Guidance Document for the Development of Site-Specific Water Quality Criteria for Metals

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ACRONYMS AND ABBREVIATIONS

ASTM	American Society for Testing and Materials
f	dissolved translator
EPA	U.S. Environmental Protection Agency
FWER	final water effect ratio
NPDES	National Pollutant Discharge Elimination System
ODEQ	Oklahoma Department of Environmental Quality
OWRB	Oklahoma Water Resources Board
ppb	part per billion
QA/QC	quality assurance and quality control
TDS	total dissolved solids
TOC	total organic carbon
TRE	toxicity reduction evaluation
TSS	total suspended solids
USGS	U.S. Geological Survey
WER	water effect ratio

FOREWORD

This document provides guidance concerning the determination of site-specific water quality criteria for metals. It is issued in support of Oklahoma's water quality standards and is considered to be guidance only. It does not establish or affect legal rights or obligations. Agency decisions regarding any specific case will be made by applying the law and regulations on the basis of specific facts when regulations are promulgated or permits are issued.

Comments, especially those accompanied by supporting data, are welcomed and should be addressed to: Derek Smithee, Oklahoma Water Resources Board, 3800 North Classen Boulevard, Oklahoma City, Oklahoma 73118.

IMPORTANT DEFINITIONS

Below are terms that are used throughout this guidance document. You can refer back to this list to help explain these terms, as necessary.

7Q2-The receiving stream flow used for determining allowable discharge load to a stream. The flow is calculated as a moving average of 7 consecutive days for each year in a given record. These 7-day, low flow values are ranked in ascending order. An order number (m) is calculated based on the number of years of record (n), with a recurrence interval (R) of 2 years, as m = (n+1)/R. A value of flow corresponding to the mth order is taken as the 7-day, 2-year (7Q2) low flow for those historical data.

Background concentration-The concentration of a metal that is present upstream from the permitted discharge or waterbody of concern.

Clean techniques-A set of procedures that are designed to prevent contamination of samples so that concentrations of trace metals can be measured accurately and precisely in the parts per billion (*μ*g/L) range.

Criterion translator (T)-The criterion translator represents the product of the final WER (FWER) and the dissolved translator (*f*) (i.e., T = FWER H *f*).

Design flow-The effluent flow used for steady-state wasteload allocation modeling.

Discharge permit limits-Numeric values that limit the amount of metal that can be discharged from a facility.

Dissolved metal-In terms of the site-specific criterion, a dissolved metal passes through either a 0.45-*μ*m or 0.40-*μ*m membrane filter.

Dissolved translator (*f*)-The dissolved translator represents the empirical ratio of dissolved-to-total recoverable metal in a sample of simulated downstream site water.

Endpoint-The concentration of a test material that is expected to cause a specified amount of adverse effect (e.g., a concentration causing a 50 percent mortality of test species [LC_{50}]).

Final WER (FWER)-The water effect ratio (WER) that is used to calculate a site-specific criterion. In Oklahoma, a FWER is derived by taking the geometric mean of four acceptable WERs.

LC_{50}-The LC_{50} is the concentration of a toxicant that causes lethal effects in 50 percent of the test population within a specified period of time (e.g., 48 hours).

Receiving water-Water in a stream that is upstream and unaffected by the discharge.

Renewal test-A toxicity test in which either the test solution in a test chamber is renewed at least once during the test or the test organisms are transferred into a new test solution of the same composition at least once during the test.

Simulated downstream site water-A sample that consists of receiving water (i.e., water that is upstream and unaffected by a site discharge) combined with effluent at a pre-specified ratio.

Site-specific criterion-A water quality criterion for aquatic life that has been derived to be specifically appropriate to the water quality characteristics at a particular location.

Static test-A test in which the solution and the organisms that are in a test chamber at the beginning of a test remain in the chamber until the end of the test.

Total recoverable metal-The metal that remains in aqueous solution after a sample has been appropriately acidified and digested by strong acid and insoluble, equipment-clogging material that has been separated either by gravity settling or by large pore filtration.

Water Effect Ratio (WER)-A numeric value that compares the outcomes of side-by-side toxicity tests. Oklahoma defines a WER as the LC_{50} of laboratory water divided by the LC_{50} of simulated downstream site water. An EPA WER is the reciprocal of an Oklahoma WER.

INTRODUCTION

In the state of Oklahoma, effluent requirements for National Pollutant Discharge Elimination System (NPDES) permits are calculated based on water quality criteria for metals. The statewide water quality criteria are based on U.S. Environmental Protection Agency (EPA) water quality criteria developed in the 1980s (U.S. EPA 1993c, 1995). EPA regulations were based on toxicity test results in which aquatic organisms were exposed to metals under laboratory conditions. The numeric limits set by EPA, and adopted by Oklahoma, are conservative estimates that are designed to be protective of aquatic communities in a wide range of water bodies. These default criteria are appropriate to use in the absence of site-specific data. However, the default criteria may, in some cases, be overprotective because they do not take into account site-specific characteristics such as water chemistry.

In response to these concerns, EPA created procedures to derive site-specific water quality criteria (U.S. EPA 1994a). The Oklahoma Water Resources Board (OWRB) has modified these procedures so that permitees or other entities in Oklahoma can also adopt site-specific criteria in lieu of using the statewide criteria. This document provides the appropriate guidance for developing water quality criteria that are specific for a waterbody. The discharger should also realize that this procedure is **intended to allow modification of a statewide criterion, not the creation of entirely new one**.

OKLAHOMA STATE WATER QUALITY STANDARDS

Oklahoma's water quality standards for metals are currently expressed as statewide total criteria, which are listed in OAC 785:45. Some of these criteria are expressed as equations, which depend on pH or hardness of the receiving stream. Numerical values for these criteria are obtained following OAC 785:46. These criteria are used to calculate the acceptable amount of metal that can be released to surface water under the NPDES program. Prior to mid-1997, Oklahoma's metals criteria were expressed only in terms of total recoverable metals. Total recoverable criteria remain scientifically defensible in terms of protecting beneficial uses, but dissolved metal criteria have been developed because the dissolved fraction is believed to better represent the biologically available portion of the metal than the total recoverable portion (U.S. EPA 1996b). To determine the new dissolved criterion, an existing total recoverable criterion is multiplied by an EPA conversion factor that is specific to each metal. These conversion factors are based on the percent of dissolved metals present in a broad range of analytical tests conducted by U.S. EPA (1995) and are found in OAC 785:45.

The use of either the total or dissolved statewide criteria directly affects the calculations that lead to final permit limits under the NPDES program. EPA regulations require that NPDES permit limits be expressed as total recoverable metals. If the statewide total recoverable criteria are used, the calculations that result in revised permit limits are fairly straightforward. Statewide dissolved criteria must be translated to site-specific total recoverable criteria to be used for permit limit calculations. Options for permittees to develop site-specific criteria are discussed in more detail below.

PERMIT LIMIT OPTIONS

A permittee continues to have the option of accepting the statewide default total or dissolved criteria. A permit limit based on the default criteria can be obtained simply by applying for a discharge permit. If, however, a permittee decides to develop site-specific criteria, three additional options to calculate the permit limit are available:

1. Use of a water effect ratio (WER)
2. Use of a dissolved translator (*f*)
3. Use of both a WER and a dissolved translator (*f*).

Figure 1 (page 32) provides a graphical summary of the options available to permittees. Option one can only be used to translate a statewide criterion for *total recoverable metal*s into a site-specific criterion for total recoverable metals. Options two and three (discussed below) are used to translate a statewide criterion for *dissolved* metals into a site-specific criterion for total recoverable metals. Each of these options is described briefly below. More detailed information about the process and requirements under each option is presented in subsequent sections of this guidance document.

Option One: Use of a Water Effect Ratio

The WER approach is based on the assumption that the toxicity of a metal will be different under site-specific conditions when compared to laboratory conditions. A WER is based, in part, on the fact that site-specific chemical and physical properties may affect the bioavailability and toxicity of the metal. The WER approach quantifies the ratio between the toxicity of a metal using site-specific samples and the toxicity of the same metal using laboratory dilution water. Very simply, a WER under the Oklahoma definition is:

$$\text{WER} = \text{LC}_{50} \text{ in laboratory water/LC}_{50} \text{ in site water}$$

This ratio of toxicity values (i.e., LC_{50}, or the concentration that is lethal to 50 percent of the test organisms) between the two conditions can then be used to translate the total statewide criterion into a total site-specific criterion. Although Option One specifically allows the translation of the state total recoverable criteria, Option Three (discussed on the following page) allows a dissolved WER to be used to translate the state dissolved criteria.

It is important to note that the Oklahoma definition is the reciprocal of the EPA definition (where WER = LC_{50} in site water/LC_{50} in laboratory water). The two forms of WER are mathematically equivalent because they reflect different and reciprocal calculation procedures used by the two agencies. Using the Oklahoma definition, if the WER is less than one, the toxicity of a metal in the site or receiving stream water is less than the toxicity in deionized laboratory water. Although this situation may exist for metals under many site-specific conditions, it is not always the case. Permittees should be aware that the WER may be higher than 1 in some circumstances (i.e., the toxicity is actually higher in site water).

Option Two: Use of a Dissolved Translator

As mentioned above, Oklahoma offers its permittees the option of using statewide dissolved metals criteria. However, discharge limits continue to be set based on total recoverable metals concentrations. Option Two allows permittees to translate the statewide dissolved criterion into a statewide total recoverable criterion using a dissolved translator (f). This translator reflects the actual site-specific dissolved-to-total recoverable metals ratio:

$$f = \text{dissolved concentration of metal/total concentration of metal}$$

This ratio is determined using a series of empirical tests that are designed to represent a spectrum of actual site conditions. In Oklahoma, empirical measurements are used in lieu of theoretical partitioning coefficients.

Option Three: Use of Both a WER and a Dissolved Translator

This approach is based on a combination of Options One and Two. The major difference between Option One (total WER) and Option Three is that the dissolved WER and the dissolved translator are used in conjunction to convert a statewide dissolved criterion into a site-specific total recoverable criterion. A criterion translator (T) is defined as:

$$T = \text{WER} \times f$$

This combination provides the most representative value for a site-specific criterion because it incorporates site-specific information about the toxicity of a particular metal in site waters, and it includes an empirical determination of the amount of dissolved (or bioavailable) metal that may be present. However, caution should be exercised when applying this option as it may lead to downstream effects from an increased wasteload. This will be discussed in some detail later.

ORGANIZATION OF GUIDANCE DOCUMENT

This guidance document illustrates the general process of developing a site-specific criterion. To meet this goal, the document is organized as follows:

- *Is A Site-Specific Approach Appropriate For Your Site?* Before deciding that a site-specific criterion is the best option, you should read this section to identify some important issues associated with this process. If the answer to this question is “Yes”, an overview of the process is provided.
- *How Should The Field Sampling Plan Be Designed?* The design of the field sampling plan will affect the success of the study. This section walks permittees through the details of the sampling process for both the receiving water and effluent.
- *How Should Laboratory Tests Be Conducted?* Defensible data must support all of the required calculations, and these data depend on properly conducted laboratory tests that follow appropriate quality control procedures. Two types of laboratory tests are conducted to determine site-specific criteria: toxicity tests to support the development of a WER and/or chemical analyses to support the development of both a WER and a dissolved translator. This section provides information on many of the details associated with both types of tests. After the data have been generated, it is important to determine whether the data can be used. This section also provides information to help you interpret the results of the tests.
- *How Are Final Site-Specific Criteria Developed?* If test data are acceptable, this section provides the calculations that are used to develop a site-specific criterion and, ultimately, a site-specific permit limit.
- *Where To Go For More Information?* This section refers you to additional resources for more information. These sources include additional Oklahoma state documents, EPA regulations, and technical papers.

In addition to the main guidance document, helpful appendices are also provided for your use. These appendices include:

- Appendix A: Work Plan Requirements
- Appendix B: Guidance Concerning the Use of Clean Techniques
- Appendix C: Procedures for Collecting Study Samples
- Appendix D: Procedures for Conducting Toxicity Tests
- Appendix E: Calculation Worksheets

- Appendix F: Reporting Requirements.

IS A SITE-SPECIFIC APPROACH APPROPRIATE FOR YOUR SITE?

ISSUES TO CONSIDER BEFORE YOU BEGIN

Oklahoma regulations have been revised to provide more flexibility to permittees, and a site-specific criterion may be beneficial in many circumstances. However, before you decide to take a site-specific approach to calculating permit limits, it is critical that you evaluate some important issues.

- **Development of a Site-Specific Criterion Costs Money-**Be prepared to invest significant resources into the project. The site-specific process includes developing a strategy that is acceptable to OWRB, the Oklahoma Department of Environmental Quality (ODEQ), and in some cases, EPA (see Appendix A). After a work plan is developed, an extensive field sampling effort is required. Laboratory costs associated with toxicity testing and chemical analyses are also expensive budget factors. At the conclusion of this process, a final report must be prepared and submitted to the agencies. Carefully weigh the costs of developing a site-specific criterion against the costs of potentially reducing the discharge of the metal, if feasible.
- **Existing Data Need to be Valid-**You may be considering a site-specific approach because your discharge appears to exceed existing or proposed discharge permit limits. Prior to investing resources into developing site-specific criteria, make sure that your existing sampling techniques are not contaminating your samples and sabotaging your compliance efforts. Quality control procedures are critical in determining where and if contamination is occurring. It generally will be much easier to verify your chemical data by using “clean techniques” for collecting, handling, storing, preparing, and analyzing samples than to develop a site-specific criterion. Clean techniques refer to the proper practices of sample handling and testing necessary to produce reliable analytical data in the parts per billion (ppb, μg/L) range. Clean techniques are discussed in greater detail in the *How Should the Field Sampling Plan Be Designed?* section later in this report and in Appendix B.
- **Be Prepared for a Wide Range of Outcomes-**Permit limits based on site-specific criteria may be less stringent (i.e., higher) than those limits based on the conservative, default statewide criteria. This does not represent back-sliding because the permit limits derived from site-specific criteria remain water-quality based. The process is designed to be environmentally protective, such that default criteria are adjusted only to a level that will not jeopardize the highest beneficial use of the receiving water body. **However, if a site-specific criterion is lower (more stringent) than the default statewide criterion, the site-specific criterion will be used in determining permit limits**. Typical ranges for total WERs (as defined by U.S. EPA [1992b], using a site water endpoint divided by a laboratory water endpoint) are summarized below:

Metal	Typical EPA Total WER Range	Typical OWRB Total WER Range
Cadmium	1.0-15	0.067-1.0
Chromium	1.0-6.0	0.167-1.0
Copper	1.0-15	0.067-1.0
Lead	1.0-6.0	0.167-1.0
Nickel	1.0-6.0	0.167-1.0
Zinc	0.7-3.0	0.333-1.43

It should be noted, however, that these are typical ranges only and actual values may be lower or higher. Therefore, a decision to develop a WER does not necessarily ensure that a higher permit limit will be attained. The discharger should also be aware that receiving water with very low hardness will typically not allow much (if any) relief from the statewide limits.

