AWWQRP Special Studies Report:

Use of the EPA Recalculation Procedure with the Copper Biotic Ligand Model

and

Relative Role of Sodium and Alkalinity vs. Hardness in Controlling Acute Ammonia Toxicity

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ACRONYMS

ACR	acute to chronic ratio
AWQC	Ambient Water Quality Criteria
AWWQRP	Arid West Water Quality Research Project
AWS-MDRs	arid West stream minimum database requirements
BLM	Biotic Ligand Model
Cu	copper
DOC	dissolved organic carbon
FACR	final acute-to-chronic ratio
FAV	final acute value
FCV	final chronic value
GMAV	genus mean acute value
GMCV	genus mean chronic value
LA50	Lethal Accumulation associated with 50% effect
MDRs	minimum data requirements
NaCl	sodium chloride
PCWMD	Pima County Wastewater Management Department
RWG	Regulatory Working Group
SAG	Scientific Advisory Group
SMAV	species mean acute value
SMCV	species mean chronic value
USEPA	U.S. Environmental Protection Agency
WER	water-effect-ratio
WQC	water quality criteria
WQCR	water quality criteria ratio
WWTP	wastewater treatment plant

FOREWORD

The Arid West Water Quality Research Project (AWWQRP or "Project") was established in 1995 as a result of a federal appropriation (Public Law 103-327) and the establishment of an Assistance Agreement between the U.S. Environmental Protection Agency (USEPA) and Pima County Wastewater Management Department (PCWMD), Tucson, Arizona. The establishment of this Agreement provided a significant opportunity for western water resource stakeholders to (1) work cooperatively to conduct scientific research to recommend appropriate water quality criteria, standards and uses for effluent-dependent and ephemeral waters in the arid and semi-arid regions of the West ("arid West"), and (2) improve the scientific basis for regulating wastewater and stormwater discharges in the arid West. Effluent-dependent waters are created by the discharge of treated effluent into ephemeral streambeds or streams that in the absence of effluent discharge would have only minimal flow.

With the establishment of the AWWQRP, a management infrastructure was created to support the development of peer-reviewed research products. From within the Environmental Planning Division of PCWMD, the AWWQRP Project Director, Program Manager and support staff administer the Project. A Regulatory Working Group (RWG), comprised of 15 stakeholders representing both public and private interests, works to ensure that Project research has a sound regulatory basis and that research activities focus on important regulatory concerns. The Scientific Advisory Group (SAG), comprised of scientists with experience in water quality research, makes certain that project research has a sound scientific basis and that studies are properly designed and technically sound.

This report represents part of a series of research reports produced by the AWWQRP, and builds upon already completed work. The first report in the series, *Pre-Research Survey of Municipal NPDES Dischargers in the Arid and Semi-Arid West*, resulted from an RWG recommendation that the Project survey arid West wastewater facilities to compile information about their effluent discharges and associated water quality concerns.

The second report, the *Habitat Characterization Study*, utilized the findings of the Discharger Survey. Recognizing that an understanding of the attributes of effluent-dependent waters was critical to the development of appropriate water quality criteria and standards for these waters, the RWG recommended that the AWWQRP commission a major study to describe the physical, chemical, and biological characteristics of effluent-created habitats.

The *Habitat Characterization Study* evaluated the physical, chemical and biological characteristics of effluent-dependent habitats at ten case study sites in the arid West: Santa Cruz River below Nogales and below Tucson, Arizona; Salt River below Phoenix, Arizona; Santa Ana River below San Bernardino, California; Fountain Creek below Colorado Springs, Colorado; South Platte River below Denver, Colorado; Las Vegas Wash below Las Vegas, Nevada; Santa Fe River below Santa Fe, New Mexico; Carrizo Creek below Carrizo Springs, Texas; and Crow Creek below Cheyenne, Wyoming (Figure F-1). The primary objectives of this effort were to (1) review existing physical, chemical and biological data; (2) conduct a site reconnaissance to characterize habitats using established protocols and protocols adapted for arid west conditions; (3) identify similarities and differences among sites; (4) discuss potential approaches to protect these habitats in the context of existing regulatory programs; and (5) recommend areas for additional study. The final report may be downloaded from the AWWQRP website, www.co.pima.az.us/wwm/wqrp, or obtained from the AWWQRP Office in a CD hyperlinked format.

The AWWQRP's third report, *Extant Criteria Evaluation*, evaluated the applicability of national water quality criteria, as well as the methods to modify those criteria, to effluent-dependent and ephemeral waters in the arid West. This work built upon the findings presented in the *Habitat Characterization Study* using the expertise of national water quality criteria researchers. The AWWQRP used the findings and recommendations contained in the *Extant Criteria Evaluation* as the primary driver for the selection and execution of three subsequent research projects, including evaluations of (1) the Biotic Ligand Model (BLM) of copper toxicity in arid west streams (Parametrix and HydroQual 2006), (2) use of the EPA recalculation procedure in effluent-dependent streams (Chadwick Ecological Consultants et al. 2006), and (3) potential hardness-modifications to ammonia toxicity and their implications for use of the water-effect ratio (Parametrix and Chadwick Ecological Consultants 2006).

Figure F-1. *Habitat Characterization Study* Case Study Sites

The purpose of this report, *AWWQRP Special Studies Report: Use of the EPA Recalculation Procedure with the Copper Biotic Ligand Model, and Relative Role of Sodium and Alkalinity vs. Hardness in Controlling Acute Ammonia Toxicity*, ("AWWQRP Special Studies Report") is to build upon knowledge gained from the last three AWWQRP studies, and apply them to the newest regulatory guidance from USEPA. This report is organized into three chapters, each of which addresses the following:

1. *Evaluation of Site-specific Standards Using Biotic Ligand Model-adjusted Copper Ambient Water Quality Criteria*. This chapter evaluates the applicability of the USEPA recalculation procedure to the recently released Ambient Water Quality Criteria (AWQC) for copper which incorporates the BLM for derivation of the freshwater criteria (USEPA 2007). This evaluation builds upon the previous AWWQRP recalculation procedure report (Chadwick Ecological Consultants et al. 2006) which addressed standard and modified approaches for adjusting criteria to enhance their protectiveness for the unique species assemblages often encountered in arid west ephemeral or effluent-dependent streams. The current report uses these same approaches and field case studies, but using the databases and BLM-based criteria calculations as are now officially recommended by USEPA. These results are then compared to other more traditional approaches for deriving site-specific water quality standards.

2. *Current Status and Future Trends in Biotic Ligand Models for Derivation of Aquatic Life Protection Criteria for Metals*. The objective of this chapter is to discuss the current status of BLMs for various metals and the future trends that will enhance our ability to predict the effects of metals on environmental systems. Specifically, the role of BLMs for deriving acute and chronic water quality standards is discussed in the context of current regulatory frameworks. Similarly, the limitations that exist for implementing BLMs into criteria development are also considered. Finally, existing BLMs and future trends in their development and application (e.g., metal mixtures, sediment and terrestrial BLMs, and dietary BLMs) are discussed.

3. *Relative Role of Sodium and Alkalinity versus Hardness Cations in Controlling Acute Ammonia Toxicity to Aquatic Organisms*. The hardness-ammonia studies previously conducted for AWWQRP (Parametrix and Chadwick Ecological Consultants 2006) supported the limited toxicity literature available which suggests that hardness (and/or related cations) may influence acute ammonia toxicity. However, to further elucidate the mechanisms governing these effects, major ion composition other than hardness (sodium is of particular interest) needed additional independent experimental manipulation. Additional studies were conducted (Parametrix 2006) which suggested that elevated sodium levels offer considerable protection to *H. azteca* against ammonia toxicity, especially when coupled with elevated hardness. However, it was not apparent how sodium may influence the ammonia/hardness relationship at other pH levels, intermediate hardness concentrations, or for other species. Therefore, to confirm the role of sodium in controlling acute ammonia toxicity in very hard or ion rich waters, additional acute toxicity tests were conducted with the invertebrate *Ceriodaphnia dubia*, as well as the freshwater fish *Pimephales promelas* (fathead minnow) employing the same series of sodium manipulations as were used by Parametrix (2006). In chapter 3, the results of these additional studies are summarized, and discussed in the context of previous work.

As with all AWWQRP work, this report has undergone independent technical review by the SAG. However, even though the findings of this study have received technical review, it is strongly recommended that local, state, and regional regulatory authorities should be consulted prior to using these findings to support or propose regulatory change.

The AWWQRP has made a significant effort to share Project results and their implications in a variety of technical, regulatory, industry and public interest forums, including publication in the primary scientific literature. This outreach effort is designed to create a broader understanding of water quality issues unique to the arid West and provide scientific and regulatory data in support of a regional approach to the development of water quality criteria, standards and uses. Heightened interest in arid west water quality issues has been fueled by the recognition that treated effluent can have a valuable role in the support and enhancement of riparian ecosystems, particularly in light of increasingly limited water resources. The AWWQRP looks forward to continuing its support of research that not only provides critical data to address unique western water quality issues, but also supports the development of innovative solutions.

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EXECUTIVE SUMMARY

The purpose of this report is to build upon knowledge gained from past studies conducted as part of the Arid West Water Quality Research Project (AWWQRP), and apply them to the newest regulatory guidance from USEPA. This report is organized into three chapters, the first two of which address implementation of the Biotic Ligand Model for regulatory protection of aquatic life from copper, and the third addresses the relative roles of sodium and hardness in controlling acute ammonia toxicity to aquatic organisms.

EVALUATION OF SITE-SPECIFIC STANDARDS USING BIOTIC LIGAND MODEL ADJUSTED COPPER AMBIENT WATER QUALITY CRITERIA/USE OF THE USEPA RECALCULATION PROCEDURE TO DEVELOP SITE-SPECIFIC BLM-BASED COPPER CRITERIA

We evaluated the applicability of the USEPA recalculation procedure to the recently released (2007) biotic ligand model (BLM)-based copper (Cu) Ambient Water Quality Criteria (AWQC), using the findings and arid west specific methodology developed in the Pima County AWWQRP *Evaluation of the USEPA Recalculation Procedure in the Arid West Technical Report*.

To generate BLM-based site-specific AWQC for Cu, non-resident taxa were first deleted from the national BLM adjusted Cu database for each study stream and two regions representing major trends in aquatic life community structure (Southwest and High Plains). The total number of species represented in the resulting site-specific BLM adjusted databases ranged from 7 for the Santa Cruz near Tuscon to 29 for the High Plains regional database. The recalculated BLM final acute values (FAVs; before adjusting to site water chemistry) were very similar between sites and regions. Next, mean site water chemistry and recalculated FAVs were run with the BLM to generate median lethal accumulation values (LA50s) and site-specific FAVs. Resulting BLM-based site specific acute and chronic Cu criteria ranged from 32.4 to 209.2 µg/L and 20.1 to 130.0 µg/L, respectively. These site-specific BLM-based criteria reflect both the sensitivity of species that are expected to be resident to arid west effluent-dependent/dominated streams, and expected copper complexing due to site-specific water quality characteristics.

Although the recalculated BLM-FAVs were very similar among sites, all BLM-based site-specific criteria were substantially greater than hardness modified site-specific criteria. These results suggest that Cu complexing resulting from site water characteristics other than hardness has a greater effect on criteria when using the BLM than the site-specific toxicity databases generated with the step-wise deletion process. For the arid west sites, the complexing reduces Cu toxicity and results in water quality criteria that are less stringent than hardness modified site-specific criteria, but still protective of aquatic life.

CURRENT STATUS AND FUTURE TRENDS IN BIOTIC LIGAND MODELS FOR DERIVATION OF AQUATIC LIFE PROTECTION CRITERIA FOR METALS

The development of computational models which can be used to predict the influence of environmental concentrations of metals on aquatic organisms has received considerable attention by researchers and regulators worldwide. Currently, advancements in predictive models that can account for biological effects from acute (short-term, high magnitude) and chronic (long-term, low level) exposures to metals have provided regulators in the United States and Europe with tools for confidently implementing site-specific water quality standards. The current status of Biotic Ligand Models (BLM) is presented with respect to the various metals, organisms, and test endpoints for which models have been validated. To date,

copper has received the most attention and there are currently BLMs representing several trophic levels (plants, invertebrates, and fish) and a variety of test endpoints (survival, reproduction, growth, etc.). As such, the USEPA has recently released a revised Cu criteria guidance document for a copper ambient water quality criterion which utilizes a BLM for criterion derivation purposes. In addition, the roles of BLMs for deriving additional acute and chronic water quality standards and as tools for identifying "at risk" aquatic habitats are discussed in detail. Future trends in BLM development, such as accounting for the influence of metal mixtures, effects in sediment and terrestrial habitats, and dietary exposures, are discussed relative to their use in a regulatory context and enhancing our ability to predict the effects of metals on environmental systems. In general, the use of BLMs to predict the influence of environmental concentrations of metals on aquatic organisms represents a significant departure from the current methodologies and should provide a more scientifically-defensible means of regulating effluent discharges based on site-specific water quality conditions.

RELATIVE ROLE OF SODIUM AND ALKALINITY VERSUS HARDNESS CATIONS IN CONTROLLING ACUTE AMMONIA TOXICITY TO AQUATIC ORGANISMS

The objective of this project was to build upon the hardness-ammonia studies conducted in 2006 for AWWQRP, which supported the limited toxicity literature available suggesting that hardness (and/or related cations) may influence acute ammonia toxicity. To further elucidate the mechanisms governing these relationships, sodium was independently manipulated in conjunction with hardness and alkalinity in a series of acute *Hyalella azteca*, *Ceriodaphnia dubia*, and *Pimephales promelas* toxicity tests. For each species, four reconstituted waters were made in which hardness was varied and sodium concentrations were manipulated, either by direct addition of sodium chloride (NaCl), or as a result of increasing alkalinity (sodium addition as sodium bicarbonate – $NaHCO_3$).

For the amphipod, *H. azteca*, the cladoceran, *C. dubia*, and the fathead minnow, *P. promelas*, increasing hardness ameliorated the acute toxicity of ammonia only when coupled with an increase in sodium as alkalinity. Increasing sodium alone (as either NaCl or alkalinity), without also increasing hardness, had variable effects on the acute toxicity of ammonia to these species. For example, increasing sodium alone as NaCl only resulted in a decrease in toxicity to *H. azteca* and, while for *H. azteca*, increasing sodium alone as alkalinity did not affect ammonia toxicity. For both *C. dubia* and *P. promelas*, this treatment caused the highest level of toxicity observed across all tests.

Based on these conclusions, hardness does exert a significant effect on acute ammonia toxicity, but only in hard waters where sodium (as alkalinity) is also elevated. These studies, as well as those found in the literature which were the basis for this research, corroborate the current suggestion from the latest AWQC for ammonia that water-effect-ratios (WERs) greater than 1 may be expected when a difference in ionic composition, in conjunction with pH or hardness, is present between site and laboratory waters.

1. EVALUATION OF SITE-SPECIFIC STANDARDS USING BIOTIC LIGAND MODEL ADJUSTED COPPER AMBIENT WATER QUALITY CRITERIA

1.1 INTRODUCTION

This chapter of the report evaluates the applicability of the USEPA recalculation procedure (USEPA 1994) to the recently released biotic ligand model (BLM)-based copper (Cu) Ambient Water Quality Criteria (AWQC)(USEPA 2007). This assessment took into consideration the findings and arid West specific methodology developed in the Pima County AWWQRP *Evaluation of the USEPA Recalculation Procedure in the Arid West Technical Report* (AWWQRP 2006), hereafter referred to as the 2006 AWWQRP report.

The site-specific standards produced in the 2006 AWWQRP report were largely based on the ambient water hardness-modified Cu criteria presented in 1995 Updates (USEPA 1996), as updated with appropriate and relevant Cu toxicity data. These efforts resulted in substantially updated acute and chronic toxicity databases, revised acute and chronic toxicity-hardness slopes and acute-chronic ratio, and resulting updated acute and chronic hardness-based equations. Although a draft BLM Cu criteria document was available at this time, the large differences in the number of species represented in the hardness modified databases compared to BLM database (greater than 2x the number of genera in the hardness modified toxicity database), in addition to the uncertainty with using the draft document, lead to the analysis of the 1995 Updates database at the time.

Since completion of the 2006 AWWQRP, the USEPA has finalized the BLM-based Cu criteria document (USEPA 2007) and although fewer species are represented in the BLM database, BLM-modeled changes in Cu toxicity could provide a useful tool for deriving protective site-specific standards.

This chapter of the report evaluates the application of the USEPA recalculation procedure (USEPA 1994), modified for conditions specific to the arid west (AWWQRP 2006), to the recently released BLM Cu AWQC (USEPA 2007). Similar to hardness modified AWQC, the BLM modifies the AWQC with respect to multiple site-specific water quality parameters. Although the BLM is useful for predicting changes in Cu sensitivity due to site-specific water quality characteristics, the criterion output is still calculated from the full freshwater toxicity database that may not be representative of the species that occur at the site. Applying the recalculation procedure to the BLM database takes into consideration the species that are expected to occur at the site. The resulting site-specific Cu criteria are thus modified with respect to both site water quality characteristics and resident species assemblages.

Before conducting the recalculation steps with the BLM database, we critically evaluated the 2007 BLM AWQC, and reviewed resident species lists for the same six effluent-dependent river segments and methods evaluated in the 2006 AWWQRP report. Using the refined site-specific recalculation methodology (AWWQRP 2006), we derived BLM adjusted acute and chronic AWQC for each stream segment and two biogeographical regions. Results were compared to our 2006 AWWQRP recalculation report, updated national hardness normalized Cu criteria, and subsequent site-specific criteria.

1.2 REVIEW OF THE CURRENT COPPER AWQC

The recently published USEPA AWQC for Cu (USEPA 2007) is unique from previous Cu criteria in that the criteria revision incorporates the BLM to adjust toxicity values according to multiple water quality parameters. The BLM is used to normalize Cu toxicity values to a set range of multiple chemical parameters, in place of the simpler hardness normalization, used in the previous USEPA Cu criteria (USEPA 1996, AWWQRP 2006), and provides means to characterize the complexity of Cu behavior due to various physiochemical characteristics of laboratory or natural waters.

