not report all of the water

quality parameters needed to run the BLM, the BLM toxicity database is substantially smaller than that currently used in the hardness-based AWQC (AWWQRP 2006). Application of the Recalculation Procedure to BLM-derived copper criteria represents an area for future study.

6.2 AMMONIA

The original AWQC for ammonia was published in 1985 (USEPA 1985a), after which the USEPA published a series of updates and comments (USEPA 1989, Heber and Ballentine 1992, USEPA 1996b) leading to a fully revised (freshwater only) AWQC for ammonia by 1998 (USEPA 1998). After obtaining public comment on the 1998 AWQC (USEPA 1999c), USEPA published a revised AWQC document in 1999 which now serves as the most recent national freshwater criteria for ammonia (USEPA 1999a). The 1999 update differs from the previous version most significantly in how it addresses the temperature-dependency of the formulation of the chronic criterion (CCC), and the chronic duration (i.e., averaging period) was increased to 30 days. Neither the 1998 or 1999 updates addressed the acute duration, or the frequency of allowed excursions.

Ammonia toxicity to aquatic organisms is a function of pH- and temperature-dependent chemical speciation, with toxicity generally increasing as pH and temperature increase. This is because ammonia toxicity is primarily dependent on the relative concentration of un-ionized ammonia, which is substantially more toxic than the ammonium ion under most conditions (USEPA 1999a). Ammonia toxicity can also depend on the ionic composition of test waters (Parametrix and CEC 2005), but insufficient evidence was available at the time for this to be included in the 1999 AWQC.

Because the 1984 AWQC was expressed in terms of un-ionized ammonia, both the acute and chronic criteria were mathematically adjusted for both pH and temperature. However, after critically evaluating the mechanisms of pH- and temperature-dependent toxicity of ammonia, it was determined for the 1999 AWQC that pH was the dominant factor controlling at least acute toxicity. Therefore, the acute criterion (CMC) is only expressed as a function of pH, and to control for variability in temperature-dependent ammonia speciation, the criteria are expressed in terms of total ammonia nitrogen concentrations (in units of mg N/L, rather than mg $NH_4 \cdot L^{-1}$). Both the 1984 and 1999 CMCs also take into account differences in species acute sensitivity, with different CMC values being derived for waters with vs. without salmonid fishes, which are particularly sensitive to acute ammonia exposure.

Therefore, the 1999 CMC is as follows for waters where *salmonids are present* (see Table 6-3 for examples):

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

The CMC is as follows for waters where *salmonids are not present* (see Table 6-3 for examples):

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

Table 6-3. Example CMC Concentrations from 1999 AWQC for Ammonia

pH	CMC (mg Total Ammonia-N/L)	
	Salmonids Present	***Salmonids Absent***
7	24.1	36.1
8	5.62	8.40

6.2.1 Recalculation Procedure

In the companion study to this User's Guide (AWWQRP 2006), substantial modifications to the 1999 AWQC toxicity database and criteria equations were recommended. An extensive review of published and unpublished literature added 23 genera, representing 28 species, to the current national acute/chronic database. The most noteworthy additions to the database were eight species of freshwater mussels in the Family Unionidae, which appear to be extremely sensitive to ammonia. The updated database also includes four endangered fish species found in the arid West.

After reviewing the toxicity database, uncertainties in the use of "large" rainbow trout data led us to an alternative approach of re-categorizing the updated database into two databases as either cold-water or warm-water species. The four most sensitive warmwater genera were all mussels from the Unionidae family. Given the uncertainty of the unionid distribution within the arid West, we also analyzed the warm-water database minus the Unionidae family. Acute equations were then derived for each database (i.e., cold-water, warm-water, warm-water minus Unionidae):

Updated Cold-water Ammonia Acute Criterion:

$$\mathrm{CMC}_{\mathrm{Cold}} = \frac{0.375}{1+10^{7.204\text{-pH}}} + \frac{53.3}{1+10^{\mathrm{pH}\text{-}7.204}}$$

Updated Warm-water Ammonia Acute Criterion:

$$\mathrm{CMC}_{\mathrm{Warm}} = \frac{0.081}{1+10^{7.204-\mathrm{pH}}} + \frac{11.5}{1+10^{\mathrm{pH}-7.204}}$$