- **You Will Need to Use Appropriate Background Conditions-**To preserve a safety margin associated with using site-specific criteria, actual background concentrations must be incorporated in the permit limit calculations. Background conditions reflect the fact that your upstream water may contain naturally occurring metals, one or more effluents, and metals or pollutants from non-point sources upstream. The upstream water may also contain sources of toxicity that are unrelated to metals. Background concentrations may affect the capacity of the receiving water to assimilate the metal from your discharge. Upstream water may be clearly impacted by point or non-point sources (as determined by existing background data or relevant listing of the upstream segment as per CWA Section 303(d) for the metals of concern. In this situation, WERs are generally not appropriate because Oklahoma's site-specific criteria are valid only for individual dischargers. The presence of clear upstream degradation would require some form of basin-wide water quality management. OWRB will decide whether a WER can be applied in such circumstances on a case-by-case basis.
- If adequate background data are not available for your site, a default concentration of zero is used in permit calculations (ODEQ 1994). If this is the case for your existing permit, actual background concentrations need to be validated as part of a site-specific criteria approach. These concentrations are likely to be higher than zero, which may offset a higher site-specific criterion and create a net negative effect on the final permit limit. Background sampling will involve the collection of 12 monthly samples, usually collected on the same day of each month to account for the random nature of flow and upstream load.

- **<u>IMPORTANT CAUTION</u>! If you discharge into a stream with no upstream flow, or that flow is so intermittent or rain dependent as to provide no dilution, you may not be able to create a site-specific criterion for that stream site.** Creating a site-specific criterion for this type of situation requires some special considerations. EPA has included some suggestions and requirements for these "special waters" in their guidance document on site-specific criteria development (EPA-823-B-94-001). Excerpts from this document follow:

> ***"Method 1 is intended to apply not only to ordinary rivers and streams but also to streams that some people might consider 'special., such as streams whose design flows are zero and streams that some state and/or federal agencies might refer to as 'effluent-dependent', 'habitat-creating', 'effluent-dominated', etc.*** (Due to differences between agencies, some streams whose design flows are zero are not considered 'effluent-dependent', etc., and some 'effluent-dependent' streams have design flows that are greater than zero.) The application of Method 1 (pg 17-64 of the EPA guidance) to these kinds of streams has the following implications:
>
> 1. If the design flow is zero, at least some WERs ought to be determined in 100% effluent.
>
> 2. If thunderstorms, etc., occasionally dilute the effluent substantially, at least one WER should be determined in diluted effluent to assess whether dilution by rainwater might result in under-protection by decreasing the WER faster than it decreases the concentration of the metal. This might occur, for example, if rainfall reduces hardness, alkalinity, and pH substantially. This might not be a concern if the WER demonstrates a substantial margin of safety.
>
> 3. If the site-specific criterion is substantially higher than the national criterion, there should be increased concern about the fate of the metal that has reduced or no toxicity. Even if the WER demonstrates a substantial margin of safety (e.g., if the site-specific criterion is three times the national criterion, but the experimentally determined WER is 11), it might be desirable to study the fate of the metal.
>
> 4. If the stream merges with another body of water and a site-specific criterion is desired for the merged waters, another WER needs to be determined for the mixture of the waters.
>
> 5. Whether WET testing is required is not a WER issue, although WET testing might be a condition for determining and/or using a WER.
>
> 6. A concern about what species should be present and/or protected in a stream is a beneficial-use issue, not a WER issue, although resolution of this issue might affect what species should be used if a WER is determined. (If the Recalculation Procedure is used, determining what species should be

> present and/or protected is obviously important.)
>
> 7. Human health and wildlife criteria and other issues might restrict an effluent more than an aquatic life criterion. Although there are no scientific reasons why 'effluent-dependent', etc., streams and streams whose design flows are zero should be subject to different guidance than other streams, a regulatory decision (for example, see 40 CFR 131) might require or allow some or all such streams to be subject to different guidance. For example, it might be decided on the basis of a use attainability analysis that one or more constructed streams do not have to comply with usual aquatic life criteria because it is decided that the water quality in such streams does not need to protect sensitive aquatic species. Such a decision might eliminate any further concern for site-specific aquatic life criteria and/or for WET testing for such streams. The water quality might be unacceptable for other reasons, however."

- **A Site-Specific Criterion May Not be Applicable for Future Conditions-** The WER is dependent in part on specific properties of the effluent that influence the bioavailability and toxicity of metals. Substantial changes in the quality or quantity of the effluent may affect the magnitude of a WER, and the resulting site-specific criterion. Therefore, if your existing permit contains requirements for toxicity reduction evaluations (TREs) or pollution prevention efforts, a site-specific criterion should not be developed until after these efforts have been completed. A new WER would likely have to be determined after those requirements are met because the characteristics of the effluent may significantly change (e.g., hardness, pH, TDS). In cases where the quality or quantity of an effluent changes, the burden rests on the permittee to demonstrate that the effluent characteristics are not significantly altered to a degree that would affect the validity of the WER.
- **You May Need to Revisit This Approach-**Similar to above, a WER may need to be re-evaluated periodically to reflect changes in the system that may alter the characteristics of the receiving water or effluent. These parameters include hardness or alkalinity, pH, total suspended solids (TSS), total organic carbon (TOC), nitrogenous compounds, or inorganic compounds that may affect the bioavailability of metals of concern.
- **Multiple metals considerations-** For dischargers seeking relief from permit limits for multiple metals, it should be noted that ***additional testing, beyond that normally required for site-specific criterion development, will be required*** to demonstrate that the additive or synergistic effects of the multiple metals at the site-specific concentrations requested will not cause toxicity (EPA, 1994a). Such demonstration will be required in the final report to the agencies.

These issues provide some indication of the conditions and risks associated with developing site-specific criteria. If you decide that a site-specific approach will be feasible and cost-effective for your situation, an overview of the general process is presented in the next section.

Again, the discharger should also realize that this procedure is intended to allow **modification of a statewide criterion, not the creation of entirely new one**.

GENERAL PROCESS FOR DEVELOPING SITE-SPECIFIC CRITERIA

The process for developing site-specific criteria in Oklahoma is relatively straightforward. To begin, a permittee must decide which of the three options discussed in the previous section to pursue. A generalized study flow diagram is provided in Figure 2 (page 33). The steps included in the process are as follows:

Step 1. Perform preliminary analysis
Step 2. Develop and receive approval of work plan
Step 3. Collect effluent and receiving water samples
Step 4. Conduct WER toxicity tests (if applicable) and chemical analyses on samples
Step 5. Calculate WER values (if applicable)
Step 6. Determine the ratio of dissolved-to-total recoverable metal
Step 7. Determine background concentrations of the metal (if necessary)
Step 8. Submit data to appropriate agencies to develop site-specific permit limits

Steps 1 and 2 are discussed in greater detail below. Step 3 is addressed in *How Should the Field Sampling Plan Be Designed?*; Step 4 is outlined in *How Should Laboratory Tests Be Conducted?*; and Steps 5, 6, 7, and 8 are discussed in *How Are Final Site-Specific Criteria Developed?*

Preliminary Analysis

As part of Step 1, a number of issues should be addressed in your preliminary analysis. In addition to the points raised earlier, additional issues to consider are summarized below.

Defining Your Site

In the general context of site-specific criteria, a ‘site’ may be a watershed, a water body, a segment of a water body, or a category of water body (e.g., ephemeral stream). You need to clearly understand your existing discharge permit, particularly in terms of the ‘site’ to which it applies, because a site-specific criterion can only be applied to your site. In most cases, the site will be a discrete, single discharge point associated with a mixing zone. You will need to define the spatial extent of the site to which the WER and the site-specific criterion are intended to apply.

Using Simulated Downstream Site Water

All WERs and dissolved translators will be determined using simulated downstream site water. Simulated downstream site water is simply upstream water (i.e., water that is upstream and unaffected by your discharge) combined with effluent at a pre-specified ratio. The final outcome of the study is likely to be highly dependent on this ratio because the amount of effluent generally controls the amount of metal in the sample. The mixing ratio is determined based on the dilution capacity of your receiving water body and is discussed in more detail in *How Should the Field Sampling Plan Be Designed?* The advantage of using simulated downstream site water is that mixtures can be prepared to simulate the ratio of effluent and receiving water at the point of maximum concentration on the mixing boundary (U.S. EPA 1994a).

Using Total or Dissolved WERs

WERs can be applied to either statewide total or dissolved criteria (see Figure 1). There are also two categories of WER tests: total and dissolved. Very simply, a total WER evaluates the toxicity of the total recoverable metal and a dissolved WER evaluates the toxicity of the dissolved metal. A total WER can be used to calculate a total site-specific criterion. If a dissolved WER is used to calculate a dissolved site-specific criterion, the dissolved translator must also be used in conjunction with the WER so that the final discharge permit limit is expressed as a total recoverable metal (U.S. EPA 1994a). Fortunately for permittees, both total and dissolved WERs are derived from the same side-by-side toxicity tests. This process is described in more detail in *How Should Laboratory Tests Be Conducted?* In general, you can use the approach that provides the most benefit to your specific situation.

Determining Acute or Chronic Criteria

Statewide water quality criteria are divided into two categories: acute (short-term) and chronic (long-term). In most cases, the statewide chronic criteria control final permit discharge limits because chronic values tend to be more conservative. Regardless of which criteria control your current discharge limits, the toxicity tests used as part of the WER process should be conducted under acute (48-hour) conditions. This condition is beneficial for permittees because acute toxicity tests are easier and less expensive to conduct than chronic toxicity tests.

Acute tests can be used to develop WERs that are applicable to acute or chronic criteria (U.S. EPA 1994a). This general applicability of acute test results is based on the relative sensitivities of acute and chronic tests. Different acute and chronic WER values reflect the sensitivity of a particular test. Less sensitive tests (i.e., acute tests) tend to yield higher WER values (as defined in Oklahoma) than more sensitive tests (i.e., chronic tests). Because Oklahoma criteria are divided by WER values, applying a higher WER to a statewide criterion results in a lower site-specific criterion. Therefore, it is considered environmentally protective to apply an acute WER to a chronic criterion.

Work Plan Development

Step 2 in the process brings the information you have compiled in the preliminary analysis into a coherent and concise plan of action. An important task in this step is to discuss your project with the appropriate state agencies. These agencies include OWRB and ODEQ, both of which provide input into calculating final permit limits. OWRB issues a site-specific criterion based on the outcome of your study, and ODEQ uses this criterion to calculate final discharge permit limits. Feedback from both of these agencies should be sought during development of the study.

Specific requirements for a work plan are provided in Appendix A. You should include these requirements in your work plan because they provide the level of detail needed to accurately understand your project. Both agencies need this information to approve your approach and, ultimately, your site-specific criteria.

The following two sections, *How Should the Field Sampling Plan Be Designed?* and *How Should Laboratory Tests Be Conducted?,* provide some strategic information that will help you prepare a work plan.

HOW SHOULD THE FIELD SAMPLING PLAN BE DESIGNED?

The design of the field-sampling plan will ultimately affect the success of the project. In addition to addressing identified data gaps, sampling should be performed to gather relevant data. This section walks permittees through an appropriate sampling process, and Appendix C provides protocols for collecting study samples. The sampling design for your study will depend on the option that you pursue to develop a site-specific criterion:

- **Option One: Use of a WER-**Three rounds of toxicity tests must be performed on samples that combine effluent and receiving water. Each round is defined as a pair of side-by-side tests performed using receiving water and laboratory water. A round of testing may involve one or more test species. At least one round of testing must be performed using two test species, resulting in a minimum of four acceptable tests (this issue is discussed in more depth in *How Should Laboratory Tests Be Conducted?*). These tests are performed on samples collected from three different seasons to address natural system variability.
- **Option Two: Use of a dissolved translator-**Chemical analyses must be performed on effluent samples, receiving water samples, and combined effluent and receiving water samples. These tests are performed on samples from at least 10 different sampling events that are representative of low stream flow conditions.
- **Option Three: Use of a WER and dissolved translator-**Elements of the first two options are incorporated into one sampling strategy. Overlap is acceptable (i.e., samples collected for the WER tests can be analyzed to support development of a dissolved translator).

A summary of sampling requirements for all three options is presented in Table 1 (page 33). This matrix will help you decide what types of samples should be collected. *It is very important to remember that all of these options require an accurate calculation of background concentrations.* Data to determine background concentrations may be available from STORET or other databases that have adequate and documentable quality assurance procedures. If sufficient data are not available, you must monitor the receiving water to determine the background concentrations in a total of 12 upstream monthly sampling events. Again, overlap is acceptable (e.g., upstream samples can be used so that one portion is used to determine the dissolved translator and one portion is used to determine background concentrations).

The acceptability of the data rely on rigorous, standardized field quality assurance and quality control (QA/QC) measures. In part, these measures include the use of clean techniques throughout the field sampling effort. A detailed description of field-related clean techniques is provided in Appendix B. It is only to your advantage to use these techniques when you sample both the receiving water and the effluent.

DETERMINING THE SIMULATED DOWNSTREAM SITE WATER RATIO

An important concept for the field sampling design is the use of simulated downstream site water. Simulated downstream site water means that WERs and/or dissolved translators are determined using sample(s) that contain both receiving water and effluent. These two types of samples are combined to represent the maximum concentration of a metal on the mixing zone boundary under “critical conditions”. Critical conditions reflect a worst-case scenario, where the receiving water flow is minimal and the effluent flow is at maximum capacity. As such, the ratio of the mixture of receiving water and effluent depends on site-specific upstream flows and effluent flows. Critical dilutions, expressed as percent effluent, PE, are given in OAC 785:45 Appendix E. A worksheet to determine the appropriate ratio to use for your study is contained in Appendix E of this guidance document. The ratio also controls the total volume that will be required from the receiving water and effluent samples. A worksheet to determine the appropriate volume requirements is also provided in this Appendix E.