The BLM uses multiple water quality characteristics (temperature, pH, dissolved organic carbon [DOC], % humic acid, sulfate, bicarbonate, and the concentrations of the following ions: Ca, Mg, Na, K, Cl, and S) to derive predicted toxicity values, as opposed to one or two water quality characteristics generally used to modify other metal toxicity values. For additional detail on the technical basis of the BLM and the necessary chemical input parameters see Chapter 2 of this report.

Because of the complexity of speciation and ligand binding, use of a single water quality parameter such as hardness to characterize toxicity is too simplified – especially for Cu. The separate effects of factors that affect toxicity have generally not been addressed with established USEPA water quality equation derivation methodologies, although the USEPA has established methods for characterizing site-specific water quality effects on toxicity using water-effect-ratios (WERs; USEPA 2001). Unfortunately, WER testing can be costly and provide a temporally and spatially limited scope of effects (USEPA 2007). The new BLM approach models Cu toxicity according to multiple water quality parameters that often transcend established test acceptability guidelines. Characterization of Cu toxicity can be theoretically derived using the BLM model according to a wider range of site water quality at less cost than conventional WER toxicity testing.

Application of a BLM model has many benefits in efficiently characterizing Cu toxicity, but doing so limits the number of toxicity values in the national database. All test water quality parameters necessary to run the BLM need to be measured, or have the ability to be estimated, for each study with Cu toxicity data. Such data are not reported in many of the available studies. As a result of the extensive BLM data requirements, the total number of genera in the BLM database is 27 (USEPA 2007)(Appendix A) compared to the 67 genera in the updated acute toxicity database (dataset without Koivisto et al. data; AWWQRP 2006). In addition to a less robust and possibly less representative toxicity database, this could potentially result in more conservative criteria values as a result of the "sample size" effect in criteria derivation (see discussion below). These issues, in part, influenced our decision to not use the draft BLM Cu national database in previous AWWQRP site-specific investigations. Now that the BLM Cu criteria are published and deviations from established criteria derivation guidelines were justified, site-specific criteria presented in this report could effectively replace the hardness modified criteria that were used in previous efforts.

The first step in deriving site-specific criteria is to review and update the current national criteria. Because of the recent publication of the current Cu criteria, this step was not deemed necessary. The current BLM adjusted acute Cu toxicity database contains 38 species in 19 families. These values characterize the toxicity in 22 species of fish, 15 invertebrates, and one amphibian. This assemblage of aquatic organisms satisfies the minimum data requirements (MDRs) for 95th percentile acute criterion derivation. Since the final criterion is dependent on specific BLM model parameters, a set of reference chemical exposure conditions based on moderately-hard reconstituted laboratory water were established by USEPA. This is similar to using a reference hardness value of 50 mg/L as $CaCO_3$ in the hardness-based criteria.

BLM-normalization calculated the final acute value (FAV) as 4.7 µg/L, based on the four most sensitive genera; *Daphnia*, *Lithoglyphus*, *Ceriodaphnia*, and *Gammarus*, respectively. This BLM adjusted FAV is slightly less than half that of the updated national FAV of 9.65 µg/L (AWWQRP 2006). It is important to note that an exact comparison of the two criteria is complicated by 1) differing reference chemical exposure conditions used to normalize respective criterion, 2) differing variability in the range of toxicity between the four most sensitive genera, as well as 3) the differential number of genera in the respective toxicity databases.

Because the limited number of acceptable chronic toxicity studies, USEPA MDRs were not met to derive a BLM adjusted Cu chronic criterion using the 95th percentile methodology. Therefore, the final chronic value (FCV) was derived using a final acute-chronic ratio (FACR) applied to the BLM normalized FAV. Acceptable freshwater chronic toxicity data were available for 10 fish and six invertebrate species. Comparable acute values were available for acute to chronic ratio (ACR) modeling for 18 tests (neither acute or chronic values are BLM normalized). The resulting ACRs varied from less than one for *Ceriodaphnia dubia* to 192 for the snail, *Campeloma decisum*. The FACR was calculated as the geometric mean of the species mean ACRs for five sensitive freshwater species (*C. dubia, Daphnia magna*, *D. pulex, Oncorhynchus tshawytscha,* and *O. mykiss*) and one saltwater species (*Cyprinodon variegates*). The resulting FACR is 3.22, and when applied to the FAV, results in a FCV of 1.3 µg/L. This current FACR is greater than the updated FACR of 2.9008 (AWWQRP 2006). All BLM derived criteria presented in this report utilized the current Cu criteria FACR of 3.22.

1.3 RESIDENT SPECIES LISTS

The resident fish and invertebrate taxa lists that were generated as part of the AWWQRP 2006 report were utilized in the recalculation efforts in this report. Effluent-dependent stream segments included in this analysis are 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. Segments are located on the Santa Ana River, California, Santa Cruz River near Nogales and near Tucson and the Salt/Gila River, Arizona, and Fountain Creek and the South Platte River, Colorado.

To summarize the fish resident species lists (AWWQRP 2006), a total of 73 resident fish taxa were compiled from the literature review of the arid West effluent-dependent stream segments (Appendix B). The only fish species collected historically at all six river segments was the green sunfish, *Lepomis cyanellus,* which may be native to the Colorado streams, but is non-native in most of the arid West. Two other fish species were collected at five of the six sites: the western mosquitofish, *Gambusia affinis* and the black bullhead, *Ameiurus melas*.

To summarize the invertebrate species lists (AWWQRP 2006), a total of 550 resident invertebrate taxa were compiled from the literature review of the arid West effluent-dependent stream segments (Appendix B). The only species identified at all sites was the midge, *Cricotopus bicinctus*. Several families or classes of invertebrates, including the worms, Oligochaeta, Naididae, and Tubificidae, and the aquatic insects, Baetidae, Coenagrionidae, Corixidae, Elmidae, Hydrophilidae, Chironomidae, Psychodidae, and Simuliidae, were also identified at all sites. Cladocerans were collected from some of the stream sites, but were likely present in these effluent-dependent sites as a result of outflows from WWTP ponds/lagoons (AWWQRP 2006). Therefore, these species were not included in site-specific toxicity databases.

The fish and invertebrate taxa datasets presented in Appendix B of this report provide a list of resident taxa for use in site-specific BLM adjusted Cu AWQC recalculations.

1.4 OVERVIEW OF THE RECALCULATION FOR ARID WEST EFFLUENT-DOMINATED STREAMS

The USEPA recognizes that some of the species within the national database may not reside in the site, or the site may have sensitive species that are not represented in the database. In these situations, the national or state criteria may be over or under protective for certain aquatic communities. Therefore, the USEPA has established methods for developing site-specific criteria that take into consideration the relative sensitivity of species that occur at a site. These methods are published in *The Recalculation Procedure in Interim Guidance on Determination and Use of Water-Effect Ratios for Metals* (USEPA 1994).

The 2006 AWWQRP report presented a through review of current USEPA methodologies and identified potential problems associated with the application of these methods to arid west streams and rivers. Refined methods were presented that better address the unique characteristics of effluent-dominated, arid west streams and rivers (AWWQRP 2006). A brief overview of these modifications is presented below.

1.4.1 Refined Step-Wise Process for Deletion of Non-Resident Taxa

For any recalculation, a resident species list is compiled for use in the formal USEPA (1994) deletion process. The step-wise deletion process is a decision matrix that uses the resident species lists to screen the national toxicity databases for the most relevant species by determining which species must be deleted and which species must be retained. 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 represent exact matches and must not be deleted (USEPA 1994). Such USEPA emphasis on "circled species" is very important since the circled species can override the retention of other potentially relevant taxa, while the lack of a circled species can lead to the retention of multiple taxa that are only distantly related.

Proposed revisions to the step-wise deletion process addressed in the 2006 AWWQRP refine the process so that the goal of deriving a site-specific database that contains the most closely related taxa to taxa found at the site is attained. The first step remains the same, which is "circling" all species that occur at the site. Note that a circled taxon may be at a higher level of identification than "species" if no lower level of identification is available for particular taxa identified at the site. Some of the studies used to develop the resident species lists only identified invertebrates to the order, family, or genus level. Following the initial circling process, a refined step-wise circling process (AWWQRP 2006), was developed to determine which of the remaining species in the toxicity database must be deleted and which must be retained. This is a six step process, with each step correlating with a higher level of taxonomic organization (Figure 1-1).

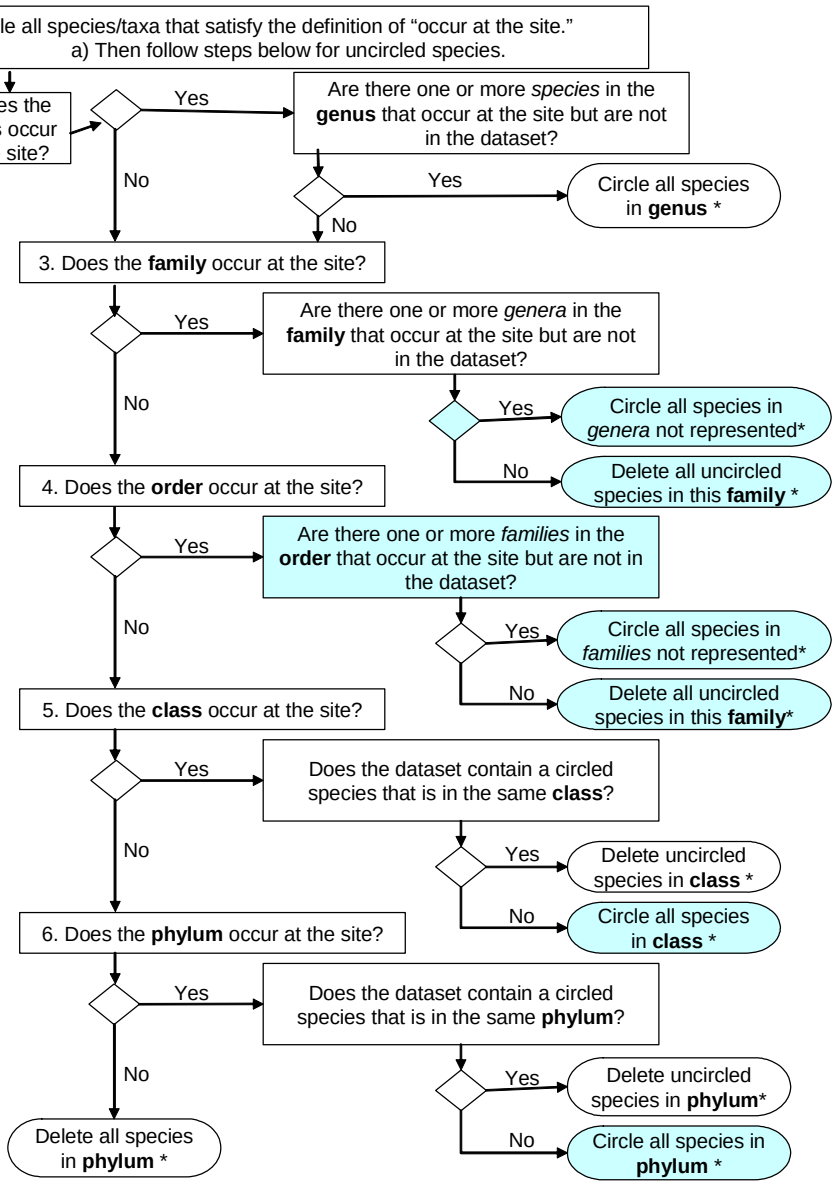

Figure 1-1. Refined Step-Wise Deletion Process Example

1.4.2 Refined Step-Wise Deletion Process Example

To better introduce the refined step-wise deletion process, an example is provided on how to generate a site-specific database using resident species lists and the 2007 BLM acute toxicity database. The taxonomic classification of each species in the database first needs to be identified to phylum. This is an important step that must be performed before the deletion process is conducted, since most deletion decisions are conducted on classification greater than the genus/species level. This example deletion process will be conducted using the Santa Ana River resident species list (AWWQRP 2006).

The first step in the deletion process is to identify exact matches and circle each species in the BLM Cu dataset that also occurs at the site. In this case, five species in the Santa Ana River resident species list also occur in the BLM database. These species include *Pimephales promelas*, *Lepomis macrochirus*, *Lumbriculus variegatus*, *Hyalella azteca*, and *Physa integra* (listed as *Physa* sp. in resident species list). These five species should be circled at the species level (Table 1-1).

Now that all of the species that occur at the site are identified, we need to evaluate each uncircled species to determine if they need to be retained as surrogates for species without toxicity data. This starts at step 2 and continues through all relevant steps for each of the uncircled species (i.e. run through the entire step-wise deletion process for each species then move the next species and repeat). Starting with *Scaphirhynchus platorynchus*, in the BLM Cu database we search the Santa Ana River database for a species in the same genus (Step 2). Since there is no *Scaphirhynchus* sp. in the Santa Ana we move Step 3. Since there is no species in the family Acipenseridae in the Santa Ana, we move Step 4. Once again, there are no species in the order Acipenseriformes in the Santa Ana, so we move Step 5. At Step 5 we accept that the class Actinopterygii does occur at the site and note that the BLM Cu dataset already contains other circled species in this class (*P. promelas* and *L. macrochirus*). Therefore, *S. platorynchus* is deleted from the Santa Ana River site-specific database at the class level. Deletions at the class level also occurred within the class Gastropoda (Table 1-1).

To demonstrate how a species is retained at a higher taxonomic level than species, we will walk through refined step-wise deletion process with the uncircled species in the order Perciformes. This order is represented by five species in two families. The one species in the family Centrarchidae is circled, which would act as a surrogate for the three centrarchids that occur at the site (Appendix B). The four uncircled species in the BLM dataset are all in the genus *Etheostoma*. Starting at Step 2, we search the Santa Ana River resident species list for a species in the genus *Etheostoma*. This genus does not occur at the site (Step 2), nor does the family Percidae (Step 3); therefore we move to Step 4. In Step, 4 we search for species in the order Perciformes. Two species are resident to the Santa Ana in the order Perciformes that are not centrachids (the one family already represented). Therefore, one or more families in this order "occur at the site" which are not in the dataset. All species in the families not already represented were circled, which explains why all the darters were retained in the site-specific BLM database.

The retention of all species in the family Percidae is an important example of how the refined step-wise deletion process differs from standard USEPA deletion methodology. At the order level, the standard step-wise process asks if the dataset contains a circled species, which only includes exact matches. In this example *L. macrochirus* occurs at the site and is in the BLM dataset, therefore all uncircled species would have been deleted. Using the standard step-wise process, only *L. macrochirus* would have been retained to represent all five species in the order Perciformes. Since the sensitivity of the unrepresented family is unknown, it is important to account for the wide range of sensitivity that could exist. Using the refined step-wise process, species in the family Percidae are retained. Retaining these additional species more adequately accounts for the potential variability in sensitivity that could exist (AWWQRP 2006).

Table 1-1. An example of the refined step-wise deletion process using the complete BLM acute Cu toxicity database and resident species list for the Santa Ana River. Species assemblages are included to show the taxonomic level (or step) that each species was either retained or deleted. Blue = deleted at the identified level, Yellow = retained at the identified level.

Phylum (6)	Class (5)	Order (4)	Family (3)	Genus (2)	Species (1)
Chordata	Actinopterygii	Acipenseriformes	Acipenseridae	*Scaphirhynchus*	*Platorynchus*
Chordata	Actinopterygii	Cyprinodontiformes	Poeciliidae	*Poeciliopsis*	*Occidentalis*
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Gila*	*Elegans*
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Acrocheilus*	*Alutaceus*
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Notemigonus*	*Crysoleucas*
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Pimephales*	*Promelas*
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Ptychocheilus*	*Oregonensis*
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Ptychocheilus*	*Lucius*
Chordata	Actinopterygii	Cypriniformes	Catostomidae	*Xyrauchen*	*Texanus*
Chordata	Actinopterygii	Perciformes	Percidae	*Etheostoma*	*Rubrum*
Chordata	Actinopterygii	Perciformes	Percidae	*Etheostoma*	*Lepidum*
Chordata	Actinopterygii	Perciformes	Percidae	*Etheostoma*	*Flabellare*
Chordata	Actinopterygii	Perciformes	Percidae	*Etheostoma*	*Nigrum*
Chordata	Actinopterygii	Perciformes	Centrarchidae	*Lepomis*	*Macrochirus*
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*Kisutch*
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*Mykiss*
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*Tshawytscha*
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*Clarki*
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*Gorbuscha*
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*Apache*
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*Nerka*
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Salvelinus*	*Confluentus*
Chordata	Amphibia	Anura	Bufonidae	*Bufo*	*Boreas*
Annelida	Clitellata	Lumbriculida	Lumbriculidae	*Lumbriculus*	*Variegates*
Arthropoda	Branchiopoda	Diplostraca*	Daphniidae	*Ceriodaphnia*	*Dubia*
Arthropoda	Branchiopoda	Diplostraca*	Daphniidae	*Daphnia*	*Pulicaria*
Arthropoda	Branchiopoda	Diplostraca*	Daphniidae	*Daphnia*	*Magna*
Arthropoda	Branchiopoda	Diplostraca*	Daphniidae	*Scapholeberis*	sp.
Arthropoda	Insecta	Diptera	Chironomidae	*Chironomus*	*Decorus*
Arthropoda	Insecta	Plexoptera	Perlidae	*Acroneuria*	*Lycorias*
Arthropoda	Malacostraca	Amphipoda	Gammaridae	*Gammarus*	*pseudolimnaeus*
Arthropoda	Malacostraca	Amphipoda	Hyalellidae	*Hyalella*	*Azteca*
Mollusca	Bivalvia	Unionoida	Unionidae	*Actinonaias*	*Pectorosa*
Mollusca	Bivalvia	Unionoida	Unionidae	*Utterbackia*	*Imbecellis*
Mollusca	Gastropoda	Architaenioglossa	Viviparidae	*Campeloma*	*Decisum*
Mollusca	Gastropoda	Basommatophora	Physidae	*Physa*	*integra***
Mollusca	Gastropoda	Neotaenioglossa	Pleuroceridae	*Juga*	*Plicifera*
Mollusca	Gastropoda	Neotaenioglossa	Hydrobiidae	*Lithoglyphus*	*Virens*

* = Diplostraca cladocerans are generally considered to be transient organisms in lotic systems and were subsequently deleted from the site-specific database (see text for more details).
** = Since *Physa* sp. was the lowest taxonomic classification identified at the site, *Physa integra* was retained at the genus/species level.