Updated Warm-water without Unionidae Ammonia Acute Criterion:

$$\mathrm{CMC}_{\mathrm{Warm\ without\ Unionidae}} = \frac{0.388}{1+10^{7.204\text{-pH}}} + \frac{55.3}{1+10^{\mathrm{pH}\text{-}7.204}}$$

Following the deletion process, recalculated ammonia CMC concentrations exhibited little variability between any of the six study sites when compared to the updated CMC equations above (Table 6-2). The similarity in results for all sites with the updated national criterion suggest that site-specific recalculations for ammonia might not be necessary, as the breakdown of warm and cold water habitats proposed in our updated ammonia criteria equations may already account for site-specific differences in arid-west streams, making further species-based recalculation efforts unnecessary.

6.2.2 Water-Effect Ratio (WER)

As we discussed in Section 3.2, WERs are only used to derive SS-AWQC when the chemical characteristics of a site water alter the bioavailability or toxicity of the chemical when compared to tests conducted in laboratory waters. For ammonia, pH and temperature are widely considered to be the most significant factors controlling toxicity, and so it has been suggested that WERs may not be a viable approach. Indeed, the 1999 AWQC reviewed available WER studies with ammonia, and concluded that WERs tended to be close to 1 (i.e., no change in the national AWQC would be achieved using this method). This conclusion was reached because few data were available, even though it was suspected that aspects of ion composition in water other than pH (e.g., hardness, or sodium) may influence toxicity.

Although the 1999 AWQC is not expressed as a function of hardness, some studies suggest that ammonia toxicity may vary as a function of hardness for both invertebrates and fish. Ankley *et al*. (1995) evaluated acute ammonia toxicity to the amphipod *Hyalella azteca* across a pH range from 6.5 – 8.5, and across a hardness range from 42 – 270 mg/L (as $CaCO_3$). As hardness increased, acute toxicity (as a function of total ammonia-N) decreased significantly, and became more pH-dependent. These results agreed with those of Borgmann (1994) who evaluated chronic ammonia toxicity in both Lake Ontario water (hardness = 130 mg/L), and Lake Ontario water that was diluted 1:10 with double-distilled water. Chronic ammonia toxicity (as a function of total ammonia-N) was significantly less in the hard water relative to the diluted soft water. Both studies further suggested that ammonia toxicity decreased at elevated hardness in response to cationic interactions with Na^+-NH_4^+ membrane exchange mechanisms. Enhanced ammonia excretion via a similar Na-related mechanism at elevated hardness has also been observed in rainbow trout (Yesaki and Iwama 1992) and Lahontan cutthroat trout (Iwama *et al.* 1997).

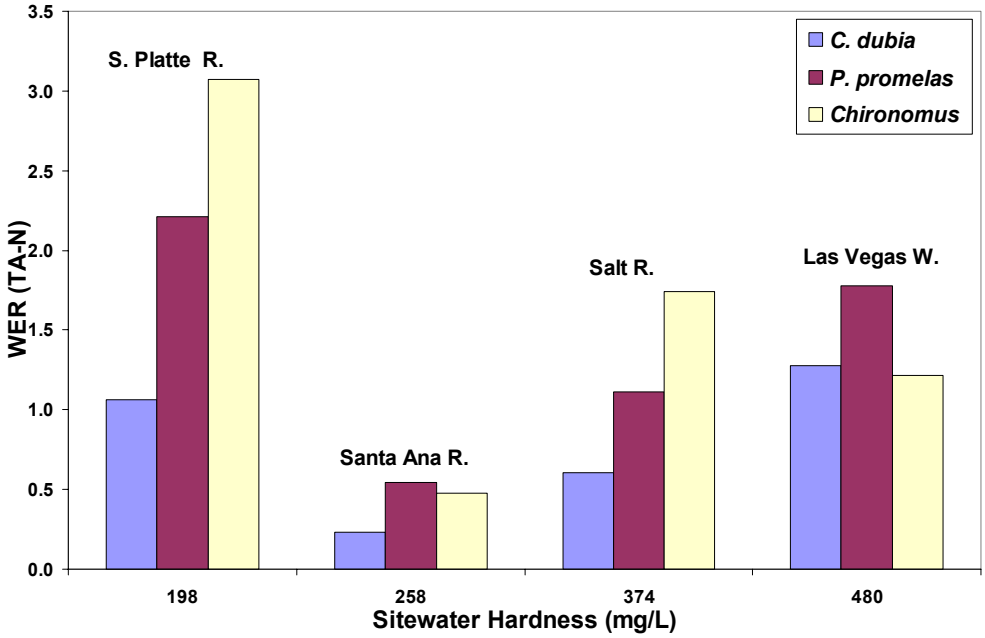
The mechanistic similarity of hardness-enhanced ammonia excretion in both amphipods and trout suggest that ammonia toxicity may indeed be hardness-dependent for a wider range of taxa. This clearly could be a significant issue for ephemeral and effluent-dependent waters in the arid West because if these ammonia/hardness relationships can be confirmed, it may be possible to use WERs to derive SS-AWQC in waters with elevated hardness (i.e., > 200 mg/L). Therefore, WER studies (Parametrix and CEC 2005) were conducted as a "proof of concept" with four effluent-dependent waters which were used in other AWWQRP studies described elsewhere in the User's Guide: Las Vegas Wash, Nevada; South Platte River, Colorado; Salt River, Arizona; and the Santa Ana River, California. WER tests were conducted with three aquatic species: the water flea (*Ceriodaphnia dubia*), fathead minnow (*Pimephales promelas*), and an aquatic insect (the midge, *Chironomus tentans*).