COLLECTING SAMPLES

Regardless of the option you choose, there are at least two types of samples:

- # **Receiving water-**Samples are collected from a river or stream segment upstream from your discharge in an area that is unaffected by the discharge
- # **Effluent-**Samples are collected from post-treatment effluent discharge (usually from the outfall immediately prior to mixing with the receiving water).

These two types of samples should be collected during the same time period for each sampling event. Appendix C contains detailed information about how to collect the required samples and includes sample collection procedures, sample handling requirements, and field documentation requirements. Each type of sample is described in more detail below.

A third type of water is required for WERs. It involves laboratory water for side by side toxicity tests.

- # **Lab water-**H_2O made in a laboratory with certain additives to reflect upstream hardness.

Receiving Water Samples

A receiving water sample will be collected in an area unaffected by and upstream from the discharge during each sampling event. The sampling location should remain consistent for all sampling events and should be accessible and safe for field personnel.

Each upstream sample should be collected in sufficient volume so the analytical laboratories can conduct the appropriate tests scheduled for each sampling event. A worksheet to determine the appropriate sampling volume requirements is provided in Appendix E.

Upstream samples must be collected during low flow events. You should sample when river conditions are close as possible to the 7Q2 (defined in *Important Definitions*) low flow values of the river, if available. It is recognized that some sampling may occur at flows higher than the 7Q2 given the requirements for seasonal sampling. However, the sampling requirements can generally be met by avoiding the season of high flows and focusing on periods of relatively low flows during other seasons. If 7Q2 values are not available, sampling should be targeted toward extreme low flow conditions. The U.S. Geological Survey (USGS) provides daily updates on stream conditions throughout Oklahoma. Statewide information can be accessed by calling the USGS office in Oklahoma City (405-853-7570) or via the Internet (http://water.usgs.gov/swr/OK).

Prior to collecting samples, the flow rate of the river for the date of sample collection must be documented. If a USGS stream gauge is located within 2 miles either upstream or downstream from the sampling location, you can use data recorded daily from this gauge to fulfill this requirement. This information can be accessed through USGS as described above. If no stream gauge is available, you must measure the flow rate of the river as part of collecting the sample. Procedures that can be used to measure the river flow rates are provided in Appendix C. If the flow rate is significantly different that the 7Q2 low flow value, sampling should be postponed until this condition can be met.

Water in a stream or river has some inherent variability because of differences in channel morphology and water flow. It is recommended that your sampling techniques attempt to capture this variability, to the extent possible, by collecting samples at various depths and points across the stream. To accomplish this goal, discrete grab samples could be collected across the width and throughout the depth of the river segment, which would then be composited. Samples could also be collected using a pumping system. To verify that the samples collected are representative of site conditions, temperature, pH, dissolved oxygen, and conductivity should be measured in the receiving water body at the specific sampling location(s). Procedures for collecting surface water samples that are representative are provided in Appendix C.

Effluent Samples

An effluent sample will be collected from post-treatment discharge during each sampling event. If there is more than one outfall in close proximity, effluent samples from the outfalls may be combined proportional to flow if the multiple discharges are jointly regulated under one permit and the permitting authority agrees with the methodology. The sampling location should remain consistent for all sampling events and should be accessible and safe for field personnel. Each effluent sample should be collected in sufficient volume so the analytical laboratories can conduct the appropriate tests scheduled for each sampling event. A worksheet to determine the appropriate sampling volume requirements is provided in Appendix E.

Effluent samples should be collected when plant flows are as close as possible to the flows used to calculate the existing permit limits (i.e., highest averaged monthly flows or the design flow). Facility discharge records can be used to document the effluent flow for the dates of sample collection. One 24-hour flow- or time-weighted composite sample will be collected during each sampling event. The effluent sample should be collected independently from the plant's daily composite sample and should be collected at sufficiently small intervals so that variations in plant conditions, plant flow, and short-term effluent quality are captured. To accomplish this goal, sampling should be performed by taking discrete time-weighted or flow-weighted samples throughout the sampling period and compositing these samples together. **At a minimum, the 24-hour composite sample should consist of at least 12 effluent portions collected at equal time intervals and combined proportional to flow.** Sampling could also be performed using an automatic sampling system (e.g., ISCO programmable samplers). To verify that the samples collected are representative of normal discharge conditions, temperature and pH should be measured in the effluent stream at the time of sample collection. Additional procedures for collecting effluent samples that are representative are provided in Appendix C.

Laboratory Water

WERs reflect differences in toxicity between water used to develop statewide criteria and site water. Site water is a mixture of effluent and receiving water. Laboratory water used for the WER test must reflect water quality similar to that used for criteria development. However, hardness-dependent criteria require lab water reflect local conditions. OAC 785:46 dictates how hardness must be handled. Specifically, it requires that toxicity variations caused by a discharge's hardness not affect criteria. **This means that lab water hardness must be similar to that of site water upstream of the discharge in order to insure that differences in hardness between lab and site water does not contribute to the WER**. OAC 785:46 restricts the mitigating effect of hardness on toxicity of certain metals to insure that effluent not be made hard to obtain less stringent permits.

Quality Control Samples

To verify that field techniques are providing high-quality samples, quality control samples should be collected during each sampling event. Types of field quality control samples are described in Appendix C.

SAMPLE HANDLING

Although complete sample handling protocols are provided in Appendix C, some important issues should be noted as part of sample handling:

- Filtering dissolved metal samples in the field should be avoided. Filtering conducted in clean laboratory environments generally reduces the possibility of cross-contamination.
- If mercury is one of the target metals, special care should be taken to cover samples and keep sample turbulence to a minimum to reduce potential volatilization.
- Toxicity tests must be initiated within 36 hours following sample collection. The end of sample collection is defined as the end of the 24-hour compositing period for the effluent samples.

HOW SHOULD LABORATORY TESTS BE CONDUCTED?

Properly conducted laboratory tests will result in defensible data that can support the development of a site-specific criterion. All of the tests used to support your study must be conducted by an Oklahoma-certified laboratory that follows appropriate quality control procedures. You can obtain a list of acceptable laboratories by calling ODEQ. The laboratory testing that is required for your study will depend on the option that you pursue.

- **Option One: Use of a WER-**Three rounds of toxicity tests must be performed on samples that combine effluent and receiving water.
- **Option Two: Use of a dissolved translator-**Ten rounds of chemical analyses must be performed on effluent samples, receiving water samples, and combined effluent and receiving water samples.
- **Option Three: Use of a WER and dissolved translator-**Elements of the first two options are incorporated into one sampling strategy. Overlap is perfectly acceptable (i.e., samples collected for the WER tests can be analyzed to support a dissolved translator).

A summary of data requirements for all three options is presented in Table 1 (page 34). This matrix will help you decide what types of tests should be performed. ***It is very important to remember that all of these options to require an accurate calculation of background concentrations***. If adequate background data do not exist, 12 monthly rounds of chemical analyses are required. Again, overlap is acceptable (e.g., upstream samples can be used so that one portion is analyzed to determine the dissolved translator and one portion is analyzed to determine background concentrations).

The acceptability of the data relies on rigorous, standardized laboratory QA/QC measures. In part, these measures include the use of clean techniques throughout the laboratory testing where feasible. A detailed description of laboratory-related clean techniques is provided in Appendix B. It is to your advantage to specify that the laboratories should use these techniques when performing toxicity tests and chemical analyses.

PERFORMING ANALYTICAL TESTS

Two types of tests will be required:

Toxicity tests-These tests measure the effects of a toxicant on a population of aquatic organisms

Chemical analyses-These tests measure the concentration of a toxicant or other chemical parameter.

Each type of test is described in more detail below.

Toxicity Tests

To develop a site-specific criterion, toxicity tests are performed to compare the toxicity of a metal in laboratory dilution water to the toxicity of the same metal in simulated downstream site water. In toxicity tests, the metal of concern is added in varying concentrations to the laboratory dilution water and the simulated downstream site water. Organisms are monitored to determine their response to the different concentrations of the metal in both tests. A complete description of the procedures used for toxicity tests is provided in Appendix D.

During at least one round of tests, toxicity must determined for two different test species. Guidance concerning the selection of species to be used in WER toxicity tests is provided in Appendix D. (If high TDS is a concern, species that are sensitive to elevated TDS should not be selected.) Oklahoma requires that WERs from four *acceptable* tests be used to calculate a final WER. To support the Oklahoma WER, two species are used to obtain at least four acceptable toxicity tests. The two species must be in different orders and should include a vertebrate and an invertebrate (U.S. EPA 1994a). One species can be used for up to three of the toxicity tests, but at least one of the four acceptable tests must be conducted on a second species. Acute toxicity tests should be performed in accordance with EPA guidance (U.S. EPA 1994a) and American Society for Testing and Materials (ASTM) guidance (ASTM 1993a,b,c,d,e,f). ASTM methods for toxicity testing are acceptable where those methods are supported by EPA protocols. Test methods or protocols that do not meet current EPA requirements are considered to be only *additional* supplemental information. Acute tests can be used to determine either an acute or chronic site-specific criterion, as discussed previously.

The WER toxicity tests results generally will be quantified using an LC_{50} endpoint, which represents the concentration of a toxicant that causes lethal effects in 50 percent of the test population within a specified period of time (e.g., 48 hours). The Oklahoma WER is calculated by dividing the endpoint of the laboratory dilution water by the endpoint of the simulated downstream site water. Based on the toxicity tests used to develop the default water quality criteria, the LC_{50} in laboratory water can generally be predicted within a reasonable range. However, the LC_{50} in simulated downstream site water is highly variable according to local water quality conditions. To determine the appropriate range of concentrations that will capture the LC_{50} given your site-specific conditions, a preliminary range-finding test is usually performed prior to the initiation of the first WER toxicity test. Your toxicity-testing laboratory can help you with this determination.

It is here that those entities performing **WERs for multiple metals should plan on additional testing for determination of synergistic or antagonistic effects**. This additional testing should be designed so as to determine the LC_{50} of the combination of all metals being tested. The first tests will likely utilize the maximum concentrations being sought through the site-specific criteria development process but a much lower concentration of some (if not all) of the constituent metals will likely be the final request. These tests must also follow the protocol for bracketing the LC_{50} as is discussed in detail in Appendix D of this document.

Chemical Tests

The following analyses will need to be conducted:

- Measure the concentrations of total recoverable and dissolved metal(s) of concern in the upstream water and effluent, as well as in the simulated downstream site water and laboratory dilution water used in each of the WER toxicity tests
- Measure conventional water quality parameters (e.g., hardness, pH, TDS including ion speciation) in the upstream water and effluent, as well as in a control sample from both the simulated downstream site water and laboratory dilution water used in each of the WER toxicity tests.

Water quality criteria for metals are generally set at ppb, or μg/L, levels, so defensible trace metal analysis is critical to the site-specific approach. This is another reason that clean techniques are so important to the process. The cost of trace metal analysis can be expensive; however, and you should not try to achieve detection limits that are lower than required to provide useful information. For metals analysis, a useful detection limit is approximately one-tenth of the existing criterion (U.S. EPA 1994a). All metals analyses should be conducted according to standard analytical EPA methods (U.S. EPA 1996a), as long as the methods meet the required detection limits. Your analytical laboratory can help you with these decisions.

Conventional water quality parameters are measured in the upstream water, the effluent, the simulated downstream site water, and the laboratory dilution water to provide a quality control check. Any other water quality characteristics, such as TDS and conductivity, that are monitored in the effluent routinely by the permittee and reported in the routine discharge monitoring report should also be measured in the upstream water, the effluent, and control samples from the simulated downstream site water and laboratory dilution water. These measurements provide information concerning the representativeness of the samples and the variability of the upstream water and effluent. If these parameters do not remain consistent between sampling events, one or more tests may be flawed and therefore unacceptable. Parameters that must be measured as part of site-specific criterion testing are summarized in Table 1 (page 33).

INTERPRETING TEST RESULTS

After data have been generated, you need to determine whether those data can be used to develop a site-specific criterion. To answer this question, you need to evaluate the toxicity tests and the chemical analyses. First, you should review the quality of the chemical analytical data. Someone outside of the analytical laboratory should validate the data according to standard methods (U.S. EPA 1994b,c). This includes evaluating the final data against the data quality objectives (e.g., accuracy, completeness, representativeness, comparability) established in the work plan.

Next, you should calculate the LC_{50} and WER values for each test. Both total and dissolved WERs can be calculated for each test. The two tests actually provide a quality control check and allow the maximum flexibility to permittees in determining an appropriate WER. The Oklahoma WER for each acceptable test is calculated as follows:

> Total WER = total LC_{50} laboratory/total LC_{50} simulated downstream
> dissolved WER = dissolved LC_{50} laboratory/dissolved LC_{50} simulated downstream

Results to be used in the calculations should also be based on the time-weighted average of the measured metal concentrations. Probit analysis must be used to calculate the LC_{50} value for both tests, unless the probit model is rejected by the goodness of fit test in one or more of the side-by-side tests. If probit analysis cannot be used, computational interpolation may be used instead. There are a number of software packages, including ToxStat® or ToxCalc®, available to assist in calculating LC_{50} values for toxicity tests using both probit and computational interpolation. Typically, the laboratory that performs the toxicity tests or an in-house statistician should complete the statistical analysis.

Next, you should review the acceptability criteria for toxicity tests listed in Appendix D. Do your tests meet all of these requirements? If not, you may not be able to use the tests in the determination of a WER. Finally, you should compare the results of your WERs with published literature values and other laboratory studies. Detailed criteria for this comparison are provided in Appendix D. If you decide not to use the results of a particular test, **you must provide the data set and explain the rationale of why you rejected the test in your final report to the agencies**.

As a general guideline, play it safe. All of your laboratory QA/QC backup materials will be required with the final report. So, if you have any questions about the usability of the data, include the test(s) unless you have strong technical arguments to support rejecting the data.

HOW IS THE FINAL SITE-SPECIFIC CRITERION DEVELOPED?

This section includes a description of the important calculations and the requirements for the final report. When submitting the report and requesting the site-specific criterion modification, it is important to remember that the ultimate goal of this exercise should be to remove or lessen the economic impact of an over-protective criterion on a discharger. The three options described herein provide the opportunity to do this but, in some cases, can provide more relief than is necessary. Dischargers are strongly encouraged to recognize this fact and **use only that amount of relief that is necessary to remove "reasonable potential" or allow that wasteload that cannot be lessened**. Creating an adverse downstream impact is unacceptable.