1.4.3 Redefining Minimum Data Requirements for Arid West Streams

A direct calculation of a criterion (not just assigning the most sensitive species in the database) requires that the toxicity database contains the MDRs of toxicity data for eight diverse families (Stephen et al. 1985). As outlined in the current recalculation procedure documents (USEPA 1994) these families must include:

1) the Family Salmonidae,
2) a family in the Class Osteichthyes,
3) a family in the Phylum Chordata,
4) a planktonic crustacean,
5) a benthic crustacean,
6) an aquatic insect,
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.

This MDR is commonly referred to as the "eight-family rule." National AWQC derived from a database that meets the eight-family rule are calculated from a series of formulas using the geometric mean toxicity values of the four most sensitive genera, and the total number of genera represented in the database. The resulting FAV represents a chemical concentration that is equivalent to the 5th percentile sensitivity of all tested organisms. Additional levels of conservatism are added (e.g., dividing the FAV by two to derive the acute criterion) prior to deriving of the acute criterion to ensure protection of most aquatic species to acute chemical exposure (Stephan et al. 1985).

Along with the clarification of the deletion process outlined above, slight modifications of the MDRs may also be warranted given the habitats present and organisms expected to occur in arid west habitats. For example, all sites under consideration for the BLM Cu AWQC 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 which would strictly be a "violation" of the "eight-family rule."

To resolve these MDR discrepancies, a revised "eight-family rule" that incorporates more typical arid west stream aquatic communities was developed (AWWQRP 2006) using suitable surrogate organisms to replace the current USEPA MDRs families expected to be non-resident in arid west effluent-dependent streams.

The arid West stream (AWS)-MDRs include:

1) an organism in the Family Centrarchidae,
2) an organism in the Family Cyprinidae,
3) a family in the Phylum Chordata,
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.

Note: 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.

1.4.4 Site-specific Criteria Derivation at the Species Level

Current AWQC are presently derived from ranked genus mean acute and chronic values (GMAV, GMCV) that are calculated as the geometric mean of species mean acute values (SMAVs) or species mean chronic values (SMCV). The 2006 AWWQRP report proposed that site-specific criteria should be derived at the species level using SMAVs rather than GMAVs since 1) the deletion process is conducted on a species level rather than a genus level, 2) toxicity of a contaminant to different species within the same genera is not always equivalent, and 3) the little overlap of arid west resident species lists and respective site-specific toxicity databases can artificially lower the criterion if derived at the GMAV level. Given each of these reasons still hold true when applying the recalculation procedure to the BLM database, proposed site-specific standards using the 2007 BLM criteria document were also calculated at the species level.

1.5 ARID WEST EFFLUENT DEPENDENT STREAM SITE-SPECIFIC BLM ADJUSTED COPPER AWQC

The current Cu AWQC document was only a few weeks old (February 2007) when this report was created; therefore, revising and updating the national criterion was not necessary.

The next steps in generating site-specific AWQC involve deletion of non-resident taxa using the national BLM adjusted Cu database and comparisons to resident species presented in the previous section of this report. Resident species deletion tables for each stream segment are presented in Appendix C. Following deletion of non-resident taxa, site-specific BLM adjusted Cu toxicity databases were developed and criteria were calculated for each study stream and the two proposed regions. The resulting toxicity databases with ranked species lists are provided in Table 1-2.

Site-specific BLM databases shared a common most sensitive species, the amphipod *Gammarus pseudolimnaeus*. The total number of species represented in the site-specific BLM adjusted databases ranged from 7 for the Santa Cruz near Tuscan to 29 for the High Plains regional database (Table 1-2). Interestingly, the most sensitive species in the hardness modified site-specific databases was also an amphipod in the genus *Gammarus* (*G. pulex*)(AWWQRP 2006).

A summary of the resulting FAVs derived using the standard USEPA 95th percentile calculations (Stephan et al. 1985) are presented in Table 1-3. The recalculated FAVs represent the acute sensitivity of an organism at 5th percentile sensitivity at BLM normalized water chemistry (USEPA 2007) and are functionally equivalent to the hardness based FAVs normalized to hardness = 50 mg/L (with the caveats noted earlier in Section 1.2).

To determine what the recalculated standard would equate to when site-specific Cu complexing is taken into consideration, the site water chemistry (Table 1-4) was run with the BLM to generate a lethal accumulation value (USEPA 2007), which was then used to generate site-specific criteria. The site specific BLM adjusted criteria would be similar to calculating the hardness-based criterion using mean site hardness.

Table 1-2. Site-specific acute toxicity databases for copper, ranked by species mean acute values

			SITE SPECIFIC RANKS						REGIONAL RANKS	
			Santa Cruz							
Species	Common Name	SMAV (ug/L)	Santa Ana	Salt/ Gila	near Nogales	near Tuscan	Fountain Creek	S Platte River	South west	High plains
Notemigonus crysoleucas	Golden shiner	107,860	21	18	15	-	27	21	20	29
Acroneuria lycorias	Stonefly	20,636	20	17	14	7	26	20	19	28
Lepomis macrochirus	Bluegill sunfish	2,231	19	16	13	6	25	19	18	27
Chironomus decorus	Midge	1,987	18	15	12	5	24	18	17	26
Acrocheilus alutaceus	Chiselmouth	216.3	17	14	11	-	23	17	16	25
Etheostoma nigrum	Johnny darter	178.3	16	13	-	-	22	16	15	24
Ptychocheilus lucius	Colorado pikeminnow	132.2	15	12	10	-	21	15	14	23
Etheostoma flabellare	Fantail darter	124.3	14	11	-	-	20	14	13	22
Etheostoma lepidum	Greenthroat darter	82.8	13	10	-	-	19	13	12	21
Xyrauchen texanus	Razorback sucker	78.66	12	9	9	-	18	12	11	20
Pimephales promelas	Fathead minnow	69.63	11	8	8	-	17	11	10	19
Salvelinus promelas	Bull trout	68.31	-	-	-	-	16	-	-	18
Gila elegans	Bonytail chub	63.22	10	7	7	-	15	10	9	17
Poeceliopsis occidentalis	Gila topminnow	56.15	9	6	6	4	14	9	8	16
Oncorhynchus nerka	Sockeye	54.82	-	-	-	-	13	-	-	15
Utterbackia imbecillis	Freshwater mussel	52.51	8	-	-	-	-	8	7	14
Lumbriculus variegatus	Worm	48.41	7	5	5	3	12	7	6	13
Oncorhynchus gorbuscha	Pink	40.13	-	-	-	-	11	-	-	12
Oncorhynchus clarki	Cutthroat trout	32.97	-	-	-	-	10	-	-	11
Oncorhynchus apache	Apache trout	32.54	-	-	-	-	9	-	-	10
Oncorhynchus tshawytscha	Chinook salmon	25.02	-	-	-	-	8	-	-	9
Oncorhynchus kisutch	Coho salmon	22.93	-	-	-	-	7	-	-	8
Etheostoma rubrum	Fountain darter	22.74	6	4	-	-	6	6	5	7
Oncorhynchus mykiss	Rainbow trout	22.19	-	-	-	-	5	-	-	6
Physa integra	Snail	20.41	5	3	4	-	4	5	4	5
Ptychocheilus oregonensis	Northern pikeminnow	14.61	4	-	3	-	3	4	-	4
Hyalella azteca	Amphipod	12.07	3	2	2	2	2	3	3	3
Actinonaias pectorosa	Freshwater mussel	11.33	2	-	-	-	-	2	2	2
Gammarus pseudolimnaeus	Amphipod	9.6	1	1	1	1	1	1	1	1

Table 1-3. Summary of the number of species, genera, and families retained for site-specific criteria calculations and resulting final acute values (FAVs) at normalized USEPA chemistry. Databases were checked to determine if USEPA and arid west stream (AWS) minimum data requirement (MDRs) were met. Site-specific FAVs and Lethal Accumulation with 50% effect (LA50s) take into consideration site-specific water quality databases (Table 1-4).

Site	Taxa Count			Meets MDRs?		Recalc		Site-Specific
	Species (N)	Genera	Families	USEPA	AWS	FAV	FAV	LA50
2007 BLM Cu Database	38	27	17	yes	yes	NA	4.7	0.0342
Recalculations								
Salt/Gila River	18	15	11	no	yes*	9.09	283.2	0.0862
Santa Ana	21	17	12	no	yes	9.89	86.4	0.1028
Santa Cruz: near Nogales	15	14	10	no	yes**	8.67	64.8	0.0830
Santa Cruz: near Tucson	7	7	7	no	no	NA	NA	NA
South Platte	21	17	11	no	yes	9.89	418.5	0.1028
Fountain Creek	27	17	12	no	yes	10.85	162.6	0.1154
Southwest Region (SW)	20	17	12	no	yes	8.89	126.4	0.0862
High Plains Region (HP)	29	19	13	no	yes	10.62	284.5	0.1154

*with the retention of the stonefly

**with the retention of expected benthic macroinvertebrates (amphipods and the stonefly)

***default to most sensitive species

Table 1-4. Mean site-specific water quality parameters used to modify the recalculated final acute values[1]. (All values in mg/L except where noted otherwise)

Site	Temp (°C)	pH	Cu	DOC	Humic Acid	Ca	Mg	Na	K	SO_4	Cl	Alkalinity
2007 BLM standardized values	20	7.5	1	0.5	10	14	12.1	26.3	2.1	81.4	1.9	65
Salt/Gila River	20	8	1	6.9	10	104.0	43.3	370.0	16.6	185.0	530.0	27.5
Santa Ana	20	8.1	1	2.5	10	55.2	11.4	45.6	6.6	48.2	46.5	32.8
Santa Cruz Nogales	20	7.7	1	3.2	10	44.3	5	22	3.3	7.3	28.8	137.5
Santa Cruz Tuscon	20	7.8	1	3.2	10	69.1	12.7	44.5	5	25.6	84.3	197.1
South Platte	20	8.2	1	9.8	10	42.5	26.3	150.0	14.1	161.0	200.0	42.0
Fountain Creek	20	8	1	4.9	10	46.7	11.7	40.9	5	23.1	89.5	135.6
Southwest Region	20	7.9	1	4.0	10	68.1	18.1	120.5	7.9	66.5	172.4	98.7
High Plains Region	20	8.1	1	7.3	10	44.6	19.0	95.5	9.6	92.1	144.8	88.8

[1] Water quality data obtained from Parametrix and HydroQual 2006, and USGS water quality databases.

Differences in site-specific BLM adjusted FAVs and the national BLM adjusted FAV can generally be explained by the lack of daphnid zooplankton in the site-specific BLM adjusted databases (Table 1-2). These site-specific FAVs better reflect the sensitivity of species expected to be resident to arid west effluent-dependent/dominated streams.

Below we discuss differences in the resulting site-specific BLM databases (Table 1-2) and the hardness modified databases (Appendix D), hereafter referred to as "hardness databases", presented in the 2006 AWWQRP report.

1.5.1 Santa Ana River

The site-specific acute BLM Cu toxicity database for the Santa Ana River contains 21 species compared to the 37 species represented in the hardness modified database. These species represent 12 families and meet the AWS-MDRs (Table 1-2). The four most sensitive species in the site-specific BLM database include *G. pseudolimnaeus, Actinonaias pectorosa, Hyalella azteca*, and *Ptychocheilus oregonensis* (Table 1-2). Two of these species are amphipods in genera that were also in the four most sensitive in the hardness site-specific database (Appendix D). The resulting FAV of 9.89 (Table 1-3) at BLM normalized water chemistry is largely driven by the sensitivity of *G. pseudolimnaeus* (SMAV = 9.6).

The resident species analysis identified one threatened species, *Catostomus santaanae*, at this site, which is in the family Catostomidae. Although this species is not in the BLM database, the database contains the catostomid *Xyrauchen texanus* (Table 1-2), which is a potential surrogate species for *C. santaanae*.

1.5.2 Santa Cruz River

Both river segments on the Santa Cruz River (near Nogales and near Tucson) did not meet the AWS-MDRs since benthic crustaceans are not resident species for these sites (Appendix B). Given that both sites contain other benthic macroinvertebrates as resident taxa, which suggests that the appropriate habitat exists, we determined benthic crustaceans "could occur" at these sites. Therefore the two amphipods (*H. azteca* and *G. pseudolimnaeus*) were retained in the Santa Cruz calculations. An additional code not previously used by the USEPA of "R" ("retained") was assigned to these species in Appendix C of this report.

1.5.2.1 Santa Cruz near Nogales

The resulting site-specific acute BLM Cu toxicity database for the Santa Cruz River near Nogales still did not meet the AWS-MDRs even after the retention of amphipods since stoneflies are not resident to the site and are not retained as a surrogate species. Like the amphipods, we anticipate stoneflies could occur at this site, or provide toxicity information for other aquatic insects that do occur but have no toxicity data (e.g., beetles). As a result, the stonefly was retained for the site-specific criteria calculations. Following the retention of expected species, the database contains 15 species that represent 10 families and meets the AWS-MDRs. The four most sensitive species in the database include *G. pseudolimnaeus, H. azteca, P. oregonensis,* and *Physa integra* (Table 1-2). The resulting FAV is 8.67 at BLM criteria normalized chemistry and represents the most sensitive FAV calculated for all the arid West sites (Table 1-3)

One endangered species, *Poeciliopsis occidentalis*, was identified as a resident in the Santa Cruz River near Nogales site. Acute Cu toxicity data are available for this species, which ranks as the 6th most sensitive of the 15 species at this site (Table 1-2).

1.5.3 Santa Cruz near Tucson

The resulting site-specific acute Cu toxicity database for the Santa Cruz River near Tucson contains 7 species in 7 different families (Table 1-3). The number of species represented in the BLM site-specific database is much less than the hardness site-specific database that contained 23 species, representing 12 families (Appendix D). The Santa Cruz near Tuscon BLM database is the most limited database of all sites under consideration for recalculation. This was also true for the hardness site-specific database. The resulting 7 families in the BLM database do not meet the AWS-MDRs, which have an eight family requirement; therefore a criterion can not be calculated. If the FAV defaults to the SMAV of the most sensitive species in the site-specific database, the resulting FAV would be 9.6 at BLM criteria normalized chemistry. No threatened or endangered species were identified for the Santa Cruz near Tucson.

1.5.4 Salt/Gila Rivers

The only documentation of benthic crustaceans for the Salt/Gila Rivers is the presence of the order Amphipoda. Therefore, both species within this order were retained in the site-specific Cu toxicity database. The resulting site-specific acute BLM Cu toxicity database for the Salt/Gila Rivers contains 18 species representing 11 families (Table 1-3). These numbers compare to 39 species, representing at least 18 families in the hardness site-specific databases (Appendix D). Like the Santa Cruz near Nogales, the stonefly was not automatically retained after completing the deletion process (Appendix C), yet could be an important surrogate for other resident species without toxicity data. With the retention of the stonefly, the site-specific BLM database meets the AWS-MDRs (Table 1-3). The four most sensitive species in the database include *G. pseudolimnaeus, H. azteca, P. integra* and *Ethostoma rubrum*. This is the only database that includes *E. rubrum* in the four most sensitive. *E. rubrum* is not a resident species at the site, yet was retained, in addition to all genera in the family Percidae, to represent species in the order Perciformes that are found at the site without BLM Cu toxicity data. The resulting FAV is 9.1 at BLM criteria normalized chemistry.

The Salt/Gila Rivers resident species list includes the most diverse group of threatened or endangered species. Five threatened or endangered species were identified including *X. texanus, Gila elegans, Rhinichthys cobitis, Cyprinodon macularus,* and *P. occidentalis* (Appendix B). Three of these species, *X. texanus, G. elegans*, and *P. occidentalis,* contain acute Cu toxicity data in the BLM database and represent the 9th, 7th and 6th most sensitive species in the site-specific BLM database, respectively (Table 1-2). Three additional fish species were also retained that could serve as surrogates for the remainder of species without toxicity data.

1.5.5 Fountain Creek

The site-specific acute Cu BLM adjusted toxicity database for the Fountain Creek contains 27 species representing 12 families (Table 1-3). These values compare to 47 species, representing at least 19 families in the hardness site-specific database. Both the BLM and hardness databases meet the AWS-MDRs (Table 1-3; AWWQRP 2006). The Fountain Creek BLM database includes the most species of all the arid West sites. The four most sensitive species in the BLM database include *G. pseudolimnaeus, H. azteca, P. oregonensis,* and *P. integra* (Table 1-2). The resulting FAV is 10.85 at BLM criteria normalized water quality and is the greatest recalculated FAV of all sites. No threatened or endangered species were identified in the Fountain Creek resident species list.

1.5.6 South Platte River

The site-specific acute Cu toxicity database for the South Platte River contains 21 species representing 11 families (Table 1-3). These values compare to the 51 species, representing at least 19 families that were included in the hardness site-specific database. Both databases meet the AWS-MDRs (Table 1-3; AWWQRP 2006). The four most sensitive species in the BLM database include *G. pseudolimnaeus, A. pectorosa, H. azteca*, and *P. oregonensis* (Table 1-2), which are identical to the four most sensitive species for the Santa Ana. The resulting site-specific FAV is 9.9 at BLM criteria normalized chemistry. No threatened or endangered species were identified in the South Platte resident species list.

1.5.7 Southwest Arid Stream Systems (CA, AZ, NV)

Combining the resident species lists for the Santa Ana River, Salt/Gila Rivers, and both sites for the Santa Cruz River resulted in a Southwest regional acute BLM Cu toxicity database containing 20 species, representing 12 families (Table 1-2). This regional database is in compliance with the AWS-MDRs. The top four most sensitive species differs from that of the individual sites and includes *G. pseudolimnaeus, A. pectorosa, H. azteca*, and *P. integra*. Combining the Southwest resident species lists resulted in a larger hardness database when compared to the individual sites (Appendix D). This was not the result when applying the refined step-wise deletion process to the BLM database. The resulting BLM adjusted FAV for the Southwest region of 8.9 at BLM criteria normalized chemistry (Table 1-3) is more restrictive than two of the three recalculated FAVs derived for this region.