Acute ammonia WERs (calculated on the basis of total ammonia nitrogen) ranged from less than 0.5 to approximately 3 (Figure 6-2), indicating that ammonia toxicity in site waters ranged from two-fold greater than to three-fold less than toxicity in standard laboratory waters of fixed hardness (100 mg/L). Results were fairly consistent among species with WERs being consistently highest for *C. tentans* among all sites (0.5 – 3), WERs being lowest for *C. dubia* at ≤ 1 for all sites, and fathead minnow WERs generally ranging from 0.5 – 2. The highest WERs were generally found in the South Platte River, the lowest WERs were generally found in the Santa Ana River. The Salt River and Las Vegas Wash WERs were intermediate. WERs were not a function of hardness at these sites given that the South Platte River had the lowest hardness (198-214 mg/L $CaCO_3$), the Santa Ana River had the second lowest hardness (258 mg/L $CaCO_3$), and the Salt River and Las Vegas Wash had the two highest hardness values measured at any of the sites (374 and 480 mg/L $CaCO_3$, respectively).

However, a companion set of acute laboratory toxicity tests suggested that water quality parameters other than hardness (i.e., alkalinity and sodium) may more directly affect the toxicity of ammonia in natural waters (Parametrix and CEC 2005). This is also consistent with suggestions made by Ankley et al. (1995) that sodium, rather than hardness cations *per se*, may have been responsible for decreases in acute ammonia toxicity to the amphipod, *H. azteca*. Therefore, the lack of a relationship between hardness and the WERs measured at these sites may be due to the fact that some other factor(s) was contributing more heavily to the toxicity of ammonia.

Figure 6-2. Ammonia WERs (as total ammonia-N) in Four Effluent-dependent Waters

6.2.3 Summary



The studies described here suggest that for ammonia, the Recalculation Procedure is not likely to modify national AWQC to a significant degree. However, at least for the sites evaluated here, WERs would result in SS-AWQC ranging from a factor of two lower than to a factor of three greater than the national AWQC. For the Recalculation Procedure, site-specific recalculations for ammonia might not be necessary, because the breakdown of warm and cold water habitats proposed in our updated ammonia criteria equations may already account for site-specific differences in arid-west streams, making further species-based recalculation efforts unnecessary. Even the 1999 AWQC salmonid-present and salmonid absent criteria are likely to take into account some of the most significant species-related factors, and so the Recalculation Procedure may be of little utility for ammonia in most cases. The only additional species group to consider would be the unionid clams, which are not adequately addressed in the current national criteria, and so would require criteria recalculations or modification such as those proposed in the companion study to this User's Guide.