PERFORMING THE APPROPRIATE CALCULATIONS

You can calculate important information using data that are acceptable. You should include all relevant calculations and backup data as part of your final report to the agencies. The appropriate calculations will depend on the option you pursue:

- **Option One: Use of a WER-**You will need to calculate a final WER (FWER) and the background concentration (if applicable).
- **Option Two: Use of a dissolved translator-**You will need to calculate a dissolved translator (*f*) and the background concentration (if applicable).
- **Option Three: Use of a WER and dissolved translator-**You will need to calculate a FWER and a dissolved translator (*f*) to obtain a final criterion translator (T). Background concentrations will also need to be calculated (if applicable).

The relationships of the various calculated values are shown in Figure 1 (page 30), which shows how the required elements fit together. Worksheets used to calculate important values are provided in Appendix E. Details of the calculations are provided below.

Preliminary Calculations

Preliminary calculations include determining a FWER, a dissolved translator, a criterion translator, and the background calculation (as appropriate). To prevent round-off errors, at least 4 significant digits must be retained in all preliminary calculations. An explanation of each preliminary calculation is presented below.

Calculating a FWER (option 1)

If the toxicity test data are acceptable, the next step is to calculate the FWER. At least four acceptable WERs must be used in the calculation of the FWER. Of the four acceptable tests, one must be from a test that was conducted on a second species. Again, if you decide not to use the results of a particular test, you must explain the rationale of why you rejected the test in your final report to the agencies. To determine the FWER, a geometric mean of the four or more acceptable WER values is calculated as follows:

$$FWER = e\ [\ (\sum Ln\ (WER_i))/n\ ]$$

where:

e = exponent
Ln = natural log
n = number of acceptable samples (≥ 4)
WER_i = acceptable WER value from *i*th test.

A dissolved FWER is calculated from at least four acceptable dissolved WER values, and a total FWER is calculated from at least four acceptable total WER values. Whether you use the total or dissolved FWER to develop a site-specific criterion depends on the option you pursue (see Figure 1, page 32). A total WER will be used under Option One, and a dissolved WER will be used under Option Three.

Calculating a Final Dissolved Translator (*f*) (option 2)

If the chemical analyses in the simulated downstream site water are acceptable for all 10 rounds of sampling, you can calculate the final dissolved translator (*f*) as follows:

$$f = e\ [\ \sum Ln\ (f_i)/n\ ]$$

where:

e = exponent
Ln = natural log
n = number of acceptable samples (≥ 10)
f_i = Cd_i / Ct_i
Cd_i = dissolved metal concentration in the *i*th simulated downstream sample
Ct_i = total recoverable metal concentration in the *i*th simulated downstream sample.

The dissolved translator will be used to express the statewide criterion as a site-specific total recoverable criterion under Option Two (see Figure 1, page 30).

Calculating a Final Criterion Translator (T) (option 3)

To determine a final criterion translator (T), you simply multiply the dissolved FWER by the dissolved translator (*f*):

$$T = FWER \times f$$

The criterion translator will be used to express the statewide criterion as a site-specific total recoverable criterion under Option Three (see Figure 1, page 30).

Calculating a Background Concentration

If adequate background data are not available, a background concentration must be calculated. Chemical analyses of the total recoverable metal concentration in the upstream water must be acceptable from 12 monthly rounds of sampling to calculate the background concentration. Background is calculated as an arithmetic average (ODEQ 1994) as follows:

$$\text{background} = [\ \sum (C\ tu_i)\ ]/n$$

where:

n = number of acceptable samples (≥ 12)

$C\ tu_i$ = total recoverable metal concentration in the *i*th upstream sample.

Criterion Calculations

Formal site-specific calculations will be made by OWRB; however, this section provides the steps for the calculations so that you have a preliminary indication of the final site-specific criterion. The criterion calculations are performed based on the option you pursue (see Figure 1, page 30):

- **Option One: Use of a WER-**The statewide total recoverable criterion is divided by the Oklahoma-defined total FWER to obtain a site-specific total recoverable criterion
- **Option Two: Use of a dissolved translator-**The statewide dissolved criterion is divided by the dissolved translator (*f*) to obtain a site-specific total recoverable criterion
- **Option Three: Use of a WER and dissolved translator-**The statewide dissolved criterion is divided by the criterion translator (T), which is the product of the Oklahoma-defined dissolved FWER and *f*, to obtain a site-specific total recoverable criterion.

An example of criterion calculations is provided in Appendix E.

SUBMITTING THE FINAL REPORT

After the preliminary calculations are performed, you should submit your final study report to the agencies for approval. The requirements for the report are contained in Appendix F of this document. Again, it is important to include all of the required information so that the agencies can approve the use of the site-specific criterion.

After the report has been reviewed and approved by all appropriate agencies, ~~as~~, a site-specific criterion can be incorporated into the Water Quality Standards (WQS). After approval of the criterion by EPA during the normal WQS revision process, ODEQ can use the criterion, in conjunction with a newly established background concentration (if applicable) to establish revised discharge permit limits for your facility. Details of how permit limits are calculated are provided by ODEQ (1994).

In order for the new permit limit to be valid for and usable in the discharge permit, the new site-specific criterion must be submitted to the ODEQ (Oklahoma's point source permitting authority) and Oklahoma Water Quality Standards revision process. The final criterion requested must be presented to the public in a public forum where comment, both for and against, is accepted. After the revision process, the criterion is submitted to EPA for review and approval/disapproval as part of the NPDES program. The final report must contain all those elements described herein and satisfy the technical scrutiny of the state agencies as well as EPA.

WHERE TO GO FOR MORE INFORMATION

Additional information is available to assist you in making important technical decisions. This information is summarized below by topic.

DISCHARGE PERMIT CALCULATION PROCESS

ODEQ. 2000 (or most recent revision). Final continuing planning process. Oklahoma Department of Environmental Quality, Oklahoma City, OK.

Existing National Pollutant Discharge Elimination System permit for the subject facility. The existing permit and associated calculations can be an excellent resource for site-specific variables that may affect the success of the site-specific criterion.

Hutcheson, M.R. 1992. Wasteload allocation for a whole effluent toxicity to protect aquatic organisms. Water Resource Res. 28(11):2989-2992.

BACKGROUND ON WATER QUALITY CRITERIA

OWRB. 2001 (or most recent revision). Water quality standards (OAC Title 785:45). Oklahoma Water Resources Board, Oklahoma City, OK.

U.S. EPA. 1993. Water quality standards handbook. Second Edition. EPA 823-B-93-002. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

U.S. EPA. 1995. Updates to water quality criteria documents for the protection of aquatic life in ambient water. EPA-820-B-96-001. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

USGS: (http://water.usgs.gov/swr/OK). USGS water quality data for Oklahoma Rivers, U.S. Geological Survey, Oklahoma City, OK.

WATER EFFECT RATIOS

U.S. EPA. 1994. Interim guidance on determination and use of water-effect ratios for metals. EPA/823/B-94/001. U.S. Environmental Protection Agency, Washington, DC.

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DISSOLVED METALS CRITERIA

Prothro, M.G. 1993. Office of Water Policy and technical guidance on interpretation and implementation of aquatic life metals criteria. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

U.S. EPA. 1993. Office of Water policy and technical guidance on interpretation and implementation of aquatic life criteria. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

U.S. EPA. 1996. The metals translator: draft guidance for calculating a total recoverable permit limit from a dissolved criterion. EPA Guide5_6.1. U.S. Environmental Protection Agency, Exposure Assessment Branch, Washington, DC. (Oklahoma only uses an empirical ratio for this determination. However, this document can be consulted for additional information on partitioning coefficients that may be applied in different jurisdictions).

TOXICITY TESTING

ASTM. 1993. Guide for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. Standard E729. American Society for Testing and Materials, Philadelphia, PA.

ASTM. 1993. Guide for conducting acute toxicity tests on aqueous effluents with fishes, macroinvertebrates, and amphibians. Standard E1192. American Society for Testing and Materials, Philadelphia, PA.

U.S. EPA. 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fourth Edition. EPA/600/4-90/027F. U.S. Environmental Protection Agency, National Technical Information Service, Springfield, VA.

CHEMISTRY DATA EVALUATION

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U.S. EPA. 1991. Methods for the determination of metals in environmental samples. EPA-600/4-91-010. U.S. Environmental Protection Agency, National Technical Information Service, Springfield, VA.

U.S. EPA. 1994. USEPA contract laboratory program national functional guidelines for inorganic data review. EPA-540/R-94-013. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC.

U.S. EPA. 1994. USEPA contract laboratory program national functional guidelines for organic data review. EPA-540/R-94-012. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC.

U.S. EPA. 1996. Methods for chemical analysis of water and wastes. EPA-600/4-79-020. U.S. Environmental Protection Agency, National Technical Information, Springfield, VA.

REFERENCES

Note: References for the entire guidance document (including appendices) are contained in this section.

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ASTM. 1993a. Guide for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. Standard E729. American Society for Testing and Materials, Philadelphia, PA.

ASTM. 1993b. Guide for conducting static acute toxicity tests starting with embryos of four species of saltwater bivalve molluscs. Standard E724. American Society for Testing and Materials, Philadelphia, PA.

ASTM. 1993c. Guide for conducting renewal life-cycle toxicity tests with *Daphnia magna*. Standard E1193. American Society for Testing and Materials, Philadelphia, PA.

ASTM. 1993d. Guide for conducting early life-stage toxicity tests with fishes. Standard E1241. American Society for Testing and Materials, Philadelphia, PA.

ASTM. 1993e. Guide for conducting three-brood, renewal toxicity tests with *Ceriodaphnia dubia*. Standard E1295. American Society for Testing and Materials, Philadelphia, PA.

ASTM. 1993f. Guide for conducting acute toxicity tests on aqueous effluents with fishes, macroinvertebrates, and amphibians. Standard E1192. American Society for Testing and Materials, Philadelphia, PA.

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Byrd, J.T., and M.O. Andreae. 1986. Dissolved and particulate tin in North Atlantic seawater. Mar. Chem. 19:193B200.

Horowitz, A.J., K.A. Elrick, and M.R. Colberg. 1992. The effect of membrane filtration artifacts on dissolved trace element concentrations. Water Res. 26:753B763.

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Sakamoto-Arnold, C.M., A.K. Hanson, Jr., D.L. Huizenga, and D.R. Kester. 1987. Spatial and temporal variability of cadmium in Gulf Stream warm-core rings and associated waters. J. Mar. Res. 45:201B230.

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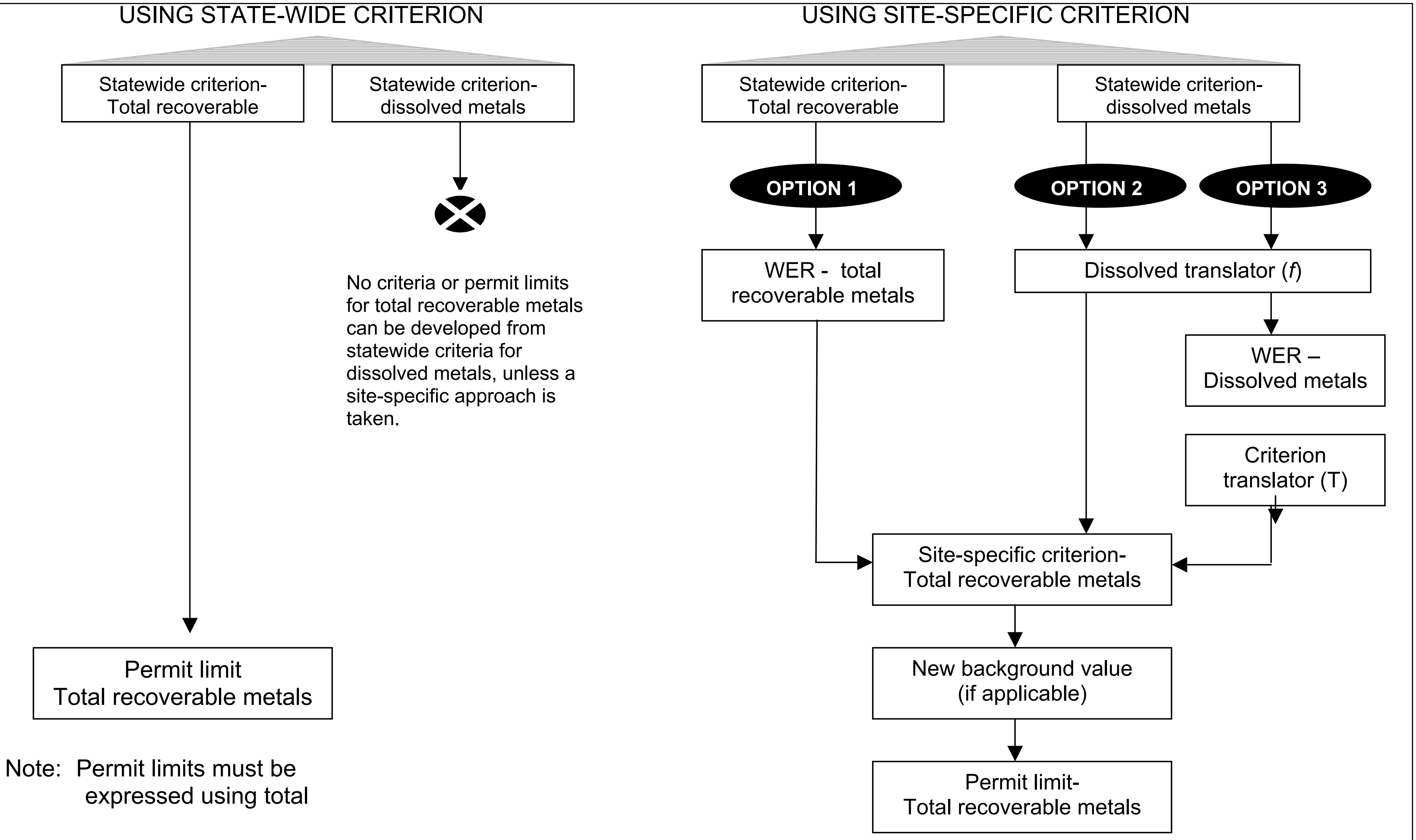

No criteria or permit limits for total recoverable metals can be developed from statewide criteria for dissolved metals, unless a site-specific approach is taken.

Note: Permit limits must be expressed using total

Figure 1: Options for deriving a permit limit.