1.5.8 High Plains Arid Stream Systems (WY, CO)

Combining the resident species lists for the Fountain Creek and the South Platte River for the High Plains region resulted in a regional acute Cu toxicity database containing 29 species, representing 13 families (Table 1-2). This regional database is in compliance with the AWS-MDRs. The slightly greater database size, when compared to the site-specific databases, and four more sensitive species that are identical the South Platte, resulted in an intermediate FAV of 10.62 at BLM criteria normalized chemistry (Table 1-3).

1.6 COMPARISON OF SITE-SPECIFIC BLM-BASED COPPER CRITERIA

Appling the recalculation procedure to the BLM Cu toxicity database resulted in site-specific and regional databases representing fewer species and families when compared to the site-specific hardness modified databases for all sites – a direct result of the smaller BLM toxicity database noted earlier. None of the resulting site-specific BLM databases were in compliance with standard USEPA MDRs, however most databases were in compliance with the AWS-MDRs after retention of species that occured or could be expected to occur at the sites.

Although differences between site-specific BLM databases exist, the recalculated BLM FAVs (before adjusting to site water chemistry) were very similar between sites and regions (Table 1-3). Similarity of the BLM FAVs among sites can be attributed to the common most sensitive species, *G. pseudolimnaeus* that has a SMAV of 9.6 µg/L at normalized USEPA water chemistry. While these site-specific BLM FAVs were considerably lower than FAVs calculated using the hardness modified databases, once again, a direct comparison is not possible given the normalization to additional water quality parameters for BLM values and different database sizes.

Although BLM recalculated FAVs are similar among sites, all BLM modified site-specific criteria are substantially greater than site-specific criteria derived using the hardness modified databases (Table 1-5). These results suggest that Cu complexing resulting from site water

chemistry has a greater effect on site-specific standards when using the BLM than the site-specific toxicity databases generated with the step-wise deletion process. For the arid West sites, the complexing reduces toxicity and results in standards that are greater than hardness modified site-specific criteria.

Table 1-5. Comparison of site-specific copper criteria derived using updated hardness modified toxicity databases (AWWQRP 2006) and the BLM database (USEPA 2007)

	Site-Specific Criteria						Regional Criteria	
		Santa Cruz						
	Santa Ana River	Near Nogales	Near Tucson	Salt/ Gila River	Fountain Creek	South Platte River	SW	HP
Mean Site Hardness (mg/L)	153	120	189	307	132	136	192	134
Acute								
Updated Hardness	23.8	18.7	29.2	47	20.6	21.2	26.7	20.9
Recalc Hardness	24.5	19.8	26.9	50.4	21.5	22.5	33.7	22.3
BLM	25	18.4	24.2	96	43.1	132.7	39.1	81.6
Recalc BLM criterion	43.2	32.4	45.7	141.6	81.3	209.2	63.2	142.2
Chronic								
Updated Hardness	10.6	9.2	12	16	9.7	9.9	12.1	9.8
Recalc Hardness	10.9	9.7	11	17.1	10.2	10.5	13.7	10.5
BLM	15.5	11.5	15.1	59.6	26.7	82.4	24.27	50.7
Recalc BLM criterion	26.8	20.1	28.4	87.95	50.5	130	39.2	88.35

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2. CURRENT STATUS AND FUTURE TRENDS IN BIOTIC LIGAND MODELS FOR DERIVATION OF AQUATIC LIFE PROTECTION CRITERIA FOR METALS

The development of computational models that can be used to predict the influence of environmental concentrations of metals on aquatic organisms has received considerable attention by researchers and regulators around the globe since the 1990s. Currently, advancements in predictive models that can account for biological effects from acute (short-term, high magnitude) and chronic (long-term, low level) exposures have provided regulators in the United States and Europe the tools for implementing site-specific water quality standards. Such predictions offer a cost-effective alternative to potentially complex toxicity testing procedures (e.g., USEPA 1994, 2001).

The objective of this section of the report is to discuss the current status of BLM for various metals and the future trends that will enhance our ability to predict the effects of metals on environmental systems. Specifically, the role of BLMs for deriving acute and chronic water quality standards is discussed in the context of current regulatory frameworks. Similarly, the limitations that exist for implementing BLMs into criteria development are also considered in a regulatory context. Finally, a discussion of the existing BLMs and the future trends (e.g., metal mixtures, sediment and terrestrial BLMs, and dietary BLMs) is presented for consideration.

2.1 BIOTIC LIGAND MODEL BASIS

Recent efforts have been made toward developing computational models that incorporate chemical reactions and metal-gill binding to better represent the complex chemical factors that influence metal bioavailability to aquatic organisms (Paquin et al. 2002a). Unlike historical hardness-based equations for predicting toxicity, these types of models (termed Biotic Ligand Models; BLM) explicitly account for individual water quality variables and are not linked to a particular correlation between measured toxicity and a single concurrently measured water quality parameter. However, the mechanistic principles underlying BLMs follow general trends of metal toxicity as related to overall water quality (i.e., the influence of hardness, alkalinity, pH, organic matter, major ions, etc.). The basic presumption is that any changes in water quality that decrease the concentration of bioavailable metal (primarily the hydrated/free metal ion) which can chemically bind to biological surfaces (i.e., the "biotic ligand") are associated with decreasing toxicity of that metal to aquatic organisms (Playle et al. 1993; Welsh et al. 1993; Erickson et al. 1996; Playle 1998; Meyer et al. 1999; Santore et al. 1999; Di Toro et al. 2001; Heijerick et al. 2002; De Schamphelaere and Janssen 2004a; De Schamphelaere et al. 2005; Villavicencio et al. 2005; USEPA 2007).

For example, increases in pH, alkalinity, or natural organic matter would all tend to decrease metal bioavailability through complexation and, ultimately, decrease metal toxicity. Metal bioavailability may also be affected by competitive interactions at the biotic ligand (e.g., critical organ of respiratory or homeostatic maintenance) by calcium, magnesium, sodium, and/or protons [H+], thereby decreasing toxic metal concentrations. The interactions between the biotic ligand and each of the dissolved chemical species with which the metal reacts are represented in the BLM by characteristic binding site densities and conditional stability constants (Playle et al. 1993; Newman and Jagoe 1994; Playle 1998; Santore et al. 1999).

Predictions of toxicity are made in the BLM by assuming that the toxic concentration of dissolved metal, which varies with ambient water chemistry, is always associated with a fixed critical level of metal accumulation at the biotic ligand. While the critical accumulation

concentration can vary based on species sensitivity (i.e., more or less metal-biotic ligand accumulation required to exert a similar toxic response), it is assumed to be constant for individual species, regardless of water quality (Newman and Jagoe 1994; Erickson et al. 1996; Playle 1998; Meyer et al. 1999; Santore et al. 1999; Di Toro et al. 2001; Paquin et al. 2002a).

The advantage of using a mechanistic model for predicting metal toxicity is that all thermodynamic constants used to simulate inorganic and organic equilibrium-based chemical reactions are empirically derived. As such, the binding constants do not change for BLM simulations involving different organisms. For example, although binding constants for copper and other cations were derived using fathead minnows and rainbow trout, the same values would apply to invertebrates and other fish (Santore et al. 1999; Di Toro et al. 2000, 2001; USEPA 2007). The interactions between some cations and the biotic ligand, however, can change between acute and chronic exposures (i.e., the importance of some cations may be less important when metals concentrations are relatively low). As such, it is necessary to characterize the empirical relationship between metal toxicity and various water quality parameters for each species and endpoint.

2.2 BLM INPUT PARAMETERS

There are currently 12 input parameters required to run existing BLMs (Table 2-1). Because the influences of water quality parameters on chemical speciation are similar among metals, the required input parameters are the same regardless of the metal of interest. Of the required parameters, temperature, pH, DOC, alkalinity, and the major ions (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, $SO4^{2-}$ and Cl^{-}) all are considered user-entered inputs to the model because they each influence metal bioavailability (Table 2-1). The two remaining parameters, % Humic Acid (HA) and sulfide, can also influence bioavailability but are considered to have relatively constant concentrations in natural waters. As such, if these parameters are not measured in test waters, default values of 10% and 0.003 mg/L are suggested for use in the model, respectively (USEPA 2007). Correlations between %HA and optical absorption of ultraviolet light have also been proposed by researchers as a means for efficiently accounting for the differential binding characteristics among DOC sources. In general, toxicity predictions using this approach are on average 35% more accurate when calculated %HA values are used as opposed to using the default value (De Schamphelaere et al. 2004a, Schwartz et al. 2004). Similarly, if test waters are from sediment elutriates, the presence of sulfides may be significant and would warrant measurement.

The concentration range for each parameter that is considered influential on metal bioavailability is different (Table 2-1). However, existing models will allow any value to be inserted as an input variable, and simply notify the user that the concentration is outside the influential range (i.e., range of concentrations which have been empirically validated). The definition of an influential range can be associated with inherent biological tolerance (e.g., pH), the limitation of geochemical influence on bioavailability (e.g., Ca and Mg), or the assumed environmental concentration range (e.g., DOC). For example, the competitive influence of calcium on acute copper toxicity would computationally be the same at a hardness of 10 or 1000 mg/L as $CaCO_3$. In reality, however, few data exist to validate the role of calcium in waters of hardness greater than 400 mg/L and so the influential range of calcium does not extend as high as 1000 mg/L hardness. However, because of the computational nature of BLMs, they are blind to the limitations of parameter input values and so the model-specific influential ranges for parameter inputs must be determined during BLM development and acknowledged by the users.

There is some evidence that other metals such as iron and aluminum can have an effect on the toxicity of the metal of interest to aquatic organisms. This influence on toxicity may be due to the same types of interactions that are observed for other cations (e.g., competition at the biotic ligand) or complexation of the metal to iron and/or aluminum colloids similar to binding to organic matter (USEPA 2007). Each of these scenarios would theoretically decrease metal bioavailability in solution. Although iron and aluminum are not currently included as routine BLM inputs, researchers have been encouraged to measure dissolved iron and aluminum as part of monitoring efforts to support possible future criteria applications (USEPA 2007).

Table 2-1. Description of required input parameters for predicting metal toxicity with existing Biotic Ligand Models.

Input Parameter	Rationale for Inclusion	Typical Units	Lower Limit	Upper Limit
Temperature	Thermodynamic control of chemical reactions	°C	10	25
pH	Cation competition (H+) and inorganic carbon complexation (OH-)	Standard Units	4.9	9.2
Dissolved Organic Carbon (DOC)	Metal complexation	mg/L	0.05	29
Humic Acid (HA)	Binding characteristics of DOC	%	10	60
Calcium (Ca)	Cation competition at biotic ligand and ionic strength adjustments	mg/L	0.3	120
Magnesium (Mg)	Cation competition at biotic ligand and ionic strength adjustments	mg/L	0.03	51
Sodium (Na)	Cation competition at biotic ligand and ionic strength adjustments	mg/L	0.2	230
Potassium (K)	Ionic strength adjustments	mg/L	0.04	150
Sulfate (SO_4)	Metal complexation and ionic strength adjustments	mg/L	0.1	270
Chloride (Cl)	Metal complexation and ionic strength adjustments	mg/L	0.4	270
Alkalinity	Inorganic carbon complexation (CO_3^{2-})	mg/L as $CaCO_3$	2	360
Sulfide (S)	Metal complexation	mg/L	-	-

2.3 CURRENT REGULATORY CONTEXT

There is a consistent regulatory strategy throughout North America, Central America, South America, Europe and Asia for deriving protective aquatic life criteria. However, although the strategy for deriving water quality criteria is similar, the toxicological data (acute versus chronic endpoints) used as the technical basis are not. In general, a criterion is based on the compilation of toxicity data that meet a minimum diversity requirement (e.g., eight or more different families representing different levels of phylogenetic organization (USEPA 1985a; European Commission 2003). Once minimum data requirements are met, the distribution of species mean or genus mean toxicity values are used to calculate the concentration associated with the 5th percentile of sensitivity (i.e., concentration that would not affect 95% of the reported test species). Because of the variation in water quality used for testing among

different laboratories, toxicity endpoints reported for a single species can vary by nearly 100-fold in some cases. As such, intra-species variability is often a significant source of error associated with determining an accurate 5th percentile sensitivity concentration and is the motivation for "normalizing" both acute and chronic toxicity data to a similar water quality condition (e.g., hardness = 50 mg/L as $CaCO_3$) prior to constructing species sensitivity distributions (SSD).

Acceptable acute toxicity (survival data obtained from short-term [48 or 96 h] exposures) data are typically reported as median-lethal concentrations (LC50) or median-effect concentrations (EC50). These data are then used to estimate critical metal-biotic ligand accumulation concentrations used for BLM modeling, which are expressed as median-lethal accumulation concentrations (LA50). Because the mechanism associated with the acute toxic mode of action for a particular metal is assumed to be similar among species (Di Toro et al. 2000, 2001; Paquin et al. 2002a), the validation of a metal-specific BLM for even just a few species provides the rationale for using a BLM for all the species within a given distribution/database.

Analogous to criteria derivation based on normalizing available toxicity data to a similar hardness (USEPA 1984, 1985b, 1985c, 1986, 1987), the BLM is used to generate water quality-normalized LA50 values for each acceptable toxicity test identified in a toxicity database (example for copper in USEPA 2007). As a result, species sensitivity is ranked relative to BLM-normalized LA50 values, and an acute criterion can be derived using various statistical models (USEPA 1985a; European Commission 2003). This in effect establishes the critical metal accumulation level (i.e., LA50) for a hypothetical organism that is more sensitive than 95% of all tested freshwater fauna. The BLM is then used to derive site-specific criteria based on the acutely toxic metal concentration for this hypothetical organism representing the 5th percentile and the actual water quality conditions in the waterbody of concern. This approach represents a significant enhancement to the current hardness-based approach, and so long as the BLM is adequately validated for a wide range of water quality conditions (Parametrix and HydroQual 2006), it should provide a more scientifically-defensible site-specific water quality criterion.

Although the current approach in the U.S. does not require a full phylogenetic characterization of metal sensitively for chronic exposures (sub-lethal data obtained from long-term [life-cycle] studies), the development and implementation of chronic BLMs that have guided criteria derivation in Europe will eventually supplement existing approaches. That is to say, entire SSDs can be constructed using only chronic toxicity data. Acceptable chronic toxicity data are reported as effect concentrations that inhibit/reduce growth, reproduction, productivity, etc. in 10% or 20% of the exposed population relative to controls (EC10 or EC20, respectively). Alternatively, the highest reported concentration that is not statistically different than control response (no observable effect concentrations) can also be used in SSDs (European Commission 2003). However, because the mechanism of the chronic toxic mode of action for a particular metal can vary relative to the test endpoint of interest, the associated critical metal-biotic ligand accumulation concentrations are often species and endpoint-specific. For example, a BLM developed and validated for predicting reproductive effects in an invertebrate may not be appropriate for predicting growth effects in fish. Although the physical binding characteristics of a given metal with biological membranes may be the same for different organisms, the site of the biotic ligand of interest will vary relative to the endpoint of concern (e.g., nervous system, endocrine system, circulatory system, etc.).

As a result, chronic BLMs should theoretically be developed specific to each organism contained in an SSD. To alleviate the obvious logistical concerns associated with generating a BLM for each organism within an SSD, the concept of trophic level BLMs is being

considered. It has been argued that classes of organisms representing major trophic levels (plants, invertebrates, fish, etc.), and the consequential test endpoints obtained from testing those classes, are generally similar. For example, BLMs generated for a species of algae (using inhibition of primary productivity as a toxicity test endpoint), a planktonic invertebrate (using reproduction as a test endpoint), and a fish (using growth as a test endpoint) should be applicable to other species within each of their respective trophic levels (De Schamphelaere et al. 2003, De Schamphelaere and Janssen 2004b, De Schamphelaere et al. 2005). If each of the species contained within an SSD is assigned to one of these organism classes/trophic levels, the number of BLMs needed for full normalization of an SSD can be minimized. However, due to physiological differences that can exist among organisms even within a similar class/trophic level (e.g., invertebrates that use parthenogenic versus sexual reproduction, organisms with exclusively aquatic versus emergent life-stages, organisms that are ionoregulators versus ionoconformers, etc.), generalizing BLM characteristics by class or trophic level may be problematic, and is the subject of ongoing debate in the scientific community.

Given that a primary goal of developing methods to normalize toxicity test results to a given water quality is to reduce the uncertainty around characterizing species sensitivity, the development of BLMs for even a few organisms within an SSD is preferred over use of a single biotic ligand characteristic for all species (as is done in the 2007 USEPA copper AWQC). Furthermore, the organisms selected as surrogates for an organism class/trophic level are often species with the most standardized available methods, and represent species within an SSD that have numerous test results with significant intra-species variability. For example, there are currently four species for which chronic nickel BLMs have been developed (Deleebeeck et al. 2005, 2007; De Schamphelaere et al. 2006a), and the toxicity results for these four species represent nearly 70% of the ecotoxicological dataset. As such, the information gained from focusing on four of the 27 species for which chronic nickel toxicity data exists, is the preferred starting point for developing site-specific criteria for nickel in natural waters

Regardless of whether normalization based on organism class/trophic level is accepted, the development of these models has provided a powerful tool for identifying aquatic habitats that deserve immediate attention and ensures the protection of the most sensitive species of aquatic biota. Similar to regulations based on acute toxicity data, this approach represents a significant departure from the current methodology and should provide a more scientifically-defensible way of regulating dischargers based on site-specific water quality.