Even though our studies suggest that WERs may derive SS-AWQC that are up to two-three fold different (both higher and lower) than the national criteria, it is difficult to generalize as to whether a WER would be useful for any other site. This may be, for the most part, because pH and temperature are

the most important water quality characteristics modifying ammonia bioavailability and toxicity (but see omission of temperature-based chronic AWQC in the companion report; AWWQRP 2006). Since both factors are already taken into account by the national AWQC equations, few additional factors may warrant selection of the WER (Section 3.3). Unfortunately, our scientific understanding of ammonia bioavailability as a function of hardness, sodium, or other factors is not as well developed as that for metals such as copper (Section 6.1). Therefore, while it is possible that waters with elevated hardness or sodium may alter acute ammonia toxicity enough to support use of a WER, empirical tests would still need to be conducted on a site-specific basis to determine whether this might be a viable approach.

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APPENDIX A

The USEPA Recalculation Procedure

The Recalculation Procedure (text reprinted from Appendix B of USEPA 1994a)

NOTE: The National Toxics Rule (NTR) does not allow use of the Recalculation Procedure in the derivation of a site-specific criterion. Thus nothing in this appendix applies to jurisdictions that are subject to the NTR.

The Recalculation Procedure is intended to cause a site-specific criterion to appropriately differ from a national aquatic life criterion if justified by demonstrated pertinent toxicological differences between the aquatic species that occur at the site and those that were used in the derivation of the national criterion. There are at least three reasons why such differences might exist between the two sets of species. First, the national dataset contains aquatic species that are sensitive to many pollutants, but these and comparably sensitive species might not occur at the site. Second, a species that is critical at the site might be sensitive to the pollutant and require a lower criterion. (A critical species is a species that is commercially or recreationally important at the site, a species that exists at the site and is listed as threatened or endangered under section 4 of the Endangered Species Act, or a species for which there is evidence that the loss of the species from the site is likely to cause an unacceptable impact on an ecologically, commercially, or recreationally important species, a threatened or endangered species, the abundance of a variety of other species, or the structure or function of the community.) Third, the species that occur at the site might represent a narrower mix of species than those in the national dataset due to a limited range of natural environmental conditions. The procedure represented here is structured so that corrections and additions can me made to the national dataset without the deletion process being used to take into account taxa that do and do not occur at the site; in effect, this procedure makes it possible to update the national aquatic life criterion.

The phrase "occur at the site" includes the species, genera, families, orders, classes, and phyla that:

a. are usually present at the site.
b. are present at the site only seasonally due to migration.
c. are present intermittently because they periodically return to or extend their ranges into the site.
d. were present at the site in the past, are not currently present at the site due to degraded conditions, and are expected to return to the site when conditions improve.
e. are present in nearby bodies of water, are not currently present at the site due to

f. degraded conditions, and are expected to be present at the site when conditions improve.

The taxa that "occur at the site" cannot be determined merely by sampling downstream and/or upstream of the site at one point in time. "Occur at the site" does not include taxa that were once present at the site but cannot exist at the site now due to permanent physical alteration of the habitat at the site resulting from dams, etc.

The definition of the "site" can be extremely important when using the Recalculation Procedure. For example, the number of taxa that occur at the site will generally decrease as the size of the site decreases. Also, if the site is defined to be very small, the permit limit might be controlled by a criterion that applies outside (e.g., downstream of) the site.

Note: If the variety of aquatic invertebrates, amphibians, and fishes is so limited that species in fewer than eight families occur at the site, the general Recalculation Procedure is not applicable and the following special version of the Recalculation Procedure **must** be used:

1. Data **must** be available for at least one species in each of the families that occur at the site.
2. The lowest Species Mean Acute Value that is available for a species that occurs at the site **must** be used as the FAV.
3. The site-specific CMC and CCC **must** be calculated as described below in part 2 of step E, which is titled "Determination of the CMC and/or CCC".

The concept of the Recalculation Procedure is to create a dataset that is appropriate for deriving a site-specific criterion by modifying the national dataset in some or all of three ways:

a. Correction of data that are in the national dataset.
b. Addition of data to the national dataset.
c. Deletion of data that are in the national dataset.

All corrections and additions that have been approved by U.S. EPA are required, whereas use of the deletion process is optional. The Recalculation Procedure is more likely to result in lowering a criterion if the net result of addition and deletion is to decrease the number of genera in the dataset, whereas the procedure is more likely to result in raising a criterion if the net result of addition and deletion is to increase the number of genera in the dataset.