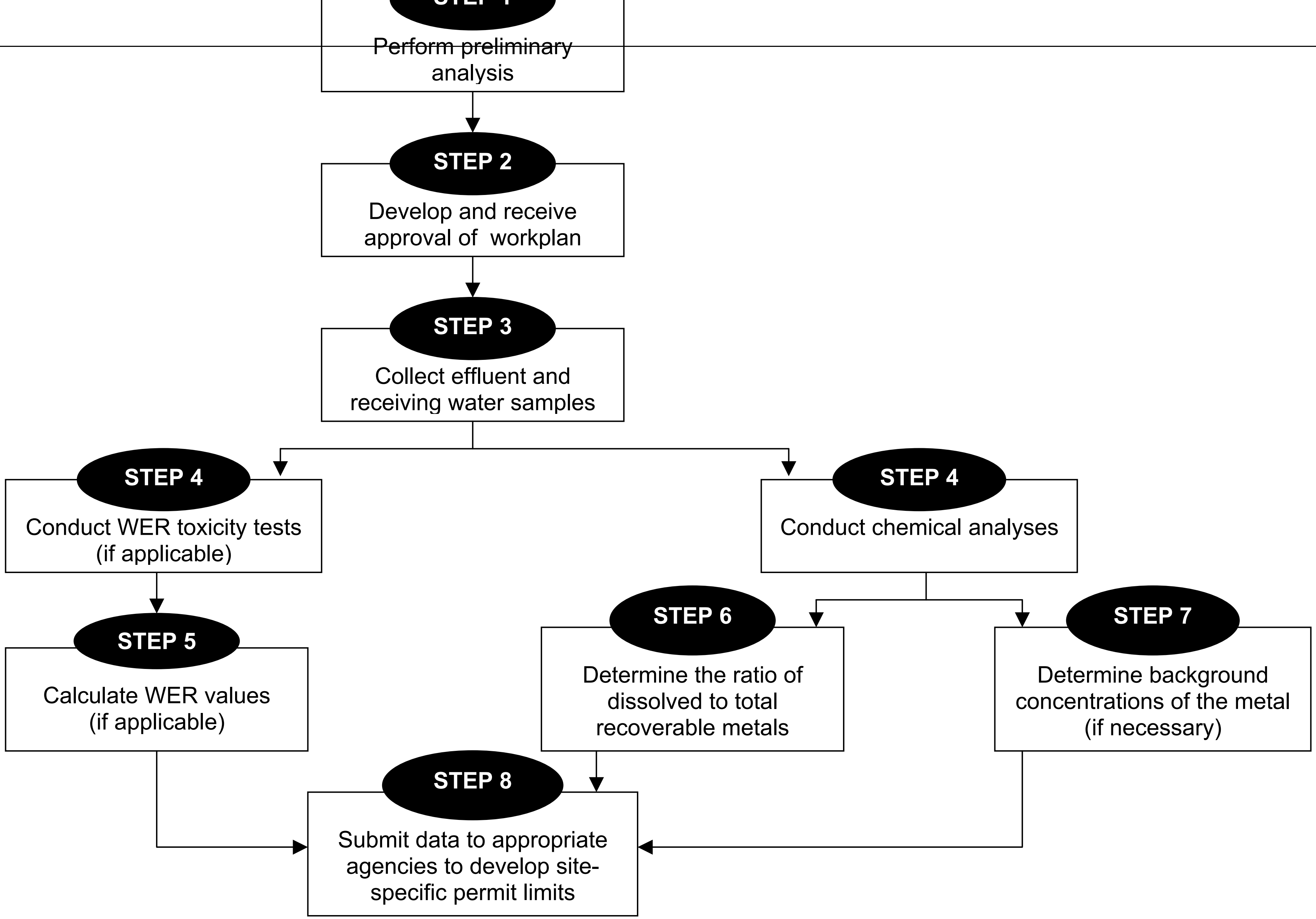

Figure 2: Generalized permit revision process.

TABLE 1. SUMMARY OF TYPES OF SAMPLES AND CHEMICAL ANALYSES REQUIRED TO SUPPORT A SITE-SPECIFIC CRITERION

			Simulated Downstream		Laboratory Dilution	
	Receiving Water	Effluent	Chemical Tests	Toxicity Tests	Chemical Tests	Toxicity Tests
Option One: Use of a WER	(a)					
Total recoverable metal	X	X	X	X	X	X
Dissolved metal	X	X	X	X	X	X
Hardness	X	X	X		X	
pH	X	X	X		X	
Alkalinity	X	X	X		X	
TSS	X	X	X		X	
TOC	X	X	X		X	
TDS (optional)	X	X	X		X	
Option Two: Use of a dissolved translator		(b)				
Total recoverable metal	X	X	X			
Dissolved metal	X	X	X			
Hardness	X	X				
pH	X	X				
Alkalinity	X	X				
TSS	X	X				
TOC	X	X				
TDS (optional)	X	X				
Option Three: Use of both a WER and a dissolved translator				(b)		
Total recoverable metal	X	X	X	X	X	X
Dissolved metal	X	X	X	X	X	X
Hardness	X	X	X		X	
pH	X	X	X		X	
Alkalinity	X	X	X		X	
TSS	X	X	X		X	
TOC	X	X	X		X	
TDS (optional)	X	X	X		X	
Background calculation (c)						
Total recoverable metal	X					

NOTE

TSS - total suspended solids
TDS - total dissolved solids
TOC - total organic carbon
WER - water effect ratio

a Includes at least three rounds of sampling from different seasons during periods that are representative of low stream flow events

b Includes at least 10 rounds of sampling during periods that are representative of low stream flow events.

c If required under any of the options, background sampling includes at least 12 rounds of sampling.

APPENDIX A
WORK PLAN REQUIREMENTS

A work plan should provide regulatory agencies with enough detail to evaluate the approach of your study and answer some fundamental questions prior to data collection. Although the organization of the work plan is flexible, the document will need to address all of the following elements:

- **Site History and Background Information-**The work plan should summarize the operating history and background information for the site. Background information should provide the rationale for conducting the study and should include available data regarding the receiving water, the effluent discharge, and the current operating permit. Data regarding the receiving water may be obtained from a number of sources, including the Oklahoma Water Resources Board (OWRB) and the U.S. Geological Survey (USGS). Historical and current discharger records generally provide available effluent data. Finally, a complete understanding of the current permit is essential to defining the proper study objectives.
- **Evaluation of Existing Data-**After the relevant data have been compiled, the data should be evaluated to determine what data gaps exist. It may be that existing data can help supplement data required as part of this approach. It may also be that existing data cannot be used to support a site-specific approach because the quality of the data may be uncertain. An example of this limitation would be historical water quality data collected from USGS gauging stations. Although it might be tempting to use these data to evaluate existing upstream background conditions because the historical records may be extensive, these data cannot be used as part of this process because the samples were probably not collected using recently established clean techniques. To determine the acceptability of existing data, ask whether the data could stand up to the standards specified in this guidance document. Discuss your existing data with the agencies as part of developing the work plan to determine the answer to this question.
- **Project Objectives and Strategy-**The project objectives should address the data gaps for the site. These objectives will be used to develop a strategy for obtaining appropriate site-specific data. A well-defined strategy will more likely result in representative data that will be approved by the agencies.

- **Field Sampling Plan-** Sample collection procedures should essentially consist of two tasks: effluent sampling and receiving water sampling. Figures showing all of the proposed sampling locations should be incorporated into the field-sampling component of the work plan. After the field procedures and locations have been described, the sampling plan should also address field sampling measurements, field documentation, sample handling and shipping, sample preservation (if any), and health and safety for field personnel. Standard U.S. Environmental Protection Agency (EPA) methods should be used for all of these procedures (see Appendix B). A section describing how clean techniques will be incorporated into field activities (see Appendix C) and a proposed field schedule should also be included in the work plan.
- **Laboratory Testing Methods-**This section should describe the proposed methods for conducting the toxicity and analytical chemistry tests, as well as identify the analytical laboratories that will be performing the work (these laboratories must be Oklahoma-certified, which can be verified by contacting the Oklahoma Department of Environmental Quality [ODEQ]). Samples should be tested using EPA or American Society for Testing and Materials (ASTM) methods, and complete protocols (which should be readily available from the testing laboratory) for the toxicity tests should be included. More information on choosing the appropriate test is contained in the *How Should Laboratory Tests Be Conducted?* section of the main text and in Appendix D.
- **Quality Assurance Project Plan-**A quality assurance project plan describes the quality assurance and quality control (QA/QC) procedures for all anticipated field and laboratories activities. This plan is important because it assures the agencies that appropriate QA/QC guidelines will be followed when performing all phases of the project. Without this assurance, the agencies cannot approve the site-specific criterion. Specifically, the data quality objectives for both toxicity tests (e.g., exposure regime, positive controls, endpoints) and chemical analyses (e.g., accuracy, completeness, representativeness, comparability) should be identified.
- **Schedule-**A project schedule, including a field sampling schedule, should be included in the work plan.

Most of the information needed to complete the work plan should be compiled during the preliminary analysis of the project. If well-written at the outset, the work plan can be used as the basis for the final report to the agencies (see Appendix F).

APPENDIX B
GUIDANCE ON THE USE OF CLEAN TECHNIQUES

GUIDANCE ON THE USE OF CLEAN TECHNIQUES

In a strict sense, the term "clean techniques" refers to techniques that reduce contamination and enable the accurate and precise measurement (in the parts per billion [ppb] range) of trace metals in surface waters. In a broader sense, the term also refers to related issues concerning detection limits, quality control, and quality assurance. The use of clean techniques for collecting, handling, storing, preparing, and analyzing samples is an important element of developing a site-specific criterion. This appendix discusses why clean techniques are important and provides specific recommendations for field sampling and laboratory analysis to ensure that clean techniques are used throughout the process. Much of the information used to develop this appendix is based on Appendix C of *Interim Guidance on Determination and Use of Water-Effect Ratios for Metals* (U.S. EPA 1994a).

BACKGROUND

Recent studies (Shiller and Boyle 1987; Windom et al. 1991) have raised questions concerning the quality of reported concentrations of trace metals in both fresh and salt (estuarine and marine) surface waters. The ranges of dissolved metals that are typical in surface waters of the United States that are away from the immediate influence of discharges (Bruland 1983; Shiller and Boyle 1985,1987; Trefry et al. 1986; Windom et al. 1991) are shown below:

Metal	Salt Water (µg/L)	Fresh Water (µg/L)
Cadmium	0.01-0.2	0.002-0.08
Copper	0.1-3.0	0.4-4.0
Lead	0.01-1.0	0.01-0.19
Nickel	0.3-5.0	1.0-2.0
Silver	0.005-0.2	not available
Zinc	0.1-15.0	0.03-5.0

U.S. EPA (1991, 1996a) has published analytical methods for monitoring metals in waters and wastewater, but these methods are inadequate for determining ambient concentrations of some metals in some surface waters. Accurate and precise measurement of these low concentrations requires appropriate attention to seven areas:

- Use of clean techniques while collecting, handling, storing, preparing, and analyzing samples to avoid contamination
- Use of analytical methods that have sufficiently low detection limits
- Avoidance of interference in the quantification (instrumental analysis) step
- Use of blanks to assess contamination

- Use of matrix spikes (sample spikes) and certified reference materials (CRMs) to assess interference and contamination
- Use of replicates to assess precision
- Use of certified standards.

Neither the "ultra-clean techniques" that might be necessary when trace analyses of mercury are performed nor safety in analytical laboratories are addressed in this appendix. Other documents should be consulted if one or both of these topics are of concern.

This appendix neither adds to nor subtracts from any regulatory requirement set forth in other U.S. Environmental Protection Agency (EPA) documents concerning analyses of metals. Although a water effects ratio (WER) can be acceptably determined without the use of clean techniques as long as the detection limits, accuracy, and precision are acceptable, any deviations from the use of clean techniques may jeopardize the acceptability of test data. No quality assurance and quality control (QA/QC) requirements beyond those that apply to measuring metals in effluents are necessary to determine WERs.

AVOIDING CONTAMINATION BY USE OF CLEAN TECHNIQUES

Measurement of trace metals in surface waters should take into account the potential for contamination during each step in the process. Ideally, a procedure for measuring concentrations of metals in surface water begins with the sampling step and continues through the quantification step. Regardless of the specific procedures used for collection, handling, storage, preparation (digestion, filtration, and/or extraction), and quantification (instrumental analysis), some general methods for controlling contamination should be applied. These methods are presented below.

Field Sampling Techniques

The first place that clean techniques should be used is during field sampling. The concentrations of many metals in ambient waters are typically very low, and collecting water samples that are representative of ambient conditions requires extreme care to prevent contamination during handling. Sampling personnel should be specifically trained on how to collect field samples, and the specific recommendations listed below should be followed:

- Prior to collecting the sample or handling the sampling equipment or sample bottles, a quick survey of the sampling area should be performed to identify sources of potential contamination to the sample (e.g., sources of dust, running engines, batteries). If there is an obvious source, it should be removed, cleaned, or isolated from the sample handling area.

- Powder-free (non-talc, class-100) latex, polyethylene, or polyvinyl chloride (PVC) gloves should be worn during all steps of sample collection. Talc seems to be a particular problem with zinc; gloves made with talc cannot be decontaminated sufficiently. Gloves should only contact surfaces that are metal-free. If there is any question as to whether the gloves are contaminated, change the gloves.
- At all times, sampling personnel should avoid touching surfaces that are not known to be clean. While sampling, samplers should be conscious of the potential chains of contamination that can occur. A chain of contamination could involve handling an object that touched another object that touched something contaminated. Unless it is known whether an object is clean (i.e., was cleaned appropriately and isolated from contaminants from the time of cleaning until the time of use), it should be assumed that it is dirty. Even contact with items in your pocket (change, keys, etc.) can cause contamination.
- The water used to prepare acidic cleaning solutions and to rinse sample containers and sampling apparatus may be prepared by distillation, de-ionization, or reverse osmosis and should be certified to be metal-free.
- The work area should be cleaned (i.e., washed and wiped dry with lint-free, class-100 wipes) frequently to remove possible contamination.
- Sample containers should never be left open to the atmosphere. Minimizing the time between cleaning and using will help minimize contamination. Similarly, reducing the time between collection and analysis of samples will reduce the chance of contamination. Sample handling should also be minimized.
- Separate sets of sample containers and sampling apparatus should be dedicated for different kinds of samples (e.g., surface water samples, effluent samples).
- Acid-cleaned plastic, such as high-density polyethylene (HDPE), low-density polyethylene (LDPE), or a fluoroplastic, should be the only material that ever contacts a sample. Sample container caps should have liners that are appropriate for metals sampling. HDPE and LDPE might not be acceptable for mercury. Even if acidified, samples and standards containing silver should be in amber containers.
- After collection, all handling of samples in the field that will expose the sample to air should be performed in a portable class-100 clean bench or glove box.
- Samples should be acidified (after filtration if dissolved metal is to be measured) to a pH of less than 2 for all metals, except mercury, which should have a pH of less than 1. This procedure ensures that the samples are properly preserved. Acidification should be done in a clean room or bench, so it might be desirable to acidify samples in a laboratory rather than in the field. (An exception would be if mercury is an analyte of interest, in which case field

acidification may prevent significant losses.) If samples are acidified in the field, metal-free acid can be transported in plastic bottles and poured into a plastic container from which acid can be removed and added to samples using plastic pipettes. Alternatively, plastic automatic dispensers can be used.