2.4 CURRENT STATUS OF BLMS

Numerous BLMs have been developed for plants, invertebrates and fish for various metals (Table 2-2). As such, the accepted BLMs encompass endpoints that are associated with survival, growth, reproduction, biomass, growth rate, etc. Currently, copper has received the most attention relative to the characterization of the influences of water quality parameters on acute and chronic toxicity to plants and animals (Table 2-2). Although only the acute BLM for copper has been implemented into a regulatory framework in the U.S. (USEPA 2007), the European Union has adopted chronic BLMs for copper, nickel and zinc into regulatory guidance. However, acute BLMs for metals such as silver, cadmium, nickel, and zinc are in the scientific validation stage (Erickson et al. 1998; De Schamphelaere et al. 2005; Deleebeeck et al. 2005) and thus have the potential to be incorporated in future US AWQC revisions for these metals. Similarly, as chronic BLMs gain support globally, their acceptance in the U.S. may also lead to their incorporation into AWQC derivation methods. Furthermore, development of marine BLMs for copper in the U.S. (*Mytilus edulis* example below) are

ongoing and will likely form the basis of site-specific copper criteria for protection of estuarine and open-ocean habitats (Arnold 2005, Arnold and Warren-Hicks 2007a, 2007b).

Other directions of BLM development include the interactions of metal mixtures, sediment and terrestrial exposures, and dietary exposure routes for metals. Similar to considering the overall water quality of an aquatic system, environmental scenarios rarely involve a single metal toxicant. This approach considers the multi-metal competitive interactions with regard to inorganic and organic complexation reactions, and interactions between multiple metals at the biotic ligand (Playle 2004). While much effort has been directed toward development of BLMs for the aquatic environment, the approach is also being considered for sediment and terrestrial environments as well. For sediments, the primary binding phase for consideration is acid-volatile sulfides as well as the importance of metal mixtures (Di Toro et al. 2005). Research in terrestrial habitats has identified the importance of considering the sustainability of microbial processes as well as insect and plant sources that can accumulate metals (Thakali et al. 2006a, 2006b; Lock et al. 2007a, 2007b). Finally, dietary considerations have been given some attention because of the predator-prey interactions between different trophic levels (De Schamphelaere et al. 2004b, 2007).

Table 2-2. List of existing Biotic Ligand Models (BLM) by species, endpoint, and metal.

Organism Class	Species	Common Name	Acute BLM	Chronic BLM	References
Plant	*Pseudokirchneriella subcapitata*	Algae	-	Cu, Ni, Zn	1,2,3
	Chlamydomonas reinhardtii	Algae	-	Cu	4
	Chlorella vulgaris	Algae	-	Cu	4
	Scenedesmus quadricauda	Algae	-	Cu	4
Invertebrate	*Ceriodaphnia dubia*	Water flea	Ag, Cd, Cu, Ni, Zn	Cu, Ni	5,6,7,8
	Daphnia magna	Water flea	Ag, Cu, Ni, Zn	Cu, Ni, Zn	2,3,5,6,7,9,10
	Daphnia pulex	Water flea	Cu	-	6
	Brachionus calyciflorus	Rotifer	-	Cu	11
	Mytilus edulis	Green Mussel	Cu	-	12
Fish	*Pimephales promelas*	Fathead Minnow	Ag, Cd, Cu, Zn	-	5,6,13,14,15
	Oncorhynchus mykiss	Rainbow Trout	Ag, Cd, Cu, Zn	Cu, Ni, Zn	7,14,15,16,17

1. De Schamphelaere et al. 2003 2. De Schamphelaere et al. 2005 3. Deleebeeck et al. 2005 4. De Schamphelaere and Janssen 2006 5. Erickson et al. 1998 6. Di Toro et al. 2001 7. Paquin et al. 2002a 8. Keithly et al. 2004 9. De Schamphelaere et al. 2002 10. De Schamphelaere and Janssen 2004c 11. De Schamphelaere et al. 2006b 12. Arnold 2005 13. Erickson et al. 1996 14. De Schamphelaere and Janssen 2004d 15. Deleebeeck et al. 2007 16. Bury et al. 1999 17. Paquin et al. 2002b

2.5 UNCERTAINTIES

All BLMs use equilibrium reactions of metal and other cations with a single, simple type of biotic ligand as the means for predicting the effects of physiochemical exposure conditions on metal toxicity. Use of these simplifications, therefore, means that BLMs can only approximate the full complex set of chemical reactions involved with environmental metal

exposures that ultimately elicit toxicity. For example, while the BLM assumes that cation effects (particularly protons H+), are best described via chemical competition with the toxic metal for binding to the biotic ligand, pH microenvironments at the gill surface can also change metal speciation (Playle et al. 1992, Playle 1998). Similarly, sensitivity parameters (i.e., critical accumulation concentrations, such as the LA50) calculated empirically from bulk gill measurements in the laboratory are likely not the same as those truly occurring on a molecular level at the biotic ligand. Moreover, because the BLM is based on the assumption of chemical equilibrium, non-equilibrium processes related to metal binding at the biotic ligand may also be an important consideration for future models. For example, the limiting factor for predicting metal-biotic ligand interactions may be the amount of time needed for metals to pass through biological membranes, and not just the equilibrium binding characteristics with the membrane (as is currently assumed).

In general, any model is a simplification of reality and the existence of uncertainties does not preclude a model from being useful and justified. Despite their simplicity, BLMs provide a reasonable mechanistic framework for the well-established effects of metal speciation, explicitly addressing the relative bioavailability of different species. BLMs also include a plausible mechanism that allows the effects of cations to be addressed and uses a comprehensive model for calculating the concentrations, and effects, of various chemical species. Even if the mechanistic descriptions are incomplete, these models allow the major empirical effects of complexing ligands and competing cations to be described in a more comprehensive and reasonable fashion than other approaches (such as use of hardness-based equations for predicting toxicity). Furthermore, BLMs are being validated under a wide range of environmental conditions, and represent a more accurate means of deriving criteria that are protective of the most sensitive organisms (Parametrix and HydroQual, 2006). As such, BLMs represent a significant technical advance for deriving regulatory standards for protection of aquatic life.

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3. RELATIVE ROLE OF SODIUM AND ALKALINITY VERSUS HARDNESS CATIONS IN CONTROLLING ACUTE AMMONIA TOXICITY TO AQUATIC ORGANISMS

3.1 BACKGROUND

The hardness-ammonia studies conducted in 2006 for AWWQRP (Parametrix and Chadwick Ecological 2006) supported the limited toxicity literature available which suggests that hardness (and/or related cations) may influence acute ammonia toxicity. However, to further elucidate the mechanisms governing these effects, that study determined that major ion composition other than hardness (sodium is of particular interest) needed additional independent experimental manipulation.

As a start to this research, a series of acute *Hyalella azteca* and *Oncorhynchus mykiss* (rainbow trout) studies were conducted wherein sodium was independently manipulated in conjunction with hardness and alkalinity (Parametrix 2006). Four reconstituted waters were made in which sodium concentrations were manipulated, either by direct addition of sodium chloride (NaCl), or as a result of increasing alkalinity (sodium addition as sodium bicarbonate – $NaHCO_3$). In the first study, hardness and alkalinity were nominally maintained at 100 and 70 mg/L as $CaCO_3$, respectively (Table 3-1). The natural amount of sodium associated with this alkalinity (32.2 mg/L) was not altered in this first experiment. Hardness and alkalinity were held at the same levels in the second study as in the first; however, 190 mg/L sodium (as NaCl) was added to mimic the natural amount of sodium associated with the nominal alkalinity of the third study (420 mg/L), in which hardness was maintained at 100 mg/L as $CaCO_3$. The fourth study was identical to the third, except that hardness was increased to 600 mg/L as $CaCO_3$. The target pH of all four studies was 8.

Table 3-1. Results of acute *H. azteca* sodium studies (Parametrix 2006)

	Nominal Na (mg/L)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	TA-N LC50 (mg N/L with 95% CI)	UA-N LC50 (mg NH3-N/L with 95% CI)
1.	32.2	104	72	58.4 (48 – 71.1)	1.9 (1.6 – 2.1)
2.	193.1*	108	78	102.1 (83.8 – 124.5)	2.2 (1.9 – 2.4)
3.	193.1	112	376	65.8 (57 – 75.9)	2.1 (1.9 – 2.4)
4.	193.1	570	360	146.2 (126.1 – 169.5)	3.4 (3.0 – 3.8)

* Sodium concentration due to NaCl addition. All other sodium concentrations due to alkalinity (as $NaHCO_3$).

The rainbow trout studies were inconclusive. However, comparing the results of the first and fourth *H. azteca* studies (Table 3-1), total ammonia toxicity decreased significantly with increasing hardness, supporting the hypothesis that increasing hardness will only decrease ammonia toxicity to the amphipod when accompanied by a concurrent increase in sodium along with alkalinity. These studies further suggested that increasing sodium as NaCl alone (i.e., without increasing hardness as well) also decreases ammonia toxicity, although increasing sodium as alkalinity (i.e., without increasing hardness) does not affect ammonia toxicity.

3.2 STUDY OBJECTIVES AND RESULTS

It is clear from these studies (Parametrix 2006; Parametrix and Chadwick Ecological 2006) that elevated sodium levels offer considerable protection to *H. azteca* against ammonia toxicity, especially when coupled with elevated hardness. However, it is not yet apparent how sodium may influence the ammonia/hardness relationship at other pH levels, intermediate hardness concentrations, or for other species. Therefore, to confirm the role of sodium in controlling acute ammonia toxicity in very hard or ion rich waters, additional acute toxicity tests were conducted with the invertebrate *Ceriodaphnia dubia*, as well as the freshwater fish *Pimephales promelas* (fathead minnow) employing the same series of sodium manipulations described above.

Results from these studies suggested that *C. dubia* and *P. promelas* responded similarly to the aforementioned treatments (Table 3-2 and Table 3-3) and were, in general, more sensitive to ammonia toxicity than *H. azteca* (Table 3-1). The patterns in effects observed for these species were similar to those for *H. azteca* in some tests, while not for others. For example, elevated hardness, when coupled with concomitantly elevated sodium/alkalinity decreased the toxicity of ammonia to all three species (*C. dubia, P. promelas,* and *H. azteca*), whereas increasing sodium alone as NaCl (i.e., without also increasing hardness) only resulted in decreased toxicity to *H. azteca*. Furthermore, while for *H. azteca*, increasing sodium alone as alkalinity (i.e. without also increasing hardness) did not affect ammonia toxicity, for both *C. dubia* and *P. promelas*, this treatment caused the highest level of toxicity observed across all tests.

Table 3-2. Results of acute *C. dubia* sodium studies

	Measured Na (mg/L)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	TA-N LC50 (mg N/L with 95% CI)	UA-N LC50 (mg NH_3-N/L with 95% CI)
1.	27.32	104	76	16.9 (14.8 – 19.4)	0.64 (0.59 – 0.69)
2.	184.7*	104	76	18.0 (15.8 – 20.3)	0.70 (0.64 – 0.76)
3.	178.2	88	392	4.4 (3.8 – 5.0)	0.45 (0.40 – 0.50)
4.	187.4	488	368	38.0 (32.1 – 45.1)	1.57 (1.37 – 1.79)

* Sodium concentration due to NaCl addition. All other sodium concentrations due to alkalinity (as $NaHCO_3$).

Table 3-3. Results of acute *P. promelas* sodium studies

	Measured Na (mg/L)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	TA-N LC50 (mg N/L with 95% CI)	UA-N LC50 (mg NH_3-N/L with 95% CI)
1.	27.32	104	76	15.7 (13.9 – 17.8)	0.57 (0.52 – 0.63)
2.	184.7*	104	76	16.6 (14.5 – 18.9)	0.58 (0.52 – 0.64)
3.	187.8	100	420	5.9 (5.2 – 6.7)	0.49 (0.44 – 0.55)
4.	187.4	488	368	27.3 (24.2 – 30.8)	1.03 (0.91 – 1.16)

* Sodium concentration due to NaCl addition. All other sodium concentrations due to alkalinity (as $NaHCO_3$).

General conclusions regarding this research are as follows:

- For the amphipod, *H. azteca*, the cladoceran, *C. dubia*, and the fathead minnow, *P. promelas*, increasing hardness ameliorates the acute toxicity of ammonia when coupled with an increase in sodium as alkalinity.

- Increasing sodium alone (as either NaCl or alkalinity), without also increasing hardness, has variable effects on the acute toxicity of ammonia to these species.

Based on these conclusions, hardness does exert a significant enough effect on acute ammonia toxicity to be used as a basis of deriving site-specific ammonia standards, but only in hard waters where sodium (as alkalinity) is also elevated. Our studies also help explain why hardness-related impacts on ammonia toxicity in *H. azteca* differed between the AWWQRP (Parametrix, and Chadwick Ecological 2006) and previously published hardness studies with the same species (Ankley et al. 1995). In the AWWQRP studies, hardness did not exert a significant impact on acute ammonia toxicity to the amphipod, but sodium and alkalinity were held constant across all hardness treatments. In contrast, sodium and alkalinity increased with increasing hardness in the Ankley et al. (1995) studies, which probably explains why increasing hardness was associated with decreasing acute ammonia toxicity. Taken together, these studies corroborate the current AWQC for ammonia's (USEPA 1999) suggestion that, ammonia WERs greater than 1 may be expected when a difference in ionic composition, in conjunction with pH or hardness, is present between site and laboratory waters.

3.3 REFERENCES

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APPENDIX A

EPA Table 3a

Table 3a. Ranked Freshwater Genus Mean Acute Values with Species Mean Acute-Chronic Ratios

Rank	GMAV	Species	SMAV (µg/L)	ACR
27	107,860	Golden shiner, *Notemigonus crysoleucas*	107,860	
26	20,636	Stonefly, *Acroneuria lycorias*	20,636	
25	3,573	Snail, *Campeloma decisum*	3,573	171.19
24	2,231	Bluegill sunfish, *Lepomis macrochirus*	2,231	40.49
23	1,987	Midge, *Chironomus decorus*	1,987	
22	216.3	Chiselmouth, *Acrocheilus alutaceus*	216.3	
21	80.38	Fantail darter, *Etheostoma flabellare*	124.3	
		Greenthroat darter, *Etheostoma lepidum*	82.80	
		Johnny darter, *Etheostoma nigrum*	178.3	
		Fountain darter, *Etheostoma rubrum*	22.74	
20	78.66	Razorback sucker, *Xyrauchen texanus*	78.66	
19	69.63	Fathead minnow, *Pimephales promelas*	69.63	11.40
18	69.63	Shovelnose sturgeon, *Scaphirhynchus platorynchus*	69.63	
17	68.31	Bull trout, *Salvelinus confluentus*	68.31	
16	63.22	Bonytail chub, *Gila elegans*	63.22	
15	56.15	Gila topminnow, *Poeciliopsis occidentalis*		56.15
14	52.51	Freshwater mussel, *Utterbackia imbecillis*	52.51	
13	48.41	Worm, *Lumbriculus variegatus*	48.41	
12	47.49	Boreal toad, *Bufo boreas*	47.49	
11	43.94	Colorado squawfish, *Ptychocheilus lucius*	132.2	
		Northern squawfish, *Ptychocheilus oregonensis*	14.61	
10	31.39	Apache trout, *Oncorhynchus apache*	32.54	
		Cutthroat trout, *Oncorhynchus clarki*	32.97	
		Pink salmon, *Oncorhynchus gorbuscha*	40.13	
		Coho salmon, *Oncorhynchus kisutch*	22.93	
		Rainbow trout, *Oncorhynchus mykiss*	22.19	2.88
		Sockeye salmon, *Oncorhynchus nerka*	54.82	
		Chinook salmon, *Oncorhynchus tshawytscha*	25.02	5.59
9	20.41	Snail, *Physa integra*	20.41	
8	12.31	Snail, *Juga plicifera*	12.31	
7	12.07	Amphipod, *Hyalella azteca*	12.07	
6	11.33	Freshwater mussel, *Actinonaias pectorosa*	11.33	
5	9.73	Cladoceran, *Scapholeberis sp.*	9.73	
4	9.60	Amphipod, *Gammarus pseudolimnaeus*	9.60	
3	6.67	Snail, *Lithoglyphus virens*	6.67	
2	5.93	Cladoceran, *Ceriodaphnia dubia*	5.93	2.85
1	4.05	Cladoceran, *Daphnia magna*	6.00	3.42
		Cladoceran, *Daphnia pulicaria*	2.73	

* Table updated as of March 2, 2007

APPENDIX B

Resident Species Lists

Table B-1. Fish Taxa Reported from Studies of Effluent-Dependent Segments of the Santa Ana River, Salt River/Gila River, Santa Cruz River, Fountain Creek, and the South Platte River

Order	Family	Genus	Species	Santa Ana River	Salt and Gila Rivers	Santa Cruz River near Nogales	Santa Cruz River near Tucson	Fountain Creek	South Platte River
Atheriniformes	Atherinopsidae	*Menidia*	*beryllina*	X					
Clupeiformes	Clupeidae	*Dorosoma*	*cepedianum*						X
			petenense		X				
Cypriniformes	Catostomidae	*Carpiodes*	*carpio*						X
		Catostomus	*catostomus*					X	X
			clarkii		X	X			
			commersonii					X	X
			fumeiventris	X					
			insignis		X	X			
			latipennis		X				
			*santaanae**	X					
		Xyrauchen	*texanus**		X				
	Cyprinidae	*Agosia*	*chrysogaster*		X	X			
		Campostoma	*anomalum*					X	X
		Carassius	*auratus*	X	X				X
		Cyprinella	*lutrensis*		X			X	X
		Cyprinus	*carpio*	X	X			X	X
		Gila	*elegans**		X				
			intermedia		X				
			orcuttii	X					
			robusta		X	X			
		Hybognathus	*hankinsoni*						X
			placitus						X
		Luxilus	*cornutus*						X
		Meda	*fulgida*		X				
		Notropis	*dorsalis*						X
			stramineus					X	X

Table B-1. Fish Taxa Reported from Studies of Effluent-Dependent Segments of the Santa Ana River, Salt River/Gila River, Santa Cruz River, Fountain Creek, and the South Platte River (continued)

Order	Family	Genus	Species	Santa Ana River	Salt and Gila Rivers	Santa Cruz River near Nogales	Santa Cruz River near Tucson	Fountain Creek	South Platte River
		Notemigonus	*crysoleucus*		X				
		Phenacobius	*mirabilis*						X
		Phoxinus	*eos*						X
		Pimephales	*promelas*	X	X			X	X
		Plagopterus	*argentissimus*		X				
		Platygobio	*gracilis*					X	
		Ptychocheilus	*lucius*		X				
		Rhinichthys	*cataractae*					X	X
			*cobitis**		X				
			osculus		X				
		Semotilus	*atromaculatus*					X	X
Cyprinodontiformes	Cyprinodontidae	*Cyprinodon*	*macularius**		X				
	Fundulidae	*Fundulus*	*sciadicus*						X
			zebrinus					X	X
	Poeciliidae	*Gambusia*	*affinis*	X	X	X	X		X
		Poecilia	sp.		X				
			latipinna	X	X				
			mexicana		X				
		Poeciliopsis	*occidentalis**		X	X			
		Xiphophorus	*variatus*		X				
Gasterosteiformes	Gasterosteidae	*Culaea*	*inconstans*					X	X
Perciformes	Centrarchidae	*Lepomis*	*cyanellus*	X	X	X	X	X	X
			gibbosus						X
			humilis						X
			macrochirus	X	X			X	X
			microlophus		X				
		Micropterus	*dolomieu*					X	X

Table B-1. Fish Taxa Reported from Studies of Effluent-Dependent Segments of the Santa Ana River, Salt River/Gila River, Santa Cruz River, Fountain Creek, and the South Platte River (continued)

Order	Family	Genus	Species	Santa Ana River	Salt and Gila Rivers	Santa Cruz River near Nogales	Santa Cruz River near Tucson	Fountain Creek	South Platte River
			salmoides	X	X				X
		Pomoxis	*annularis*						X
			nigromaculatus		X				X
	Cichlidae	*Oreochromis*	*aurea*		X				
			mossambicus	X	X				
		Tilapia	sp.	X	X				
			zillii		X				
	Moronidae	*Morone*	*mississippiensis*		X				
	Percidae	*Etheostoma*	*cragini*					X	
			exile						X
			nigrum						X
		Perca	*flavescens*						X
		Sander	*vitreus*						X
Salmoniformes	Salmonidae	*Salmo*	*trutta*					X	
Scorpaeniformes	Cottidae	*Cottus*	*asper*	X					
Siluriformes	Ictaluridae	*Ameirus*	*melas*	X	X		X	X	X
			natalis	X	X				X
		Ictalurus	*punctatus*	X	X			X	X
		Pylodictis	*olivaris*		X				
			Total Number of Taxa	**18**	**40**	**7**	**3**	**19**	**37**

* threatened or endangered species

Table B-2. Invertebrate Taxa Reported from Studies of Effluent-Dependent Segments of the Santa Ana River, Salt River, Gila River, Santa Cruz River, Fountain Creek, and the South Platte River.