The Recalculation Procedure consists of the following steps:

A. Corrections are made in the national dataset.
B. Additions are made to the national dataset.
C. The deletion process may be applied if desired.
D. If the new dataset does not satisfy the applicable Minimum Data Requirements (MDRs), additional pertinent data **must** be generated; if the new data are approved by the U.S. EPA, the Recalculation Procedure **must** be started again at step B with the addition pf the new data.
E. The new CMC or CCC or both are determined.
F. A report is written.

Each step is discussed in more detail below.

A. Correction

1. Only corrections approved by the U.S. EPA may be made.
2. The concept of "correction" includes removal of data that should not have been in the national dataset in the first place. The concept of "correction" does not include removal of a datum from the national dataset just because the quality of the datum is claimed to be suspect. If additional data are available for the same species, the U.S. EPA will decide which data should be used, based on the available guidance (U.S. EPA 1985); also, data based on measured concentrations are usually preferable to those based on nominal concentrations.
3. Two kinds of corrections are possible:
    a. The first includes those corrections that are known to and have been approved by the U.S.EPA; a list of these will be available from the U.S. EPA.
    b. The second includes those corrections that are submitted to the U.S. EPA for approval. If approved, these will be added to EPA's list of approved corrections.
4. Selective corrections are not allowed. All corrections on EPA's newest list **must** be made.

B. Additions

1. Only additions approved by the U.S. EPA may be made.
2. Two kinds of additions are possible:
    a. The first includes those additions that are known to and have been approved by the U.S.EPA; a list of these will be available from the U.S. EPA.
    b. The second includes those additions that are submitted to the U.S. EPA for approval. If approved, these will be added to EPA's list of approved additions.
3. Selective additions are not allowed. All additions on EPA's newest list **must** be made.

C. The Deletion Process

The basic principles are:

1. Additions and corrections **must** be made as per steps A and B above, before the deletion process is performed.
2. Selective deletions are not allowed. If any species is to be deleted, the deletion process described below **must** be applied to all species in the national dataset, after any necessary corrections and additions have been made to the national dataset. The deletion process specifies which species **must** be deleted and which species **must not** be deleted. Use of the deletion process is optional, but no deletions are optional when the deletion process is used.

3. Comprehensive information **must** be available concerning what species occur at the site; a species cannot be deleted based on incomplete information concerning the species that do and do not satisfy the definition of "occur at the site".
4. Data might have to be generated before the deletion process is begun:
    a. Acceptable pertinent toxicological data **must** be available for at least one species in each class of aquatic plants, invertebrates, amphibians, and fish that contains a species that is a critical species at the site.
    b. For each aquatic plant, invertebrate, amphibian, and fish species that occurs at the site and is listed as threatened or endangered under section 4 of the Endangered Species Act, data **must** be available or generated for an acceptable surrogate species. Data for each surrogate species **must** be used as if they are data for species that occur at the site.

    If additional data are generated using acceptable procedures (U.S. EPA 1985) and they are approved by the U.S. EPA, the Recalculation Procedure **must** be started again at step B with the addition of the new data.
5. Data might have to be generated after the deletion process is completed. Even if one or more species are deleted, there still are MDRs (see step D below) that **must** be satisfied. If the data remaining after deletion do not satisfy the applicable MDRs, additional toxicity tests **must** be conducted using acceptable procedures (U.S. EPA 1985) so that all MDRs are satisfied. If the new data are approved by the U.S. EPA, the Recalculation Procedure **must** be started again at step B with the addition of the new data.
6. Chronic tests do not have to be conducted because the national Final Acute-Chronic Ratio (FACR) may be used in the derivation of the site-specific Final Chronic Value (FCV). If acute-chronic ratios (ACRs) are available or are generated so that the chronic MDRs are satisfied using only species that occur at the site, a site-specific FACR may be derived and used in place of the national FACR. Because a FACR was not used in the derivation of the freshwater CCC for cadmium, this CCC can only be modified the same way as a FAV; what is acceptable will depend on which species are deleted.

If any species are to be deleted, the following deletion process **must** be applied:

a. Obtain a copy of the national dataset, i.e., tables 1, 2, and 3 in the national criteria document (see Appendix E).
b. Make corrections in and/or additions to the national dataset as described in steps A and B above.
c. Group all the species in the dataset taxonomically by phylum, class, order, family, genus, and species.
d. Circle each species that satisfies the definition of "occur at the site" as presented on the first page of this appendix, and including any data for species that are surrogates of threatened or endangered species that occur at the site.
e. Use the following step-wise process to determine which of the uncircled species **must** be deleted and which **must not** be deleted:

1. Does the genus occur at the site?

   If "No", go to step 2.