- Things such as probes and thermometers should not be put into samples that are to be analyzed for metals. In particular, pH electrodes and mercury-in-glass thermometers should not be used if mercury is to be measured. If pH is measured, it should be done on a separate aliquot.
- Samples should be stored in the dark, preferably between 0^oC and 4^oC, with no air space in the sample container.

Laboratory Techniques

Once samples have been collected, the next place that clean techniques should be used is during laboratory testing. The specific recommendations listed below should be followed:

- Powder-free (non-talc, class-100) latex, polyethylene, or PVC gloves should be worn during all steps from sample collection to analysis. Talc seems to be a particular problem with zinc; gloves made with talc cannot be decontaminated sufficiently. Gloves should only contact surfaces that are metal-free. If there is any question as to whether the gloves are contaminated, change the gloves.
- The acid used to acidify samples for preservation and digestion and to acidify water for final cleaning of labware, sampling apparatus, and sample containers should be metal-free. The quality of the acid used should be better than reagent-grade. Each lot of acid should be analyzed for the metal(s) of interest before use.
- The water used to prepare acidic cleaning solutions and to rinse labware, sample containers, and sampling apparatus may be prepared by distillation, deionization, or reverse osmosis and should be certified to be metal-free.
- The work area, including bench tops and hoods, should be cleaned (i.e., washed and wiped dry with lint-free, class-100 wipes) frequently to remove possible contamination.
- All handling of samples in the laboratory, including filtering and analysis, should be performed in a class-100 clean bench or a glove box fed by particle-free air or nitrogen. Ideally, the clean bench or glove box should be located within a class-100 clean room.
- Labware, reagents, sampling apparatus, and sample containers should never be left open to the atmosphere. They should be stored in a class-100 bench, covered with plastic wrap, stored in a plastic box, or turned upside down on a clean surface. Minimizing the time between cleaning and using will help minimize contamination. All samples should also be analyzed as soon as possible after collection to minimize contamination.

- Separate sets of sample containers, labware, and sampling apparatus should be dedicated for different kinds of samples (e.g., surface water samples, effluent samples).

- To avoid contamination of clean rooms, samples that contain very high concentrations of metals and do not require use of clean techniques should not be brought into clean rooms.

- Acid-cleaned plastic, such as HDPE, LDPE, or a fluoroplastic, should be the only material that ever contacts a sample, except possibly during digestion for the total recoverable measurement. HDPE and LDPE might not be acceptable for mercury. Even if acidified, samples and standards containing silver should be in amber containers.

- All labware, sample containers, and sampling apparatus should be acid-cleaned before use or reuse as follows:

- Sample containers, sampling apparatus, tubing, membrane filters, filter assemblies, and other labware should be soaked in acid until metal-free. The amount of cleaning necessary might depend on the amount of contamination and the length of time the item will be in contact with samples. For example, if an acidified sample will be stored in a sample container for 3 weeks, the container should have been soaked in an acidified metal-free solution for at least 3 weeks.

- It might be desirable to perform initial cleaning, for which reagent-grade acid may be used, before the items are taken into a clean room. For most metals, items should be either soaked in 10 percent concentrated nitric acid at 50°C for at least 1 hour, or soaked in 50 percent concentrated nitric acid at room temperature for at least 2 days. For arsenic and mercury, soaking for up to 2 weeks at 50°C in 10 percent concentrated nitric acid might be required. For plastics, such as polycarbonate and possibly HDPE and LDPE, that might be damaged by strong nitric acid, soaking in 10 percent concentrated hydrochloric acid, either in place of or before soaking in a nitric acid solution, might be desirable.

- Chromic acid should not be used to clean items that will be used in analysis of metals.

- Final soaking and cleaning of sample containers, labware, and sampling apparatus should be performed in a class-100 clean room using metal-free acid and distilled, de-ionized, or reverse osmosis-purified water. The solution in the acid bath should be analyzed periodically to demonstrate that it is metal-free.

- Labware, sampling apparatus and sample containers should be stored appropriately after cleaning as follows:

- After the labware and sampling apparatus are cleaned, they may be stored in a clean room in a weak acid bath prepared using metal-free acid and water. Before use, the items should be rinsed at least three times with metal-free water. After the final rinse, the items should be moved immediately, with the open end pointed down, to a class-100 clean bench. Items may be dried on a class-100 clean bench, but they should not be dried in an oven or with laboratory towels. The sampling apparatus should be assembled in a class-100 clean room or bench and double-bagged in metal-free, polyethylene, zip-type bags for transport to the field; new bags are usually metal-free.

- After sample containers are cleaned, they should be filled with metal-free water that has been acidified to a pH of 2 with metal-free nitric acid (about 0.5 mL/L) for storage until use.
- Labware, sampling apparatus, and sample containers should be rinsed as follows with sample as necessary to prevent bias of analytical results because acid-cleaned plastic will sorb some metals from un-acidified solutions:
- Because samples for measuring dissolved metals are not acidified until after filtration, all sampling apparatus, sample containers, labware, filter holders, membrane filters, and other equipment that contact the sample before or during filtration should be rinsed with a portion of the sample. That portion should then be discarded.
- For the total recoverable measurement, labware and other equipment that contact the sample only before it is acidified should be rinsed with sample, whereas items that contact the sample after it is acidified should not be rinsed. For example, the sampling apparatus should be rinsed because the sample will not be acidified until it is in a sample container, but the sample container should not be rinsed if the sample will be acidified in the sample container.
- If the total recoverable and dissolved measurements are to be performed on the same sample (rather than on two samples obtained at the same time and place), all the apparatus and labware, including the sample container, should be rinsed before the sample is placed in the sample container; then an un-acidified aliquot should be removed for the total recoverable measurement and an un-acidified aliquot should be removed for the dissolved measurement.
- Samples should be acidified (after filtration if dissolved metals are to be measured) to a pH of less than 2 for all metals, except mercury, which should have a pH of less than 1. Acidification should be done in a clean room or bench.
- Things such as probes and thermometers should not be put into samples that are to be analyzed for metals. In particular, pH electrodes and mercury-in-glass thermometers should not be used if mercury is to be measured. If pH is measured, it should be done on a separate aliquot.
- Sample handling should be minimized. For example, instead of pouring a sample into a graduated cylinder to measure the volume, the sample can be weighed after being poured into a tared container.
- Each reagent used should be verified to be metal-free. If metal-free reagents are not commercially available, removal of metals will probably be necessary.

- For the total recoverable measurement, samples should be digested in a class-100 bench, not in a metallic hood. If feasible, digestion should be done in the sample container by acidification and heating (using only those sample containers that can withstand such rigorous digestion procedures).
- Samples should be stored in the dark, preferably between 0EC and 4EC, with no air space in the sample container. The elapsed time between collection and analysis of samples should be minimized.

The following subsections discuss ways to lower detection limits, increase accuracy, and/or increase precision, including increasing the sensitivity of the analytical methods, decreasing contamination, and decreasing interference.

Achieving Low Detection Limits

Extraction of the metal from the sample can be extremely useful if it simultaneously concentrates the metal and eliminates potential matrix interferences. For example, ammonium l-pyrrolidine-dithiocarbamute and/or diethyl-ammonium diethyldithio-carbamate can extract cadmium, copper, lead, nickel, and zinc (Bruland et al. 1979; Nriagu et al. 1993). The detection limit should be less than 10 percent of the lowest concentration that is to be measured.

Avoiding Interferences

Potential interferences should be assessed for the specific instrumental analysis technique used and for each metal to be measured. If direct analysis is used, the salt present in high-salinity saltwater samples is likely to cause interference in most instrumental techniques. As stated above, extraction of the metal from the sample is particularly useful because it simultaneously concentrates the metal and eliminates potential matrix interferences.

Using Blanks to Assess Contamination

A laboratory (also known as a procedural or method) blank consists of filling a sample container with analyzed metal-free water and processing (e.g., filtering, acidifying) the water through the laboratory procedure in exactly the same way as a sample. A laboratory blank should be included in each set of 10 or fewer samples to check for contamination in the laboratory and should contain less than 10 percent of the lowest concentration that is to be measured. Separate laboratory blanks should be processed for the total recoverable and dissolved measurements, if both measurements are performed.

A field rinsate blank consists of filling a sample container with analyzed metal-free water in the laboratory, taking the container to the site, processing the water through the field equipment used, collecting the water in a sample container, and acidifying the water the same as a field sample. A field blank should be processed for each sampling trip. Separate field blanks should be processed for the total recoverable and dissolved measurements, if filtering is performed at the site. Field blanks should be processed in the laboratory the same as laboratory blanks. Additional information regarding quality control samples is included in Appendix C.

Assessing Accuracy

A calibration curve should be determined for each analytical run, and the calibration should be checked about every tenth sample. Calibration solutions should be traceable back to a certified standard from EPA or the National Institute of Science and Technology (NIST). A blind standard or a blind calibration solution should be included in each group of about 20 samples.

At least one of the following should also be included in each group of about 20 samples:

- A matrix spike (also known as a spiked sample or the method of known additions).
- A CRM, if one is available in a matrix that closely approximates that of the samples. Values obtained for the CRM should be within the published values. The concentrations in blind standards and solutions, spikes, and CRMs should not be more-than 5 times the median concentration expected to be present in the samples.

Assessing Precision

A sampling replicate should be included with each set of samples collected at each sampling location. The volume of all samples should be large enough to ensure a replicate analysis of at least 1 sample along with each group of about 10 samples.

Special Considerations Concerning Dissolved Metals Measurements

Whereas total recoverable measurements are especially subject to contamination during digestion, dissolved measurements are subject to both loss and contamination during filtration. Because acid-cleaned plastic sorbs metals from un-acidified solutions and because samples for the dissolved measurement are not acidified before filtration, all sampling apparatus, sample containers, labware, filter holders, and membrane filters that contact the sample before or during filtration should be conditioned by rinsing with a portion of the sample and discarding that portion.

Filtration should be performed using acid-cleaned plastic filter holders and acid-cleaned membrane filters. Samples should not be filtered through glass fiber filters, even if the filters have been cleaned with acid. If positive-pressure filtration is used, the air or gas should be passed through a 0.2-*F*m in-line filter; if vacuum filtration is used, it should be performed on a class-100 bench. Plastic filter holders should be rinsed and/or dipped between filtration, but they do not have to be soaked between filtration if all the samples contain about the same concentrations of metal. It is best to filter samples from low to high concentrations. A membrane filter should not be used for more than one filtration. After each filtration, the membrane filter should be removed and discarded, and the filter holder should be either rinsed with metal-free water or dilute acid and dipped in a metal-free acid bath or rinsed at least twice with metal-free dilute acid; finally, the filter holder should be rinsed at least twice with metal-free water. For each sample to be filtered, the filter holder and membrane filter should be conditioned with the sample (i.e., an initial portion of the sample should be filtered and discarded).

The accuracy and precision of the dissolved measurement should be assessed periodically. A large volume of a buffered solution (such as aerated 0.05 N sodium bicarbonate for analyses in fresh water and a combination of sodium bicarbonate and sodium chloride for analyses in salt water) should be spiked so that the concentration of the metal of interest is in the range of the low concentrations that are to be measured. Sufficient samples should be taken alternately for 1) acidification in the same way as after filtration in the dissolved method and 2) filtration and acidification using the procedures specified in the dissolved method, until 10 samples have been processed in each way. The concentrations of metals in each of the 20 samples should then be determined using the same analytical procedure. The means of the two groups of 10 measurements should be within 10 percent, and the coefficient of variation for each group of ten should be less than 20 percent. Any values deleted as outliers should be acknowledged.

Reporting Results

To document the quality of the data, a description of the blanks, spikes, CRMs, replicates, and standards that were run; the number run; and the results obtained should be reported. If potential outliers are present, the analysis may be verified and repeated, including duplicating accuracy and precision tests. All values deleted as outliers should be acknowledged.

ADDITIONAL INFORMATION

Additional information that might be useful in conducting clean techniques can be found in Patterson and Settle (1976), Zief and Mitchell (1976), Bruland et al. (1979), Moody and Beary (1982), Moody (1982), Bruland (1983), Adeloju and Bond (1985), Berman and Yeats (1985), Byrd and Andreae (1986), Taylor (1987), Sakamoto-Arnold (1987), Tramontano et al. (1987), Puls and Barcelona (1989), Windom et al. (1991), U.S. EPA (1992a), Horowitz et al. (1992), and Nriagu et al. (1993).

APPENDIX C
PROCEDURES FOR COLLECTING STUDY SAMPLES

PROCEDURES FOR COLLECTING STUDY SAMPLES

Procedures for collecting receiving water and effluent samples can have a great impact on the quality of the study. Rigorous, standardized procedures should be followed for each sampling event. This appendix provides a generalized description of sample collection techniques. If a particular study requires tasks that are not presented here, common sense should be used in identifying the procedures that will provide you with representative, high quality samples. This appendix provides information on the following topics:

- Measuring flow
- Collecting samples
- Handling samples
- Documenting field efforts.

MEASURING FLOW

The flow rate of the receiving water for the date of sample collection must be documented. If a U.S. Geological Survey (USGS) stream gauge is located within 2 miles either upstream or downstream of the sampling location, you can use data recorded daily from this gauge to fulfill this requirement. Statewide information can be accessed by calling the USGS office in Oklahoma City (405-853-7570) or via the Internet (http://water.usgs.gov/swr/OK). If no stream gauge is available, you must measure the flow rate of the receiving water as part of sample collection.

Streamflow measurements of shallow and relatively slow-moving streams can be accomplished by wading into the stream. Two persons are required to collect the streamflow measurement. One person, identified as the sampler, works in the water, setting up the transect tape and taking flow measurements. The second person, the data recorder, remains on shore to record the data called out by the sampler.