								Santa Cruz River			
Family	**Class**	**Order**	**Family**	**Genus**	**Species**	**Santa Ana**	**Salt/Gila**	**Near Nogales**	**Near Tucson**	**Fountain Creek**	**South Platte River**
Bryozoa						X					
Cnidaria	Hydrozoa	Hydroida								X	
			Hydridae	*Hydra*	*americana*				X		
Nematoda							X	X		X	X
	Adenophorea	Dorylaimida	Dorylaimidae	*Dorylaimus*	*sp.*			X			
		Mermithida	Mermithidae					X			
Nematomorpha										X	
Nemertea						X			X	X	
	Enopla	Hoplonemertea	Tetrastemmatidae	*Prostoma*	*sp.*	X					
Platyhelminthes	Turbellaria					X	X			X	X
		Tricladida	Planariidae	*Girardia*	*sp.*	X				X	X
					dorotocephala	X					
					tigrina					X	X
				Polycelis	*coronata*					X	
		Macrostomida	Macrostomidae	*Macrostomum*	*sp.*						X
Annelida	Clitellata (Subclass Hirudinae)						X	X		X	X
								X			X
		Arhynchobdellidae	Erpobdellidae	*Dina*	*dubia*						X
				Erpobdella	*punctata*			X		X	X
				Mooreobdella	*fervida*	X					
					microstoma	X				X	X
		Rhynchobdellida	Glossiphoniidae					X			X
				Gloiobdella	*elongata*						X
				Helobdella	*fusca*						X
					stagnalis					X	X
	Clitellata (Subclass Oligochaeta)					X	X	X	X	X	X
		Branchiobdellida	Branchiobdellidae			X					
		Haplotaxida				X					
			Enchytraeidae			X		X		X	X
			Lumbricidae					X			
				Eiseniella	*tetraedra*	X				X	X
			Naididae			X	X	X	X		X
				Dero	*sp.*			X			
				Homochaeta	*naidini*	X				X	
				Nais	*sp.*	X				X	X
					behningi					X	
					bretscheri					X	X
					communis			X		X	
					communis/variabilis					X	
					elinguis					X	
					pseudobtusa					X	
				Ophidonais	*sp.*					X	
					serpentina	X		X		X	X

Table B 2. Invertebrate Taxa Reported from Studies of Effluent-Dependent Segments of the Santa Ana River, Salt River, Gila River, Santa Cruz River, Fountain Creek, and the South Platte River (continued)

Family	Class	Order	Family	Genus	Species	Santa Ana	Salt/Gila	Santa Cruz River		Fountain Creek	South Platte River
								Near Nogales	Near Tucson		
				Paranais	*sp.*	X					
				Pristina	*sp.*	X		X		X	X
					longiseta	X					
				Slavina	*sp.*	X					
				Stephensoniana	*tandyi*	X				X	
				Uncinais	*uncinata*					X	X
			Tubificidae			X	X	X	X	X	X
				Aulodrilus	*americanus*	X					
				Ilyodrilus/Tubifex	*sp.*					X	X
				*Isochaetides**	*sp.*						X
				Limnodrilus		X				X	X
					hoffmeisteri					X	X
					udekemianus					X	
				Tubifex	*tubifex*					X	
		Lumbriculida	Lumbriculidae			X		X		X	
				Eclipidrilus/ Rhynchelmis	*sp.*					X	
				Lumbriculus	*variegatus*	X				X	
				Rhynchelmis	*sp.*					X	
	Polychaeta	Aeolosomatida	Aeolosomatidae	*Aeolosoma*	*sp.*	X					
Mollusca	Gastropoda					X	X	X			X
		Basommatophora	Ancylidae	*Ferrissia*	*sp.*	X					X
			Lymnaeidae					X			
				Fossaria	*sp.*	X				X	
				Stagnicola	*sp.*	X					
			Physidae					X		X	
				Physa	*sp.*	X	X	X		X	X
				Physella	*sp.*	X					X
			Planorbidae	*Gyraulus*	*sp.*	X					X
				Menetus	*sp.*	X					
	Bivalvia					X					
		Veneroida	Corbidulidae			X					
				Corbicula	*fluminea*	X					X
			Pisidiidae	*Sphaerium*	*sp.*	X					
Arthropoda	Arachnida	Acari				X	X	X			X
			Eylaidae	*Eylais*	*sp.*			X			
			Hygrobatidae	*Atractides*	*sp.*			X			X
			Lebertiidae	*Lebertia*	*sp.*			X			
			Limnesiidae	*Limnesia*	*sp.*			X			
				Tyrrellia	*sp.*			X		X	
			Sperchontidae	*Sperchon*	*sp.*			X			
				Sperchon/Sperchonopsis	*sp.*	X				X	X
Branchiopoda	Diplostraca**					X	X		X		X
			Bosminidae	*Bosmina*	*sp.*				X		

Table B 2. Invertebrate Taxa Reported from Studies of Effluent-Dependent Segments of the Santa Ana River, Salt River, Gila River, Santa Cruz River, Fountain Creek, and the South Platte River (continued)

								Santa Cruz River			
Family	**Class**	**Order**	**Family**	**Genus**	**Species**	**Santa Ana**	**Salt/Gila**	**Near Nogales**	**Near Tucson**	**Fountain Creek**	**South Platte River**
			Chydoridae	*Alona*	*costata*		X				
				Chydorus	*sphaericus*		X				
				Kurzia	*latissima*		X				
			Daphnidae	*Ceriodaphnia*	*sp.*		X				
				Daphnia	*sp.*			X	X		X
			Macrothricidae	*Macrothrix*	*rosea*		X				
			Moinidae	*Moina*	*sp.*		X	X	X		
					micrurus		X				
	Malacostraca	Amphipoda					X			X	
			Gammaridae	*Gammarus*	*lacustris*	X				X	X
			Hyalellidae	*Hyalella*	*azteca*	X				X	X
		Decapoda	Astacidae	*Pacifastacus*	*leniusculus*	X					
			Cambaridae								X
				Orconectes	*sp.*					X	
				Procambarus	*clarkii*	X					
		Isopoda								X	X
			Asellidae	*Asellus*	*sp.*					X	X
				Caecidotea	*sp.*	X				X	X
					communis					X	
					intermedius						X
Insecta	Collembola					X	X	X	X	X	
			Entomobryidae	*Willowsia*	*sp.*	X					
			Hypogastruridae	*Hypogastrura*	*sp.*	X				X	
				Odontella	*sp.*			X			
			Isotomidae	*Isotomurus*	*sp.*	X					
					palustris	X				X	
					tricolor	X					
		Ephemeroptera				X					X
			Ameletidae	*Ameletus*	*sp.*					X	
			Baetidae			X	X		X	X	X
				Acentrella	*insignificans*					X	X
				Apobaetis	*indeprensus*	X					
				Baetis	*sp. 1*	X		X		X	X
					sp. 2						X
					bicaudatus	X				X	X
					magnus					X	
					tricaudatus	X				X	X
				Callibaetis	*sp.*	X	X	X		X	X
					californicus	X					
					pictus	X					
				Camelobaetidius	*sp.*	X					
					similis	X					
					warreni	X					

Table B 2. Invertebrate Taxa Reported from Studies of Effluent-Dependent Segments of the Santa Ana River, Salt River, Gila River, Santa Cruz River, Fountain Creek, and the South Platte River (continued)

Family	Class	Order	Family	Genus	Species	Santa Ana	Salt/Gila	Santa Cruz River		Fountain Creek	South Platte River
								Near Nogales	Near Tucson		
				Cloeodes	*macrolamellus*	X					
				Fallceon	*quilleri*	X		X		X	X
				Labiobaetis	*sp.*	X				X	
				Paracloeodes	*sp.*	X		X		X	
			Caenidae				X				
				Caenis	*sp.*	X					X
					amica	X					
			Ephemerellidae	*Ephemerella*	*inermis*					X	
					dorothea					X	
			Heptageniidae			X				X	X
				Cinygmula	*sp.*					X	
				Epeorus	*sp.*						X
				Heptagenia	*sp.*					X	X
			Leptophlebiidae			X				X	
				Paraleptophlebia	*sp.*	X				X	
			Siphlonuridae	*Siphlonurus*	*sp.*					X	
			Tricorythidae	*Leptohyphes*	*sp.*			X			
				Tricorythodes	*sp.*	X		X		X	X
					minutus			X		X	X
		Odonata (Anisoptera)				X					
			Aeshnidae	*Aeshna*	*sp.*					X	
				Anax	*junius*						X
			Corduliidae	*Neurocordulia*	*sp.*	X					
			Gomphidae			X		X		X	
				Erpetogomphus	*sp.*			X			
				Ophiogomphus	*sp.*			X		X	X
					severus					X	X
				Progomphus	*sp.*	X		X			
					borealis	X		X			
			Libellulidae					X			
				Brechmorhoga	*mendax*	X		X		X	
				Libellula	*subornata*						X
					lineatipes	X		X			
				Pantala	*hymenaea*						X
		Odonata (Zygoptera)				X	X				
			Calopterygidae			X	X	X			
				Calopteryx	*sp.*			X			
				Hetaerina	*sp.*	X		X			X
					americana	X				X	
			Coenagrionidae			X		X		X	X
				Amphiagrion	*sp.*						X
				Argia	*sp.*	X	X	X		X	X
					alberta	X					

Table B 2. Invertebrate Taxa Reported from Studies of Effluent-Dependent Segments of the Santa Ana River, Salt River, Gila River, Santa Cruz River, Fountain Creek, and the South Platte River (continued)

Family	Class	Order	Family	Genus	Species	Santa Ana	Salt/Gila	Santa Cruz River		Fountain Creek	South Platte River
								Near Nogales	Near Tucson		
					vivida	X					
				Coenagrion	*sp.*			X			
					resolutum	X		X			
				Coenagrion/Enallagma	*sp.*	X		X		X	X
				Enallagma	*sp.*	X	X				X
				Hesperagrion	*sp.*			X			
					heterodoxum			X			
				Ischnura	*sp.*	X	X	X	X		
				Zoniagrion	*sp.*	X		X			
			Lestidae	*Archilestes*	*sp.*		X				
		Plecoptera									X
			Capniidae							X	
			Chloroperlidae							X	
				Alloperla	*sp.*					X	
				Sweltsa	*sp.*	X			X	X	
				Triznaka	*sp.*					X	
			Nemouridae								X
				Amphinemura	*sp.*					X	
				Zapada	*cinctipes*	X					
			Perlodidae							X	
				Isoperla	*sp.*					X	
					fulva					X	
			Pteronarcyidae	*Pteronarcella*	*badia*					X	
			Taeniopterygidae							X	
				Taenionema	*sp.*	X					
		Hemiptera				X		X	X		
			Belostomatidae				X	X			
				Abedus	*sp. 1*	X		X			
					sp. 2			X			
					herberti			X			
				Belostoma	*sp.*	X			X		
					flumineum	X		X			
			Corixidae			X	X	X	X	X	
				Callicorixa	*sp.*					X	
				Cenocorixa	*sp.*					X	
				Corisella	*sp.*	X					X
					decolor	X					
					edulis			X			
					inscripta	X					
				Graptocorixa	*sp.*			X			
					abdominalis			X			
					serrulata			X			
				Hesperocorixa	*sp.*	X					

Table B 2. Invertebrate Taxa Reported from Studies of Effluent-Dependent Segments of the Santa Ana River, Salt River, Gila River, Santa Cruz River, Fountain Creek, and the South Platte River (continued)

Family	Class	Order	Family	Genus	Species	Santa Ana	Salt/Gila	Santa Cruz River		Fountain Creek	South Platte River
								Near Nogales	Near Tucson		
				Pseudocorixa	*beameri*				X		
				Sigara	*sp.*	X				X	
					alternata	X					
				Trichocorixa	*sp.*	X				X	
					calva	X					
			Gelastocoridae	*Gelastocoris*	*sp.*	X					
			Gerridae			X					
				Gerris	*sp.*	X			X	X	X
				Trepobates	*sp.*	X					
			Hebridae	*Hebrus*	*sp.*	X					
				Merragata	*hebroides*	X					
			Macroveliidae	*Macrovelia*	*sp.*	X				X	
			Mesoveliidae	*Mesovelia*	*sp.*	X					
					mulsanti			X		X	
			Naucoridae	*Ambrysus*	*sp.*	X		X		X	
			Nepidae	*Ranatra*	*sp.*			X			
					quadridentata			X			
			Notonectidae	*Notonecta*	*sp.*	X		X			X
			Ochteridae	*Ochterus*	*sp.*					X	
			Saldidae	*Salda*	*sp.*	X					
				Saldula	*sp.*	X					
			Veliidae			X	X				
				Microvelia	*sp.*	X		X		X	X
				Rhagovelia	*sp.*	X		X		X	
					distincta	X					
		Megaloptera						X	X		
			Corydalidae					X			
				Corydalus	*sp.*			X			
		Hymenoptera				X					
		Coleoptera							X		
			Carabidae								X
			Chrysomelidae	*Donacia*	*sp.*					X	
			Curculionidae			X		X		X	
			Dryopidae			X		X			
				Helichus	*sp.*	X				X	
					suturalis	X		X			
				Postelichus	*sp.*	X		X		X	
					confluentus			X			
					immsi	X		X			
					productus	X					
			Dytiscidae					X			
				Agabus	*sp.*			X		X	X
					semivittatus			X			

Table B 2. Invertebrate Taxa Reported from Studies of Effluent-Dependent Segments of the Santa Ana River, Salt River, Gila River, Santa Cruz River, Fountain Creek, and the South Platte River (continued)

Family	Class	Order	Family	Genus	Species	Santa Ana	Salt/Gila	Santa Cruz River		Fountain Creek	South Platte River
								Near Nogales	Near Tucson		
				Agabinus	*sp.*	X					
				Copelatus	*chevrolati*			X			
				Desmopachria	*mexicana*			X			
				Dytiscus	*sp.*	X				X	
				Hydaticus	*sp.*	X					
				Hydroporus	*sp.*	X					
					occidentalis	X					
				Hydrovatus	*sp.*	X					
				Ilybius	*sp.*					X	
				Laccophilus	*sp.*	X		X		X	
					fasciatus			X			
					maculosus	X		X		X	
					mexicanus			X			
					pictus			X			
					salvini			X			
				Liodessus	*sp.*	X					
					affinis cx.			X			
				Liodessus/Neoclypeodytes	*sp.*	X					
				Oreodytes	*sp.*					X	
				Rhantus	*sp.*	X		X			
					gutticollis			X		X	
				Stictotarsus	*sp.*			X			
					aequinoctialis			X			
					corpulentus			X			
					funereus	X					
					roffi			X			
				Thermonectus	*nigrofasciatus*			X			
				Uvarus	*sp.*	X					
					amandus			X			
			Elmidae				X				X
				Dubiraphia	*sp.*						X
				Heterelmis	*glaber*				X		
				Heterlimnius	*sp.*						X
					corpulentus	X				X	
				Macronychus	*sp.*	X					
				Microcylloepus	*sp.*	X		X			
					pusillus	X		X			
				Neoelmis	*sp.*			X			
				Optioservus	*sp.*					X	X
					divergens	X					
					quadrimaculatus					X	
				Phanocerus	*sp.*					X	
				Zaitzevia	*parvula*					X	X

Table B 2. Invertebrate Taxa Reported from Studies of Effluent-Dependent Segments of the Santa Ana River, Salt River, Gila River, Santa Cruz River, Fountain Creek, and the South Platte River (continued)