   If "Yes", are there one or more species in the genus that occur at the site but are not in the dataset?

If "No", go to step 2.

If "Yes", delete† the uncircled species.*

2. Does the family occur at the site?

   If "No", go to step 3.

   If "Yes", are there one or more genera in the family that occur at the site but are not in the dataset?

   If "No", go to step 3.

   If "Yes", delete† the uncircled species.*

3. Does the order occur at the site?

   If "No", go to step 4.

   If "Yes", does the dataset contain circled species that is in the same order?

   If "No", retain the uncircled species.*

   If "Yes", retain the uncircled species.*

4. Does the class occur at the site?

   If "No", go to step 5.

   If "Yes", does the dataset contain a circled species that is in the same class?

   If "No", retain the uncircled species.*

   If "Yes", retain the uncircled species.*

5. Does the phylum occur at the site?

   If "No", delete the uncircled species.

   If "Yes", does the dataset contain a circled species that is in the same phylum?

   If "No", retain the uncircled species.*

   If "Yes", retain the uncircled species.*

* = Continue the deletion process by starting at step 1 for another uncircled species unless all uncircled species in the dataset have been considered.

The species that are circled and those that are retained constitute the site-specific dataset. (An example of the deletion process is given in Figure B1).

This deletion process is designed to ensure that:

a. Each species that occurs both in the national dataset and at the site also occurs in the site-specific dataset.

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† Changed from "retained" in updated USEPA guidance (Stephan and Hansen 1997).

b. Each species that occurs at the site but does not occur in the national dataset is represented in the site-specific dataset by all species in the national dataset that are in the same genus.
c. Each genus that occurs at the site but does not occur in the national dataset is represented in the site-specific dataset by all genera in the national dataset that are in the same family.
d. Each order, class, and phylum that occurs both in the national dataset and at the site is represented in the site-specific dataset by the one or more species in the national dataset that are most closely related to a species that occurs at the site.

D. Checking the Minimum Data Requirements

The initial MDRs for the Recalculation Procedure are the same as those for the derivation of a national criterion. If a specific requirement cannot be satisfied after deletion because that kind of species does not occur at the site, a taxonomically similar species **must** be substituted in order to meet the eight MDRs:

> If no species of the kind required occurs at the site, but a species in the same order does, the MDR can only be satisfied by data for a species that occurs at the site and is in that order; if no species in the order occurs at the site, but a species in the class does, the MDR can only be satisfied by data for a species that occurs at the site and is in that class. If no species in the same class occurs at the site, but a species in the phylum does, the MDR can only be satisfied by data for a species that occurs at the site and is in the phylum. If no species in the same phylum occurs at the site, any species that occurs at the site and is not used to satisfy a different MDR can be used to satisfy the MDR. If additional data are generated using acceptable procedures (U.S. EPA 1985) and they are approved by the U.S. EPA, the Recalculation Procedure **must** be started again at step B with the addition of the new data.

If fewer than eight families of aquatic invertebrates, amphibians, and fishes occur at the site, a Species Mean Acute Value **must** be available for at least one species in each of the families and the special version of the Recalculation Procedure described on the second page of this appendix **must** be used.

E. Determining the CMC and/or CCC

1. Determining the FAV:
   a. If the eight family MDRs are satisfied, the site-specific FAV **must** be calculated from Genus Mean Acute Values using the procedure described in the national aquatic life guidelines (U.S. EPA 1985).
   b. If fewer than eight families of aquatic invertebrates, amphibians, and fishes occur at the site, the lowest Species Mean Acute Value that is available for a species that occurs at the site **must** be used as the FAV, as per the special version of the Recalculation Procedure described on the second page of this appendix.
2. The site-specific CMC must be calculated by dividing the site-specific FAV by 2. The site-specific FCV must be calculated by dividing the site-specific FAV by the national FACR (or by a site-specific FACR if one is derived). (Because a FACR was not used to

derive the national CCC for cadmium in fresh water, the site-specific CCC equals the site-specific FCV).