Flow (velocity) meters are used to measure the velocity along a selected stream transect. The transect should have a uniform depth and velocity. An area with still or reverse currents, such as eddies behind boulders or areas with fast chutes, should be avoided. Measurements are to be taken using a top-setting flow rod and portable flow meter or other acceptable method detailed in the workplan. Instantaneous cross sectional flows may be taken at various fixed-distance intervals depending upon overall stream width but no less than 15 fixed-distance points. This means using the same interval for the entire width of the stream. Utilizing instantaneous velocity (feet/second), distance from the shore and depth at each point, a volume may be calculated in cubic feet per second (cfs). This most effectively accomplished by using a tape measure stretched and anchored across the stream.

Results of the distance, depth and velocity measurements are then inserted into the following formula.

$$D = \sum_{i=1}^{n} (w_i - w_{i+1}) \frac{v_1 + v_{i+1}}{2} \frac{d_i + d_{i+1}}{2}$$

where:

n = the total number of individual sections

w_i = horizontal distance from initial point

d_i = water depth at location i

v_i = measured velocity at location i

For larger streams and rivers where high discharges are encountered, flow measurements can be taken from a bridge or boat. Bridge measurements are taken from the deck of a bridge, and boat measurements are taken from a lightweight boat guided across the current by fixed cable lines anchored to the shore. These sampling procedures for taking flow and depth measurements are similar to the wading technique, in that depth and velocity readings must be taken at points that are perpendicular to the shoreline. Care must be taken when conducting flow measurements from a bridge to avoid disruption of flow streamlines caused by piers. If flow is not perpendicular to the bridge, note or sketch the angle of deviation.

COLLECTING SAMPLES

Receiving water and effluent samples should be collected from the same general time period for each sampling event. When receiving water and effluent samples are collected for the determination of water effect ratios (WERs), samples should be collected in three different seasons. Quality control samples should also be collected during each event. Each type of sample is described in more detail below.

Receiving Water Samples

A receiving water sample will be collected upstream in an area unaffected by the discharge during each sampling event. The distance upstream from the discharge should be as small as possible, while still ensuring that the discharge will not bias the sampling location. The sampling location should remain consistent for all sampling events and should be accessible and safe for field personnel.

Upstream samples must be collected during low flow events so that they are not unduly affected by recent runoff events (or other erosion or re-suspension events). Samples should be collected when river conditions are as close as possible to the 7Q2 low flow values of the river, if available. If 7Q2 values are not available, sampling should be targeted toward extreme low flow conditions. USGS provides daily updates on stream conditions throughout Oklahoma. Statewide information can be accessed by calling the USGS office in Oklahoma City or via the Internet, as described above. Another way to ensure that samples are representative of low flow conditions is to wait at least 72 hours after the end of a storm event to conduct any sampling. This period allows the river to equilibrate back to conditions that are more representative of normal stream flow. If the flow rate is significantly different that the 7Q2 low flow value, sampling should be postponed until this condition can be met.

Water in a stream or river has some inherent variability because of differences in channel morphology and water flow. It is recommended that your sampling techniques attempt to capture this variability, to the extent possible, by collecting samples at various depths and points across the stream. To accomplish this goal, discrete grab samples could be collected across the width and throughout the depth of the river segment, and then be composited. Samples could also be collected using a pumping system. To verify that the samples collected are representative of site conditions, temperature, pH, dissolved oxygen, and conductivity should be measured in the receiving water body at the specific sampling location(s).

Effluent Samples

A sample will be collected from post-treatment discharge (i.e., effluent) during each sampling event. The effluent sample used in the determination of a WER must be representative of normal operating conditions. Selection of the date and time of effluent sampling should take into account the discharge pattern of the discharger. The intake for the sample collection should be placed in the effluent at a location that is downstream of the final treatment area. If there is more than one outfall in close proximity, effluent samples from the outfalls may be combined proportional to flow. The sampling location should remain consistent for all sampling events and should be accessible and safe for field personnel.

Effluent samples should be collected when plant flows are as close as possible to the values used to calculate the existing permit limits (i.e., highest averaged monthly flows or the design flow). Facility discharge records can be used to document the effluent flow for the dates of sample collection. The plant effluent will likely have some inherent variability based on the time of day and plant operations. Sampling techniques should attempt to capture this variability, to the extent possible. One 24-hour composite, time-weighted or flow-weighted sample should be collected during each sampling event. The effluent sample should be collected independently from the plants daily composite sample and should be collected at sufficiently small intervals so that variations in plant conditions, plant flow, and short-term effluent quality are captured. To accomplish this goal, discrete samples should be collected throughout the sampling period and then composited. At a minimum, the 24-hour composite sample should consist of at least 12 effluent portions collected at equal time intervals and combined proportional to flow. Sampling could also be accomplished using an automatic sampling system (e.g., ISCO programmable samplers). To verify that the samples collected are representative of site conditions, temperature and pH should be measured in the effluent stream.

Quality Control Samples

Field quality control samples are incorporated into the field-sampling program at predetermined frequencies to verify that field techniques are providing high-quality samples.

Samples that can be prepared by sampling personnel in the field and submitted to the analytical laboratory for quality control include:

- **Field Replicates -** A field replicate sample is a separately collected sample from the same station as either the receiving water or effluent sample. Replicates aid in determining precision by analyzing at least two samples from the same sample source. The entire process of sample collection is repeated.
- **Field Split -** A split receiving water or effluent sample is collected from the same sample composite as the primary sample. Splits aid in determining accuracy by analyzing subsamples from the same sample.
- **Field Blanks -** Field blanks will be used to identify possible contamination from the sampling environment. The field blank is a sample bottle prepared in the laboratory containing deionized water and preservative. This blank is carried to each sample site with the other filled sample bottles. The contents should be exposed to the atmosphere in close proximity to the sampling area during sample collection. The field blank should receive similar treatment as the other samples and should accompany the sample containers throughout sample collection, handling, and shipping.
- **External Contamination Blanks -** These blank samples isolate sources of external contamination of trace metals and other contaminants by testing the water used during decontamination (e.g., clean laboratory distilled/deionized water).
- **Equipment Rinsate Blanks -** Rinsate blanks are collected to ensure that decontamination activities are being performed correctly. After a piece of sampling equipment has been decontaminated, de-ionized water is poured over/through the decontaminated sampling tool and into a decontaminated stainless-steel bowl, and then poured into the appropriate sample container.

The number, types, and frequency of field quality control samples will generally depend on the objectives for the sampling event.

HANDLING SAMPLES

Receiving water and effluent samples must be collected, transported, handled, and stored as recommended by U.S. EPA (1993a). These methods are described in 40 CFR Part 136 and U.S. EPA (1996a). Chain-of-custody procedures (U.S. EPA 1993a) should be used for all receiving water, effluent, and quality control samples, especially if the data might be involved in a legal proceeding.

If acute and/or chronic toxicity tests are to be conducted with daphnids and if the receiving water sample contains predators, the site water must be filtered through a 37-μm sieve or screen to remove predators. This filtering should occur at the testing laboratory. It is also recommended that field samples be combined at the laboratory to achieve the simulated downstream water sample. Finally, sample volumes collected for toxicity tests should *never* be acidified. All associated sample containers should be rinsed with a portion of the sample or receiving water, not acid, prior to collection.

Samples must be stored at 0^{o}C to 4^{o}C in the dark with no air space in the sample container. Toxicity tests must begin within 36 hours after effluent and/or receiving water samples are collected. In some cases, tests may be begun more than 36 hours after the collection of the samples if it would require an inordinate amount of resources to transport the samples to the laboratory and begin the tests within 36 hours. Any exceedances of the 36-hour time limit should be minimal and only occur in unusual circumstances. The end of sample collection is defined as the end of the 24-hour compositing period for the effluent samples.

DOCUMENTING FIELD EFFORTS

Proper record-keeping and chain-of-custody procedures should be used throughout each sampling event. Field forms that could be used to document sampling activities and measurements include field log forms, field logbooks, and chain-of-custody forms. In addition, any changes from the work plan should be documented. These changes will need to be included in the final report submitted to the agencies.

APPENDIX D
PROCEDURES FOR CONDUCTING TOXICITY TESTS

INTRODUCTION

To support development of a site-specific criterion, toxicity tests must be conducted under rigorous laboratory conditions. This appendix describes specific requirements of toxicity tests needed to calculate a water effect ratio (WER). Much of the information used to develop this appendix is based on *Interim Guidance on Determination and Use of Water-Effect Ratios for Metals* (U.S. EPA 1994a).

This appendix is divided into the following sections:

- **Before Tests are Performed̃**This section provides details on test selection, preliminary range-finding tests, dilution factors, and laboratory dilution water standards.
- **Conducting Tests-**This section provides information on acquiring organisms, preparing stock solutions, preparing the toxicity tests, and monitoring the tests. Detailed chemical monitoring requirements are also presented.
- **Calculating and Interpreting the Results-**This section presents specific criteria that are used to determine whether or not tests are acceptable, as well as information on calculating the endpoints for the tests.

Detailed guidance on toxicity tests is provided in U.S. EPA (1993a) and ASTM (1993a,b,c,d,e,f). Complete references are provided at the end of this appendix.

BEFORE TESTS ARE PERFORMED

Prior to performing WER toxicity tests, the project team will need to make a number of strategic decisions, including:

- What toxicity tests should be used?
- Do preliminary range-finding tests need to be performed?
- What dilution factor should be used for testing?
- What laboratory dilution water should be used in the tests?

The project team should work closely with the toxicity testing laboratory during this process. Strategic decisions should be discussed in the project work plan. These questions are evaluated in more detail below.

Test Selection

To answer the question of what toxicity tests should be used to determine the WER, you should evaluate the following three issues:

- Test species selection
- Test sensitivity
- Comparability.

Test Species Selection

In Oklahoma, acute toxicity tests conducted to support whole effluent toxicity (WET) testing generally use daphnids (e.g., *Daphnia pulex, Ceriodaphnia dubia*) or fathead minnows (*Pimephales promelas*). However, you can use species that are not normally used for WET testing or species that do not occur at the site because it might be difficult to determine which of the site-specific species are sensitive to the metal and are adaptable to laboratory conditions.

To support calculation of the Oklahoma WER, two species must be used to obtain at least four acceptable toxicity tests. Two species are used to provide information concerning the validity of the WER. The two species must be in different orders and should include a vertebrate and an invertebrate. Either species chosen can be used for the majority of the toxicity tests, but at least one of the four acceptable tests must be conducted on a second species. It may be appropriate to conduct the second test simultaneously with the first test because of the difference in species-specific responses.

Recommended specific acute toxicity tests are listed in Table D-1. There are advantages to using the recommended tests in Table D-1 because reliable sensitivity data are available. The predictable sensitivities help ensure that the endpoint in laboratory dilution water is appropriate, and aids in selecting metal concentrations to be used. Reliance on test organisms without EPA-published protocols and methods is not generally recommended.

Additional issues to consider in selecting the appropriate test species include the following:

- The relative sensitivities of test species vary substantially from metal to metal. The sensitivity of a species to a metal usually depends on both the life stage and the kind of test used.

- The test organisms (i.e., species and life stage) should be readily available throughout the testing period.
- A test in which the test organisms are not fed might give a different WER than a test in which the organisms are fed, just because of the presence of the food. This result might depend on the metal, the type and amount of food, and whether a total recoverable or dissolved WER is determined.

Test Sensitivity

The magnitude of an experimentally determined WER is likely to depend on the sensitivity of the toxicity test used. This relationship between the magnitude of the WER and the sensitivity of the toxicity test is due to the aqueous chemistry of metals and is not related to the test organisms or the type of test. Available data indicate that WERs determined with different tests do not differ greatly if the tests have about the same sensitivities. The data also support the generalization that less sensitive toxicity tests usually give larger WERs (as defined in Oklahoma) than more sensitive tests.

Comparability

For the determination of WERs, the most important aspect of toxicity tests is the quality of the laboratory dilution water because the dilution water is the most important difference between the two side-by-side tests from which the WER is calculated. Therefore, it is important to be able to judge the quality of the results in laboratory dilution water. To judge the quality, the test data should be compared with data reported in the literature for the same metal. This comparison should be reported in the final laboratory report.

Range-Finding Tests

Once the appropriate tests have been selected, the concentration of metal to be used in test solutions needs to be determined. Specifically, the effects of the metal on the selected organisms in site water should be known. If such data are not available, a range-finding test can be conducted to determine the concentrations that should be used to bracket the endpoint in the definitive test. The range-finding test will also provide information on whether the control survival will be acceptable.

If range-finding tests are necessary, you should conduct those tests before the first set of toxicity tests to obtain additional information concerning the effluent, dilution water, and organisms before each set of side-by-side tests begin. The range-finding test may be conducted in either of two ways:

- The test may be conducted using the effluent and receiving water samples that will be used in the first definitive test. In this case, the duration of the range-finding test should be as long as possible except that the definitive test must begin within 36 hours after the samples of effluent and receiving water were collected.
- The test may also be conducted using one set of samples of effluent and receiving water that are collected prior to the definitive tests. In this case, the range-finding test might give better results because it can last longer, but there is the possibility that the quality of the effluent and/or receiving water might change. Chemical analyses for hardness and pH might indicate whether any major changes occur between the range-finding test and the first definitive test.

Your toxicity testing laboratory can help you with this evaluation.

Dilution Factors

The dilution factor controls the number of treatments required to determine the endpoint for the test. A dilution factor of 0.75 means that second highest total recoverable metal concentration is 75 percent of the highest concentration, and so on until the control concentration is reached. Use of concentrations that are close together will reduce the uncertainty in the WER but will require more test concentrations to cover a range of potentially toxic concentrations. The selected endpoints (e.g., EC_{50}) must be obtained in both dilution waters (i.e., simulated downstream site water and laboratory dilution water) whenever a set of side-by-side tests are conducted. In Oklahoma, the dilution factor for total recoverable metal in acute toxicity tests must be 0.75 (ODEQ 1994), which is within the U.S. Environmental Protection Agency (EPA)-recommended range of between 0.65 and 0.99 (U.S. EPA 1994a).