Family	Class	Order	Family	Genus	Species	Santa Ana	Salt/Gila	Santa Cruz River		Fountain Creek	South Platte River
								Near Nogales	Near Tucson		
			Georyssidae	*Georyssus*	*sp.*	X					
			Haliplidae					X			
				Haliplus	*sp.*	X					
				Peltodytes	*sp.*	X		X		X	
					callosus	X					
			Helophoridae	*Helophorus*	*sp.*	X		X		X	
			Hydraenidae	*Gymnochthebius*	*fossatus*			X			
				Ochthebius	*sp.*			X		X	
			Hydrophilidae				X	X			
				Anacaena	*sp.*	X					
				Berosus	*sp.*					X	X
					peregrinus			X			
				Cheatarthria	*sp.*	X		X			
				Enochrus	*sp.*	X		X		X	
					carinatus			X			
					pectoralis	X					
					pygmaeus			X			
				Helochares	*sp. 1*	X					
					sp. 2			X			
					normatus			X			
				Hydrobius	*sp.*					X	
				Hydrophilus	*triangularis*						X
				Laccobius	*sp.*	X				X	
					mexicanus			X			
				Paracymus	*sp.*	X				X	
				Tropisternus	*sp. 1*	X		X	X	X	
					sp. 2			X			
					ellipticus	X		X			
					lateralis			X		X	
			Hydroscaphidae	*Hydroscapha*	*natans*	X			X		
			Lampyridae								X
			Noteridae	*Pronoterus*	*sp.*				X		
			Staphylinidae								X
				Stenus	*sp.*	X					
		Lepidoptera	Noctuidae	*Bellura*	*sp.*	X					
			Pyralidae			X				X	
				Crambus	*sp.*	X					
				Parapoynx	*sp.*	X					
				Petrophila	*sp.*	X					X
		Trichoptera							X		
			Brachycentridae							X	
				Brachycentrus	*americanus*					X	
					occidentalis						X

Table B 2. Invertebrate Taxa Reported from Studies of Effluent-Dependent Segments of the Santa Ana River, Salt River, Gila River, Santa Cruz River, Fountain Creek, and the South Platte River (continued)

Family	Class	Order	Family	Genus	Species	Santa Ana	Salt/Gila	Santa Cruz River		Fountain Creek	South Platte River
								Near Nogales	Near Tucson		
			Glossosomatidae			X					
			Helicopsychidae	*Helicopsyche*	*sp.*	X					X
					borealis						X
			Hydropsychidae			X					
				Ceratopsyche	*bronta*						X
					cockerelli					X	
					oslari					X	
					slossonae						X
				Cheumatopsyche	*sp.*	X				X	X
				Hydropsyche	*sp.*	X				X	X
					californica	X					
					occidentalis	X					X
			Hydroptilidae			X	X				X
				Agraylea	*sp.*	X					
				Hydroptila	*sp.*	X				X	X
					icona	X					
					pecos						X
				Leucotrichia	*sp.*					X	X
					pictipes						X
				Ochrotrichia	*sp.*						X
				Orthotrichia	*sp.*	X					
				Oxyethira	*sp.*	X					
			Leptoceridae					X			
				Nectopsyche	*sp.*					X	
				Oecetis	*sp.*						X
			Limnephilidae							X	
				Hesperophylax	*sp.*					X	
				Limnephilus/ Philarctus	*sp.*					X	
			Philopotamidae	*Chimarra*	*utahensis*						X
				Wormaldia	*sp.*	X					
			Rhyacophilidae	*Rhyacophila*	*sp.*					X	
					brunnea gr.	X					
					sibirica gr.	X					
		Diptera				X		X		X	X
			Ceratopogonidae			X		X	X	X	X
				Genus 1				X			
				Genus 2				X			
				Atrichopogon	*sp.*			X		X	
				Bezzia	*sp.*	X					
				Bezzia/Palpomyia	*sp.*						X
				Ceratopogon	*sp.*					X	
				Culicoides	*sp.*					X	
				Dasyhelea	*sp.*	X		X		X	

Table B 2. Invertebrate Taxa Reported from Studies of Effluent-Dependent Segments of the Santa Ana River, Salt River, Gila River, Santa Cruz River, Fountain Creek, and the South Platte River (continued)

Family	Class	Order	Family	Genus	Species	Santa Ana	Salt/Gila	Santa Cruz River		Fountain Creek	South Platte River
								Near Nogales	Near Tucson		
				Forcipomyia	*sp.*			X		X	
				Mallochohelia	*sp.*	X		X		X	
				Palpomyia	*sp.*						X
				Palpomyia cx.	*sp.*					X	
				Sphaeromias	*sp.*			X			
			Chaoboridae	*Chaoborus*	*sp.*			X			X
			Chironomidae			X	X	X	X	X	X
			Chironominae (Subfamily)			X	X	X	X		X
				Chironomus	*sp.*	X	X	X	X	X	X
				Cladotanytarsus	*sp.*	X					
				Cryptochironomus	*sp.*	X		X		X	X
				Demicryptochironomus	*sp.*	X					
				Dicrotendipes	*sp.*	X		X		X	X
					fumidus						X
				Einfeldia	*sp.*	X				X	X
				Endochironomus	*sp.*			X			X
				Glyptotendipes	*sp.*	X				X	X
					amplus						X
				Goeldichironomus	*sp.*			X			
				Lauterborniella	*sp.*			X			X
				Microchironomus	*sp.*						X
				Micropsectra	*sp.*	X				X	X
				Micropsectra/ Tanytarsus	*sp.*	X					
				Microtendipes	*sp.*	X		X		X	X
				Nilothauma	*sp.*	X					
				Parachironomus	*sp.*	X		X			X
				Paracladopelma	*sp.*	X				X	
				Paralauterborniella	*sp.*	X				X	
				Paratanytarsus	*sp.*	X				X	X
				Paratendipes	*sp.*					X	
				Phaenopsectra	*sp.*			X		X	X
				Polypedilum	*sp.*	X		X		X	X
					convictum gr.						X
					fallax gr.					X	
					illinoense gr.						X
				Pseudochironomus	*sp.*	X		X			X
				Rheotanytarsus	*sp.*	X	X	X		X	X
				Saetheria	*sp.*	X		X		X	X
				Stempellinella	*sp.*	X					
				Stictochironomus	*sp.*	X		X		X	
				Tanytarsus	*sp.*	X		X		X	X
				Tribelos	*sp.*					X	
			Diamesinae (Subfamily)	*Diamesa*	*sp.*			X		X	X

Table B 2. Invertebrate Taxa Reported from Studies of Effluent-Dependent Segments of the Santa Ana River, Salt River, Gila River, Santa Cruz River, Fountain Creek, and the South Platte River (continued)

Family	Class	Order	Family	Genus	Species	Santa Ana	Salt/Gila	Santa Cruz River		Fountain Creek	South Platte River
								Near Nogales	Near Tucson		
				Pagastia	*sp.*					X	
				Potthastia	*sp.*					X	
			Orthocladinae (Subfamily)			X		X		X	X
				Brillia	*sp.*					X	X
				Cardiocladius	*sp.*	X				X	X
				Chaetocladius	*sp.*					X	
				Corynoneura	*sp.*	X		X		X	
				Cricotopus	*sp.*	X		X		X	X
					bicinctus gr.	X	X	X	X	X	X
					cylindraceus						X
					festivellus	X					X
					fuscus						X
					nostocicola	X					X
					tremulus	X					X
					trifascia	X				X	X
				Cricotopus (Isocladius)	*sp.*	X					
					sylvestris	X					X
				Cricotopus/Orthocladius	*sp.*	X	X	X		X	X
				Doncricotopus	*sp.*	X					
				Endotribelos	*sp.*	X		X			
					hesperium	X					
				Eukiefferiella	*sp.*	X		X	X		X
				Claripennis gr.	*sp. A*						X
					devonica						X
					pseudomontana						X
				Euryhapsis	*sp.*			X			
				Heleniella	*sp.*					X	
				Heterotrissocladius	*sp.*	X				X	
				Hydrobaenus	*sp.*	X		X		X	X
				Limnophyes	*sp.*			X		X	X
				Mesosmittia	*sp.*			X			
				Nanocladius	*sp.*	X		X		X	X
				Orthocladius	*sp.*	X				X	X
				Orthocladius (Euorthocladius)	*sp.*	X				X	X
				Paracricotopus	*sp.*	X					
				Parakiefferiella	*sp.*					X	X
				Parametriocnemus	*sp.*			X		X	X
				Parorthocladius	*sp.*			X			
				Paraphaenocladius	*sp.*	X		X		X	X
				Pseudosmittia	*sp.*			X		X	
				Rheocricotopus	*sp.*	X		X		X	X
				Rheosmittia	*sp.*			X			
				Smittia	*sp.*					X	

Table B 2. Invertebrate Taxa Reported from Studies of Effluent-Dependent Segments of the Santa Ana River, Salt River, Gila River, Santa Cruz River, Fountain Creek, and the South Platte River (continued)

Family	Class	Order	Family	Genus	Species	Santa Ana	Salt/Gila	Santa Cruz River		Fountain Creek	South Platte River
								Near Nogales	Near Tucson		
				Synorthocladius	*sp.*						X
				Thienemanniella	*sp.*	X		X		X	X
				Tvetenia	*sp.*	X				X	X
			Prodiamesinae (Subfamily)	*Odontomesa*	*sp.*					X	
				Prodiamesa	*sp.*					X	
			Tanypodinae (Subfamily)			X		X			X
				Ablabesmyia	*sp.*	X		X		X	
				Alotanypus	*sp.*					X	
				*Apsectrotanypus**	*sp.*					X	
				Brundiniella	*sp.*	X					
				Conchapelopia/Thienemannmyia gr.	*sp.*					X	X
				Labrundinia	*sp.*	X		X			
				Larsia	*sp.*	X		X		X	X
				Natarsia	*sp.*	X					
				Paramerina	*sp.*	X		X		X	
				Pentaneura	*sp.*	X		X			X
				Procladius	*sp.*			X		X	X
				Radotanypus	*sp.*					X	
				Tanypus	*sp.*	X					
				Thienemannimyia gr.	*sp.*	X		X		X	X
				Zavrelimyia	*sp.*	X					
			Culicidae					X			
				Aedes	*sp.*			X			X
				Anopheles	*sp.*			X			
				Culex	*sp.*	X		X	X	X	X
			Dixidae	*Dixella*	*sp.*			X		X	
			Dolichopodidae			X		X	X	X	X
				Hydrophorus	*agalma*					X	
			Empididae			X					
				Chelifera	*sp.*					X	X
				Clinocera	*sp.*					X	
				Hemerodromia	*sp.*	X		X		X	X
				Neoplasta	*sp.*					X	
				Oreogeton	*sp.*	X					
			Ephydridae			X		X		X	X
				Ephydra	*sp.*	X		X			
				Hydrellia	*sp.*			X			
				Scatella	*sp.*					X	
			Muscidae			X	X	X		X	X
				Limnophora	*sp.*	X		X		X	X
			Psychodidae			X	X	X	X	X	X
				Maruina	*sp.*	X					
				Pericoma	*sp.*	X		X	X	X	

Table B 2. Invertebrate Taxa Reported from Studies of Effluent-Dependent Segments of the Santa Ana River, Salt River, Gila River, Santa Cruz River, Fountain Creek, and the South Platte River (continued)

Family	Class	Order	Family	Genus	Species	Santa Ana	Salt/Gila	Santa Cruz River		Fountain Creek	South Platte River
								Near Nogales	Near Tucson		
				Pericoma/Telmatoscopus	*sp.*				X		
				Psychoda	*sp.*			X	X	X	X
			Ptychopteridae	*Bittacomorphella*	*sp.*						X
			Sarcophagidae								X
			Simuliidae			X	X	X	X	X	X
				Prosimulium	*sp.*					X	X
				Simulium	*sp.*	X		X		X	X
					arcticum					X	
					vittatum cx.			X		X	X
			Stratiomyidae								X
				Allognosta	*sp.*					X	
				Caloparyphus	*sp.*	X		X		X	X
				Euparyphus	*sp.*	X		X		X	
				Nemotelus	*sp.*			X		X	
				Odontomyia	*sp.*	X		X			
				Stratiomys	*sp.*			X			
			Syrphidae						X		
			Tabanidae							X	
				Hybomitra	*minusculus*	X					
				Tabanus	*sp.*			X	X	X	
					punctifer	X					
			Tanyderidae							X	
				Protanyderus	*sp.*					X	
					margarita					X	
			Tipulidae			X		X		X	
				Antocha	*sp.*	X				X	
				Dicranota	*sp.*	X					
				Erioptera	*sp.*	X		X		X	
				Gonomyia	*sp.*	X				X	X
				Hexatoma	*sp.*					X	
				Limonia	*sp.*	X		X		X	
				Ormosia	*sp.*				X	X	
				Rhabdomastix	*sp.*						X
				Tipula	*sp.*	X		X		X	X
				Number of Taxa		283	43	211	41	251	192

* Identification was uncertain in the original documentation

** Diplostracans (cladocerans) are generally considered to be transient organisms in lotic systems

APPENDIX C

Site-specific Deletion Tables - BLM database

Table C-1. Deletion process for the Santa Ana River site-specific BLM adjusted copper database

Phylum	Class	Order	Family	Genus	Species	Code
Chordata	Actinopterygii	Acipenseriformes	Acipenseridae	*Scaphirhynchus*	*platorynchus*	D
Chordata	Actinopterygii	Cyprinodontiformes	Poeciliidae	*Poeciliopsis*	*occidentalis*	F
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Gila*	*elegans*	G
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Acrocheilus*	*alutaceus*	F
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Notemigonus*	*crysoleucas*	F
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Pimephales*	*promelas*	S
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Ptychocheilus*	*oregonensis*	F
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Ptychocheilus*	*lucius*	F
Chordata	Actinopterygii	Cypriniformes	Catostomidae	*Xyrauchen*	*texanus*	F
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*rubrum*	O
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*lepidum*	O
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*flabellare*	O
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*nigrum*	O
Chordata	Actinopterygii	Perciformies	Centrarchidae	*Lepomis*	*macrochirus*	S
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*kisutch*	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*mykiss*	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*tshawytscha*	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*clarki*	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*gorbuscha*	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*apache*	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*nerka*	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Salvelinus*	*confluentus*	D
Chordata	Amphibia	Anura	Bufonidae	*Bufo*	*boreas*	D
Annelida	Clitellata	Lumbriculida	Lumbriculidae	*Lumbriculus*	*variegatus*	S
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Ceriodaphnia*	*dubia*	D
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Daphnia*	*pulicaria*	D
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Daphnia*	*magna*	D
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Scapholeberis*	sp.	D
Arthropoda	Insecta	Diptera	Chironomidae	*Chironomus*	*decorus*	G
Arthropoda	Insecta	Plectoptera	Perlidae	*Acroneuria*	*lycorias*	F
Arthropoda	Malacostraca	Amphipoda	Gammaridae	*Gammarus*	*pseudolimnaeus*	G
Arthropoda	Malacostraca	Amphipoda	Hyalellidae	*Hyalella*	*azteca*	S
Mollusca	Bivalvia	Unionoida	Unionidae	*Actinonaias*	*pectorosa*	C
Mollusca	Bivalvia	Unionoida	Unionidae	*Utterbackia*	*imbecillis*	C
Mollusca	Gastropoda	Architaenioglossa	Viviparidae	*Campeloma*	*decisum*	D
Mollusca	Gastropoda	Basommatophora	Physidae	*Physa*	*integra*	G
Mollusca	Gastropoda	Neotaenioglossa	Pleuroceridae	*Juga*	*plicifera*	D
Mollusca	Gastropoda	Neotaenioglossa	Hydrobiidae	*Lithoglyphus*	*virens*	D

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 data set

F = retained because there is a genus in this Family that occurs at the site, but not in the national data set

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

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

Table C-2. Deletion process for the Salt/Gila rivers site-specific BLM adjusted copper database

Phylum	Class	Order	Family	Genus	Species	Code
Chordata	Actinopterygii	Acipenseriformes	Acipenseridae	*Scaphirhynchus*	*platorynchus*	D
Chordata	Actinopterygii	Cyprinodontiformes	Poeciliidae	*Poeciliopsis*	*occidentalis*	S
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Gila*	*elegans*	S
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Acrocheilus*	*alutaceus*	F
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Notemigonus*	*crysoleucas*	S
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Pimephales*	*promelas*	S
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Ptychocheilus*	*oregonensis*	D
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Ptychocheilus*	*lucius*	S
Chordata	Actinopterygii	Cypriniformes	Catostomidae	*Xyrauchen*	*texanus*	S
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*rubrum*	O
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*lepidum*	O
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*flabellare*	O
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*nigrum*	O
Chordata	Actinopterygii	Perciformies	Centrarchidae	*Lepomis*	*macrochirus*	S
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*kisutch*	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*mykiss*	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*tshawytscha*	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*clarki*	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*gorbuscha*	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*apache*	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*nerka*	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Salvelinus*	*confluentus*	D
Chordata	Amphibia	Anura	Bufonidae	*Bufo*	*boreas*	D
Annelida	Clitellata	Lumbriculida	Lumbriculidae	*Lumbriculus*	*variegatus*	C
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Ceriodaphnia*	*dubia*	D
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Daphnia*	*pulicaria*	D
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Daphnia*	*magna*	D
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Scapholeberis*	sp.	D
Arthropoda	Insecta	Diptera	Chironomidae	*Chironomus*	*decorus*	G
Arthropoda	Insecta	Plectoptera	Perlidae	*Acroneuria*	*lycorias*	R
Arthropoda	Malacostraca	Amphipoda	Gammaridae	*Gammarus*	*pseudolimnaeus*	O
Arthropoda	Malacostraca	Amphipoda	Hyalellidae	*Hyalella*	*azteca*	O
Mollusca	Bivalvia	Unionoida	Unionidae	*Actinonaias*	*pectorosa*	D
Mollusca	Bivalvia	Unionoida	Unionidae	*Utterbackia*	*imbecillis*	D
Mollusca	Gastropoda	Architaenioglossa	Viviparidae	*Campeloma*	*decisum*	D
Mollusca	Gastropoda	Basommatophora	Physidae	*Physa*	*integra*	G
Mollusca	Gastropoda	Neotaenioglossa	Pleuroceridae	*Juga*	*plicifera*	D
Mollusca	Gastropoda	Neotaenioglossa	Hydrobiidae	*Lithoglyphus*	*virens*	D

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G = retained because there is a species in this genus that occurs at the site, but not in the national data set

F = retained because there is a genus in this Family that occurs at the site, but not in the national data set

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

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

R = non-resident species retained due to potential occurrence at the site

Table C-3. Deletion process for the Santa Cruz River near Nogales (NOG) and near Tucson (TUC) site-specific BLM adjusted copper databases.