3. The calculated FAV, CMC, and/or CCC must be lowered, if necessary, to (1) protect an aquatic plant, invertebrate, amphibian, or fish species that is a critical species at the site, and (2) ensure that the criterion is not likely to jeopardize the continued existence of any endangered or threatened species listed under section 4 of the Endangered Species Act or result in the destruction or adverse modification of such species' critical habitat.

F. Writing the Report

The report of the results of use of the Recalculation Procedure **must** include:

1. A list of all species of aquatic invertebrates, amphibians, and fishes that are known to "occur at the site", along with the source of the information.
2. A list of all aquatic plant, invertebrate, amphibian, and fish species that are critical species at the site, including all species that occur at the site and are listed as threatened or endangered under section 4 of the Endangered Species Act.
3. A site-specific version of Table 1 from a criteria document produced by the U.S. EPA after 1984.
4. A site-specific version of Table 3 from a criteria document produced by the U.S. EPA after 1984.
5. A list of all species that were deleted.
6. The new calculated FAV, CMC, and/or CCC.
7. The lowered FAV, CMC, and/or CCC, if one or more were lowered to protect a specific species.

Reference

U.S. EPA. 1985. 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.

Figure B1: An Example of the Deletion Process Using Three Phyla

SPECIES THAT ARE IN THE THREE PHYLA AND OCCUR AT THE SITE

Phylum	Class	Order	Family	Species
Annelida	Hirudin.	Rhynchob.	Glossiph.	*Glossip. complanata*
Bryozoa	(No species in this phylum occur at the site.)			
Chordata	Osteich.	Cyprinif.	Cyprinid.	*Carassius auratus*
Chordata	Osteich.	Cyprinif.	Cyprinid.	*Notropis anogenus*
Chordata	Osteich.	Cyprinif.	Cyprinid.	*Phoxinus eos*
Chordata	Osteich.	Cyprinif.	Catostom.	*Carpiodes carpio*
Chordata	Osteich.	Salmonif.	Osmerida.	*Osmerus mordax*
Chordata	Osteich.	Percifor.	Centrarc.	*Lepomis cyanellus*
Chordata	Osteich.	Percifor.	Centrarc.	*Lepomis humilis*
Chordata	Amphibia	Caudata	Ambystom.	*Ambystoma gracile*

SPECIES THAT ARE IN THE THREE PHYLA AND IN THE NATIONAL DATASET

Phylum	Class	Order	Family	Species	Code
Annelida	Oligoch.	Haplotax.	Tubifici.	*Tubifex tubifex*	P
Bryozoa	Phylact.	---	Lophopod.	*Lophopod. carteri*	D
Chordata	Cephala.	Petromyz.	Petromyz.	*Petromyzon marinus*	D
Chordata	Osteich.	Cyprinif.	Cyprinid.	*Carassius auratus*	S
Chordata	Osteich.	Cyprinif.	Cyprinid.	*Notropis hudsonius*	G
Chordata	Osteich.	Cyprinif.	Cyprinid.	*Notropis stramineus*	G
Chordata	Osteich.	Cyprinif.	Cyprinid.	*Phoxinus eos*	S
Chordata	Osteich.	Cyprinif.	Cyprinid.	*Phoxinus oreas*	D
Chordata	Osteich.	Cyprinif.	Cyprinid.	*Tinca tinca*	D
Chordata	Osteich.	Cyprinif.	Catostom.	*Ictiobus bubalus*	F
Chordata	Osteich.	Salmonif.	Salmonid.	*Oncorhynchus mykiss*	O
Chordata	Osteich.	Percifor.	Centrarc.	*Lepomis cyanellus*	S
Chordata	Osteich.	Percifor.	Centrarc.	*Lepomis macrochirus*	G
Chordata	Osteich.	Percifor.	Percidae	*Perca flavescens*	D
Chordata	Amphibia	Anura	Pipidae	*Xenopus laevis*	C

Explanation of Codes:

S = retained because this Species occurs at the site.

G = retained because there is a species in this Genus that occurs at the site but not in the national dataset.

F = retained because there is a genus in this Family that occurs at the site but not in the national dataset.

O = retained because this Order occurs at the site and is not represented by a lower taxon.

C = retained because this Class occurs at the site and is not represented by a lower taxon.

P = retained because this Phylum occurs at the site and is not represented by a lower taxon.

D = deleted because this species does not satisfy any of the requirements for retaining species.