Phylum	Class	Order	Family	Genus	Species	NOG Code	TUC Code
Chordata	Actinopterygii	Acipenseriformes	Acipenseridae	*Scaphirhynchus*	*platorynchus*	D	D
Chordata	Actinopterygii	Cyprinodontiformes	Poeciliidae	*Poeciliopsis*	*occidentalis*	F	F
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Gila*	*elegans*	G	D
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Acrocheilus*	*alutaceus*	F	D
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Notemigonus*	*crysoleucas*	F	D
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Pimephales*	*promelas*	F	D
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Ptychocheilus*	*oregonensis*	F	D
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Ptychocheilus*	*lucius*	F	D
Chordata	Actinopterygii	Cypriniformes	Catostomidae	*Xyrauchen*	*texanus*	F	D
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*rubrum*	D	D
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*lepidum*	D	D
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*flabellare*	D	D
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*nigrum*	D	D
Chordata	Actinopterygii	Perciformies	Centrarchidae	*Lepomis*	*macrochirus*	G	G
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*kisutch*	D	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*mykiss*	D	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*tshawytscha*	D	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*clarki*	D	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*gorbuscha*	D	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*apache*	D	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*nerka*	D	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Salvelinus*	*confluentus*	D	D
Chordata	Amphibia	Anura	Bufonidae	*Bufo*	*boreas*	D	D
Annelida	Clitellata	Lumbriculida	Lumbriculidae	*Lumbriculus*	*variegatus*	F	F
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Ceriodaphnia*	*dubia*	D	D
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Daphnia*	*pulicaria*	D	D
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Daphnia*	*magna*	D	D
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Scapholeberis*	sp.	D	D
Arthropoda	Insecta	Diptera	Chironomidae	*Chironomus*	*decorus*	G	G
Arthropoda	Insecta	Plectoptera	Perlidae	*Acroneuria*	*lycorias*	R	O
Arthropoda	Malacostraca	Amphipoda	Gammaridae	*Gammarus*	*pseudolimnaeus*	R	R
Arthropoda	Malacostraca	Amphipoda	Hyalellidae	*Hyalella*	*azteca*	R	R
Mollusca	Bivalvia	Unionoida	Unionidae	*Actinonaias*	*pectorosa*	D	D
Mollusca	Bivalvia	Unionoida	Unionidae	*Utterbackia*	*imbecillis*	D	D
Mollusca	Gastropoda	Architaenioglossa	Viviparidae	*Campeloma*	*decisum*	D	D
Mollusca	Gastropoda	Basommatophora	Physidae	*Physa*	*integra*	G	D
Mollusca	Gastropoda	Neotaenioglossa	Pleuroceridae	*Juga*	*plicifera*	D	D
Mollusca	Gastropoda	Neotaenioglossa	Hydrobiidae	*Lithoglyphus*	*virens*	D	D

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 data set

F = retained because there is a genus in this Family that occurs at the site, but not in the national data set

O = retained because this Order 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

R = non-resident species retained due to potential occurrence at the site

Table C-4. Deletion process for Fountain Creek site-specific BLM adjusted copper database

Phylum	Class	Order	Family	Genus	Species	Code
Chordata	Actinopterygii	Acipenseriformes	Acipenseridae	*Scaphirhynchus*	*platorynchus*	D
Chordata	Actinopterygii	Cyprinodontiformes	Poeciliidae	*Poeciliopsis*	*occidentalis*	O
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Gila*	*elegans*	F
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Acrocheilus*	*alutaceus*	F
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Notemigonus*	*crysoleucas*	F
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Pimephales*	*promelas*	S
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Ptychocheilus*	*oregonensis*	F
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Ptychocheilus*	*lucius*	F
Chordata	Actinopterygii	Cypriniformes	Catostomidae	*Xyrauchen*	*texanus*	F
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*rubrum*	G
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*lepidum*	G
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*flabellare*	G
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*nigrum*	G
Chordata	Actinopterygii	Perciformies	Centrarchidae	*Lepomis*	*macrochirus*	S
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*kisutch*	F
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*mykiss*	F
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*tshawytscha*	F
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*clarki*	F
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*gorbuscha*	F
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*apache*	F
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*nerka*	F
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Salvelinus*	*confluentus*	F
Chordata	Amphibia	Anura	Bufonidae	*Bufo*	*boreas*	D
Annelida	Clitellata	Lumbriculida	Lumbriculidae	*Lumbriculus*	*variegatus*	S
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Ceriodaphnia*	*dubia*	D
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Daphnia*	*pulicaria*	D
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Daphnia*	*magna*	D
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Scapholeberis*	sp.	D
Arthropoda	Insecta	Diptera	Chironomidae	*Chironomus*	*decorus*	F
Arthropoda	Insecta	Plectoptera	Perlidae	*Acroneuria*	*lycorias*	G
Arthropoda	Malacostraca	Amphipoda	Gammaridae	*Gammarus*	*pseudolimnaeus*	G
Arthropoda	Malacostraca	Amphipoda	Hyalellidae	*Hyalella*	*azteca*	S
Mollusca	Bivalvia	Unionoida	Unionidae	*Actinonaias*	*pectorosa*	D
Mollusca	Bivalvia	Unionoida	Unionidae	*Utterbackia*	*imbecillis*	D
Mollusca	Gastropoda	Architaenioglossa	Viviparidae	*Campeloma*	*decisum*	D
Mollusca	Gastropoda	Basommatophora	Physidae	*Physa*	*integra*	G
Mollusca	Gastropoda	Neotaenioglossa	Pleuroceridae	*Juga*	*plicifera*	D
Mollusca	Gastropoda	Neotaenioglossa	Hydrobiidae	*Lithoglyphus*	*virens*	D

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

Table C-5. Deletion process for the South Platte River site-specific BLM adjusted copper database

Phylum	Class	Order	Family	Genus	Species	Code
Chordata	Actinopterygii	Acipenseriformes	Acipenseridae	*Scaphirhynchus*	*platorynchus*	D
Chordata	Actinopterygii	Cyprinodontiformes	Poeciliidae	*Poeciliopsis*	*occidentalis*	F
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Gila*	*elegans*	F
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Acrocheilus*	*alutaceus*	F
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Notemigonus*	*crysoleucas*	F
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Pimephales*	*promelas*	S
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Ptychocheilus*	*oregonensis*	F
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Ptychocheilus*	*lucius*	F
Chordata	Actinopterygii	Cypriniformes	Catostomidae	*Xyrauchen*	*texanus*	F
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*rubrum*	G
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*lepidum*	G
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*flabellare*	G
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*nigrum*	S
Chordata	Actinopterygii	Perciformies	Centrarchidae	*Lepomis*	*macrochirus*	S
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*kisutch*	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*mykiss*	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*tshawytscha*	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*clarki*	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*gorbuscha*	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*apache*	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*nerka*	D
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Salvelinus*	*confluentus*	D
Chordata	Amphibia	Anura	Bufonidae	*Bufo*	*boreas*	D
Annelida	Clitellata	Lumbriculida	Lumbriculidae	*Lumbriculus*	*variegatus*	F
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Ceriodaphnia*	*dubia*	D
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Daphnia*	*pulicaria*	D
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Daphnia*	*magna*	D
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Scapholeberis*	sp.	D
Arthropoda	Insecta	Diptera	Chironomidae	*Chironomus*	*decorus*	G
Arthropoda	Insecta	Plectoptera	Perlidae	*Acroneuria*	*lycorias*	O
Arthropoda	Malacostraca	Amphipoda	Gammaridae	*Gammarus*	*pseudolimnaeus*	G
Arthropoda	Malacostraca	Amphipoda	Hyalellidae	*Hyalella*	*azteca*	S
Mollusca	Bivalvia	Unionoida	Unionidae	*Actinonaias*	*pectorosa*	C
Mollusca	Bivalvia	Unionoida	Unionidae	*Utterbackia*	*imbecillis*	C
Mollusca	Gastropoda	Architaenioglossa	Viviparidae	*Campeloma*	*decisum*	D
Mollusca	Gastropoda	Basommatophora	Physidae	*Physa*	*integra*	G
Mollusca	Gastropoda	Neotaenioglossa	Pleuroceridae	*Juga*	*plicifera*	D
Mollusca	Gastropoda	Neotaenioglossa	Hydrobiidae	*Lithoglyphus*	*virens*	D

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Table C-6. Deletion process for the derivation of the southwest (SW) and high plains (HP) arid stream region BLM adjusted copper databases

Phylum	Class	Order	Family	Genus	Species	SW Code	HP Code
Chordata	Actinopterygii	Acipenseriformes	Acipenseridae	*Scaphirhynchus*	*platorynchus*	D	D
Chordata	Actinopterygii	Cyprinodontiformes	Poeciliidae	*Poeciliopsis*	*occidentalis*	S	F
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Gila*	*elegans*	S	F
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Acrocheilus*	*alutaceus*	F	F
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Notemigonus*	*crysoleucas*	S	F
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Pimephales*	*promelas*	S	S
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Ptychocheilus*	*oregonensis*	D	F
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Ptychocheilus*	*lucius*	S	F
Chordata	Actinopterygii	Cypriniformes	Catostomidae	*Xyrauchen*	*texanus*	S	F
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*rubrum*	O	G
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*lepidum*	O	G
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*flabellare*	O	G
Chordata	Actinopterygii	Perciformies	Percidae	*Etheostoma*	*nigrum*	O	S
Chordata	Actinopterygii	Perciformies	Centrarchidae	*Lepomis*	*macrochirus*	G	S
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*kisutch*	D	F
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*mykiss*	D	F
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*tshawytscha*	D	F
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*clarki*	D	F
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*gorbuscha*	D	F
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*apache*	D	F
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*nerka*	D	F
Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Salvelinus*	*confluentus*	D	F
Chordata	Amphibia	Anura	Bufonidae	*Bufo*	*boreas*	D	D
Annelida	Clitellata	Lumbriculida	Lumbriculidae	*Lumbriculus*	*variegatus*	S	S
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Ceriodaphnia*	*dubia*	D	D
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Daphnia*	*pulicaria*	D	D
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Daphnia*	*magna*	D	D
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	*Scapholeberis*	sp.	D	D
Arthropoda	Insecta	Diptera	Chironomidae	*Chironomus*	*decorus*	G	G
Arthropoda	Insecta	Plectoptera	Perlidae	*Acroneuria*	*lycorias*	F	F
Arthropoda	Malacostraca	Amphipoda	Gammaridae	*Gammarus*	*pseudolimnaeus*	G	G
Arthropoda	Malacostraca	Amphipoda	Hyalellidae	*Hyalella*	*azteca*	S	S
Mollusca	Bivalvia	Unionoida	Unionidae	*Actinonaias*	*pectorosa*	C	C
Mollusca	Bivalvia	Unionoida	Unionidae	*Utterbackia*	*imbecillis*	C	C
Mollusca	Gastropoda	Architaenioglossa	Viviparidae	*Campeloma*	*decisum*	D	D
Mollusca	Gastropoda	Basommatophora	Physidae	*Physa*	*integra*	G	G
Mollusca	Gastropoda	Neotaenioglossa	Pleuroceridae	*Juga*	*plicifera*	D	D
Mollusca	Gastropoda	Neotaenioglossa	Hydrobiidae	*Lithoglyphus*	*virens*	D	D

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APPENDIX D

Ranked Hardness Modified Sites-specific toxicity database from the 2006 AWWQRP report

Appendix D. Ranked Hardness Modified Sites-specific toxicity database from the 2006 AWWQRP report.

			Site Specific						Regional	
				Santa Cruz						
Species	**Common name**	**SMAV**	**Santa Ana**	**below Nogales**	**near Tuscan**	**Salt/Gila**	**Fountian Creek**	**S Platte River**	**South west**	**High plains**
Notemigonus crysoleucas	Golden shiner	59017.19		41		43	47	51	52	52
Acroneuria lycorias	Stonefly	9407.86	37		23		46	50	51	51
Corbicula manilensis	Asiatic clam	7484.64	36					49	50	50
Trichoptera spp	Caddisfly	6200.00	35	40	22	42	45	48	49	49
Morone americana	White perch	5842.52				41			48	
Zygoptera spp.	Damselfly	4600.00	34	39	21	40	44	47	47	48
Procambarus clarkii	Red crayfish	2072.67	33	38	20			46	46	
Lepomis macrochirus	Bluegill	1394.46	32	37	19	39	43	45	45	47
Orconectes rusticus	Rusty crayfish	1363.30		36	18		42	44		46
Crangonyx pseudogracilis	Amphipod	1290.00				38				
Chironomus decorus	Midge	837.64	31	35	17	37	41	43	44	45
Gambusia affinis	Mosquitofish	795.54	30	34	16	36		42	43	44
Fundulus diaphanus	Banded killifish	788.32					40	41		43
Cyprinus carpio	Common carp	746.62	29	33		35	39	40	42	42
Oreochromis mossambicus	Tilapia	746.62	28			34			41	
Lepomis gibbosus	Pumpkinseed	619.00	27	32	15	33	38	39	40	41
Chironomus tentans	Midge	452.70	26	31	14	32	37	38	39	40
Ephemerella subvaria	Mayfly	362.71	25	30	13	31	36	37	38	39
Notropis chrysocephalus	Striped shiner	314.86		29		30	35	36	37	38
Carassius auratus	Goldfish	288.69	24	28		29	34	35	36	37
Ictalurus punctatus	Channel catfish	249.80	23		12	28	33	34	35	36
Etheostoma spectabile	Orangethroat darter	218.44					32	33		35
Jordanella floridae	Flagfish	189.81				27			34	
Etheostoma nigrum	Johnny darter	159.55					31	32		34
Acrocheilus alutaceus	Chiselmouth	132.61		27		26	30	31	33	33
Pectinatella magnifica	Bryozoan	128.27	22						32	

Appendix D. Ranked Hardness Modified Sites-specific toxicity database from the 2006 AWWQRP report. (continued)

			Site Specific						Regional	
				Santa Cruz						
Species	Common name	SMAV	Santa Ana	below Nogales	near Tuscan	Salt/Gila	Fountian Creek	S Platte River	South west	High plains
Chironomus plumosus	Midge	124.86	21	26	11	25	29	30	31	32
Ptychocheilus lucius	Colorado pikeminnow	117.29		25		24	28	29	30	31
Salmo salar	Atlantic salmon	114.58					27			30
Etheostoma flabellare	Fantail darter	107.94					26	28		29
Morone saxatillis	Striped bass	95.34				23			29	
Nais spp	Worm	90.00	20	24	10	22	25	27	28	28
Hybognathus amarus	Rio Grande silvery minnow	86.59		23		21	24	26	27	27
Rhinichthys atratulus	Blacknose dace	82.24		22		20	23	25	26	26
Etheostoma caeruleum	Rainbow darter	82.24					22	24		25
Poecilia reticulata	Guppy	81.76	19	21		19			25	
Xyrauchen texanus	Razerback sucker	81.02				18		23	24	24
Pimephales promelas	Fathead minnow	80.66	18	20		17	21	22	23	23
Etheostoma lepidum	Greenthroat darter	79.74					20	21		22
Semotilus atromaculatus	Creek chub	79.67		19		16	19	20	22	21
Campostoma anomalum	Central stoneroller	74.53		18		15	18	19	21	20
Ictalurus nebulosus	Brown bullhead	66.22			9			18		19
Catostomus latipinnis	Flannelmouth sucker	62.06	17	17		14	17	17	20	18
Gila elegans	Bonytail chub	59.25	16	16		13	16	16	19	17
Gyraulus circumstriatus	Snail	54.75	15	15			15	15	18	16
Limnodrilus hoffmeisteri	Worm	51.71	14	14	8	12	14	14	17	15
Poeciliopsis occidentalis	Gila topminnow	49.07		13		11			16	
Lumbriculus variegatus	Worm	46.40	13	12			13		15	14
Physa integra	Snail	43.28	12	11		10	12	13	14	13
Ptychocheilus oregonensis	Northern pikeminnow	40.14		10			11	12	13	12
Gammarus spp	Amphipod	39.00	11	9	7	9	10	11	12	11
Plumatella emarginata	Bryozoan	35.21	10						11	
Lophopodella carteri	Bryozoan	35.10	9						10	

Appendix D. Ranked Hardness Modified Sites-specific toxicity database from the 2006 AWWQRP report. (continued)

			Site Specific						Regional	
				Santa Cruz						
Species	**Common name**	**SMAV**	**Santa Ana**	**below Nogales**	**near Tuscan**	**Salt/Gila**	**Fountian Creek**	**S Platte River**	**South west**	**High plains**
Physa heterostropha	Snail	34.98	8	8		8	9	10	9	10
Tubifex tubifex	Worm	33.28	7	7	6	7	8	9	8	9
Chironomus spp	Midge	30.00	6	6	5	6	7	8	7	8
Physella gyrina	Snail	27.26	5					7	6	7
Gammarus pseudolimnaeus	Amphipod	22.72	4	5	4	5	6	6	5	6
Brachydanio rerio	Zebrafish	22.27		4		4	5	5	4	5
Ephoron virgo	Mayfly	19.35	3	3	3	3	4	4	3	4
Etheostoma rubrum	Fountain darter	18.40					3	3		3
Hyalella azteca	Amphipod	16.35	2	2	2	2	2	2	2	2
Gammarus pulex	Amphipod	15.22	1	1	1	1	1	1	1	1
	Final Acute Value (ug/L) =		17.03	17.48	15.22	17.72	17.3	17.59	18.76	17.67
	Final Chronic Value (ug/L) =		5.87	6.03	5.25	6.11	5.97	6.06	6.47	6.09

APPENDIX E

***P. promelas* Toxicity Test Reports**
(Included on separate CD)

APPENDIX F

***C. dubia* Toxicity Test Reports**
(Included on separate CD)