Laboratory Dilution Water

Prior to test initiation, laboratory dilution water must satisfy the requirements given by U.S. EPA (1993a) or ASTM (1993a,b,c,d,e,f). These requirements include the following:

- The laboratory dilution water must be groundwater, surface water, reconstituted water, diluted mineral water, or dechlorinated tap water that has been demonstrated to be non-toxic to aquatic organisms.
- If surface water is used for acute or chronic tests with daphnids and if predators are observed in the sample of the water, the sample must be filtered through a 37μm sieve or screen to remove the predators.
- Water prepared by treatments such as deionization and reverse osmosis must not be used as the laboratory dilution water unless salts, mineral water, hypersaline brine, or sea salts are added as recommended by U.S. EPA (1993a) or ASTM (1993a).
- The concentrations of both total organic carbon (TOC) and total suspended solids (TSS) must each be less than 5 mg/L.
- The hardnesses should be between 50 and 200 mg/L. However, if the criterion of the metal is hardness-dependent (e.g., cadmium or lead), the hardness of the laboratory dilution water should be adjusted to the same hardness as the upstream site water.
- The alkalinity and pH of the laboratory dilution water must be appropriate for its hardness. Values for alkalinity and pH that are appropriate for some hardnesses are given by U.S. EPA (1993a) and ASTM (1993a); other corresponding values should be determined by interpolation.

Alkalinity should be adjusted using sodium bicarbonate, and pH should be adjusted using aeration, sodium hydroxide, and/or sulfuric acid.

Toxicity tests conducted to compare results of the same tests from other laboratories should be conducted in the laboratory dilution water before any receiving water or effluent samples are collected. These tests should be performed at the hardness, alkalinity, and pH specified above.

CONDUCTING TESTS

After all of the issues described above have been addressed, toxicity testing can proceed. The sections below present procedures for conducting toxicity tests and specify chemical measurements that are required as part of the testing.

Toxicity Tests

Facilities for conducting toxicity tests should be set up, and test chambers should be selected and cleaned as recommended by U.S. EPA (1993a) and/or ASTM (1993a,b,c,d,e,f). The main criterion for a successful toxicity test is that there must be no differences between the side-by-side tests other than the composition of the dilution water and the concentrations of metal tested. It cannot be stressed enough that the acceptability of the data rely on rigorous, standardized laboratory quality assurance and quality control (QA/QC) measures. In part, these measures include the use of clean techniques throughout the laboratory testing where feasible. A detailed description of laboratory-related clean techniques is provided in Appendix B.

Acquiring and Acclimating Test Organisms

Test organisms should be obtained, cultured, held, acclimated, fed, and handled as recommended by U.S. EPA (1993a) and/or by ASTM (1993a,b,c,d,e,f). All test organisms must be acceptably acclimated to laboratory dilution water. An appropriate number of the organisms may be randomly or impartially removed from laboratory dilution water and placed in the simulated downstream site water when it becomes available to acclimate the organisms to the simulated downstream site water for a while just before tests are begun. Organisms used in a pair of side-by-side tests must be drawn from the same population and tested under identical conditions.

Preparing Stock Solutions

To add the appropriate metal to the toxicity tests, a stock solution should be prepared using an inorganic salt that is highly soluble in water. Recommended salts for use when determining a WER are provided in Table D-2. The salt used should meet American Chemical Society (ACS) specifications for reagent-grade, if such specifications are available. No salt should be used until information concerning safety and handling has been read. The stock solution may be acidified (using metal-free nitric acid) only as necessary to force the metal into solution. (If the solution is acidified, then the pH should be measured just prior to test initiation to ensure that the pH levels are appropriate for test organisms.) The same stock solution must be used to add metal to all tests conducted at one time.

Preparing the Tests

For the test using simulated downstream water, one of the following procedures should be used to prepare the test solutions for the test chambers and the chemistry controls:

- Thoroughly mix the sample of the effluent and place the same known volume of the effluent in each test chamber; add the necessary amount of metal, which will be different for each treatment; mix thoroughly; let stand for 2-4 hours; add the necessary amount of upstream water to each test chamber; mix thoroughly; let stand for 1-3 hours.
- Add the necessary amount of metal (using the pre-prepared stock solution) to a large sample of the effluent and also maintain an unspiked sample of the effluent; perform serial dilution using a graduated cylinder and the well-mixed spiked and unspiked samples of the effluent; let stand for 2-4 hours; add the necessary amount of upstream water to each test chamber; mix thoroughly; let stand for 1-3 hours.
- Prepare a large volume of simulated downstream water by mixing effluent and upstream water in the desired ratio; place the same known volume of the simulated downstream water in each test chamber; add the necessary amount of metal, which will be different for each treatment; mix thoroughly and let stand for 1-3 hours.
- Prepare a large volume of simulated downstream water by mixing effluent and upstream water in the desired ratio; divide it into two portions; prepare a large volume of the highest test concentration of metal using one portion of the simulated downstream water; perform serial dilution using a graduated cylinder and the well-mixed spiked and unspiked samples of the simulated downstream water; let stand for 1-3 hours.

Procedures 3 and 4 allow the metal to equilibrate somewhat with the effluent before the solution is diluted with upstream water.

For the test using the laboratory dilution water, either of the following procedures may be used to prepare the test solutions for the test chambers and the chemistry controls:

- Place the same known volume of the laboratory dilution water in each test chamber; add the necessary amount of metal, which will be different for each treatment; mix thoroughly; let stand for 1-3 hours.
- Prepare a large volume of the highest test concentration in the laboratory dilution water; perform serial dilution using a graduated cylinder and the well-

mixed spiked and unspiked samples of the laboratory dilution water; let stand for 1-3 hours.

The test organisms, which have been acclimated as described above, must be added to the test chambers for the site-by-side tests at the same time. If daphnid toxicity tests are used, simulated downstream site water must be filtered through a 37-μm sieve or screen to remove the predators prior to introducing the organisms. **The number of test organisms exposed to each treatment (i.e., each concentration for each test), including the controls, should be at least 20. The organisms should be distributed between two or more test chambers per treatment.** Traditionally, 4 test chambers of 5 organisms each are used. If test organisms are not randomly assigned to the test chambers, they must be assigned impartially (U.S. EPA 1993a; ASTM 1993a) between all test chambers for a pair of side-by-side tests. The test chambers should be assigned a location in a totally random arrangement or in a randomized block design. The time at which the test organisms are placed in the test chambers is defined as the beginning of the tests and must be within 36 hours of the final collection of the samples.

Monitoring the Tests

Observe the test organisms and record the effects and symptoms as specified by U.S. EPA (1993a) and/or ASTM (1993a,b,c,d,e,f). Especially note whether the effects, symptoms, and time course of toxicity are the same in the side-by-side tests. Recommendations concerning temperature, loading, feeding, dissolved oxygen, aeration, disturbance, and controls given by U.S. EPA (1993a) and/or ASTM (1993a,b,c,d,e,f) must be followed. The same procedures must be used in both of the side-by-side tests.

Renewing Acute Tests

During the toxicity testing period, some of the tests may need to be renewed with additional simulated downstream site and/or laboratory water. The following guidance applies to all tests that are conducted for the determination of WERs:

- The renewal technique must be used for tests that last longer than 48 hours.
- If the concentration of dissolved metal decreases by more than 50 percent in 48 hours, the test solutions must be renewed every 24 hours. Similarly, if the concentration of dissolved oxygen becomes too low, the test solutions must be renewed every 24 hours. If one test in a pair of tests is a renewal test, both tests must be renewal tests.
- When test solutions are to be renewed, the new test solutions must be prepared from the original unspiked effluent and water samples that have been stored at 0^oC to 4^oC in the dark with no air space in the sample container.

- Whenever solutions are renewed, sufficient solution should be prepared to allow for chemical analyses.

A static test does not require renewal. The static technique may be used for tests that do not last longer than 48 hours, unless the above specifications require use of the renewal technique. If a test is used that is not suggested in Table D-1, the duration and technique recommended for a comparable test using the most closely related species to those identified in Table D-1 should be used.

Chemical Measurements

As part of the WER testing, chemical analyses must be conducted on the side-by-side toxicity tests. These analyses include total and dissolved metals and conventional parameters such as pH and dissolved oxygen. To reduce the possibility of contamination of test solutions before or during tests, thermometers and probes for measuring pH and dissolved oxygen must not be placed in test chambers that will provide data concerning effects on test organisms or data concerning the concentration of the metal. Therefore, measurements of pH, dissolved oxygen, and temperature before or during a test must be performed either on chemistry controls that contain test organisms and are fed the same as the other test chambers or on aliquots that are removed from the test chambers.

Total and Dissolved Metal

You must measure metals in the receiving water, the effluent, the simulated downstream site water, and the laboratory dilution water. It may be beneficial to measure both total recoverable and dissolved metals so that you can calculate and use either a total or dissolved WER, providing the greatest flexibility. The analytical measurements should be sufficiently sensitive and precise that variability in analyses will not greatly increase the variability of the WERs. Trace-metal EPA methods (U.S. EPA 1993a, 1996a) should be used for both total recoverable and dissolved measurements. The detection limit of the analytical method must not be greater than one-tenth of the criterion that is to be adjusted. If the detection limit of the analytical method used is above the endpoint in simulated downstream site water, a WER cannot be determined. Replicates, matrix spikes, and other QA/QC checks must be performed as required by U.S. EPA (1993a, 1996a).

You may analyze the metal concentration in all test solutions, or you may store samples and then analyze only those that are needed to calculate the results of the toxicity tests. For dichotomous data (e.g., data concerning survival), the metal concentrations in the following treatments must be analyzed:

- All concentrations in which some, but not all, of the test organisms were adversely affected
- The highest concentration that did not adversely affect any test organisms

- The lowest concentration that adversely affected all of the test organisms
- The test controls.

In each treatment in which the concentration of metal is to be measured, both the total recoverable and dissolved concentrations must be measured. Nominal concentrations (i.e., concentrations that are expected or predicted to be in the tests) cannot be used in WER determinations in lieu of measured concentrations.

Samples must be collected for analysis of the total recoverable metal concentration at least once for a static test. The total recoverable metal concentration must also be measured at least once for each renewal test; samples should be collected after the organisms have been transferred to the new test solutions. When the total recoverable metal concentration is measured in a test chamber, the whole solution in the chamber must be mixed before the sample is taken for analysis. The solution in the test chamber must be acidified after it is placed in the sample container.

The dissolved metal concentration must be measured at the beginning and end of each static test. In a renewal test, the dissolved metal concentration must be measured at the beginning of the test and just before the solution is renewed the first time. When the dissolved metal concentration is measured in a test chamber, the whole solution in the test chamber must be mixed before a sufficient amount is removed for filtration. The sample must be filtered using an appropriate method-specific pore size and filter media within 1 hour after it is collected, and the filtrate must be acidified after filtration.

Conventional Parameters

Hardness, pH, alkalinity, TSS, and TOC must be measured in the receiving water, the effluent, the simulated downstream site water, and the laboratory dilution water. Measurement of conductivity and/or total dissolved solids is also recommended.

Dissolved oxygen, pH, and temperature must be measured during the test at the times specified by U.S. EPA (1993a) and/or ASTM (1993a,b,c,d,e,f). The measurements must be performed on the same schedule for both of the side-by-side tests. Measurements also must be performed on both the chemistry controls and actual test solutions at the end of the test.

CALCULATING AND INTERPRETING THE RESULTS

After all of the tests have been conducted, you must evaluate the toxicity tests individually to determine if the data are acceptable. If the procedures used deviated from those specified above, particularly in terms of acclimation, randomization, temperature control, measurement of metal, and/or disease or disease-treatment, the test should be rejected. If deviations were numerous and/or substantial, the test must be rejected.

To determine the acceptability of a toxicity test used to support development of a WER, the following criteria must be met:

- Most tests are unacceptable if more than 10 percent of the organisms in the controls were adversely affected, but the limit is higher for some tests. For the tests recommended in Table D-1, the references given should be consulted.
- The percent of the organisms that were adversely affected must have been less than 50 percent, and should have been less than 37 percent, in at least one treatment other than the control.
- In the laboratory dilution water, the percent of the organisms that were adversely affected must have been greater than 50 percent, and should have been greater than 63 percent, in at least one treatment. In simulated downstream site water, the percent of the organisms that were adversely affected should have been greater than 63 percent in at least one treatment.
- If there was an inversion in the data (i.e., if a lower concentration killed or affected a greater percentage of the organisms than a higher concentration). The inverted data must not have involved more than two concentrations that killed or affected between 20 and 80 percent of the test organisms.
- The concentration of dissolved metal must not have decreased by more than 50 percent from the beginning to the end if a static test was conducted or more than 50 percent from the beginning to the end during each phase if a renewal test was conducted.

The effects, symptoms, and time course of toxicity should also be compared in the side-by-side tests in the simulated downstream site water and the laboratory dilution water. For example, did mortality occur in one acute test, but immobilization in the other? Did most deaths occur before 24 hours in one test, but after 24 hours in the other? If the effects, symptoms, and/or time course of toxicity were different, it might indicate that the test is questionable.

To calculate the results of each test, the endpoint must be calculated as an LC_{50} using methods described by U.S. EPA (1993a) or ASTM (1993a). The same computational method must be used for both side-by-side tests used in the calculation of a WER. Both total recoverable and dissolved endpoints should be calculated for each test. The exposures should also be based on the time-weighted average measured metal concentrations. The following guidance should be followed to calculate an LC_{50}:

- If no treatment killed or affected more than 50 percent of the test organisms and the test was otherwise acceptable, the LC_{50} should be reported to be greater than the highest test concentration

- If no treatment other than the control killed or affected less than 50 percent of the test organisms and the test was otherwise acceptable, the LC_{50} should be reported to be less than the lowest test concentration
- If the detection limit of the analytical method used to measure the metal is above the endpoint in laboratory dilution water, the detection limit must be used as the endpoint, resulting in a lower WER than would be obtained if the actual concentration had been measured.

Probit analysis must be used to calculate results of both tests in a side-by-side pair, unless the probit model is rejected by the goodness-of-fit test in one or both of the acute tests. If probit analysis cannot be used because the model does not fit the data, computational interpolation must be used; graphical interpolation must not be used to calculate an LC_{50}. The endpoints for both tests must be calculated using the same analysis (i.e., an LC_{50} from a probit analysis cannot be compared to an LC_{50} from interpolation). There are a number of software packages available, including ToxStat® or ToxCalc®, to assist in calculating LC_{50} values for toxicity tests using both probit and computational interpolation.

You must also compare the results of tests with other reported data to provide a check on all aspects of the test procedure. The study data and the data from comparable studies must be carefully evaluated to determine whether the laboratory dilution water used in the WER determination was acceptable. The quality of the laboratory dilution water is paramount because all other aspects of the side-by-side WER tests must be the same. This is particularly important if the new endpoints are (after taking into account any known effect of hardness on toxicity):

- More than a factor of 1.5 higher than the respective means of the values from other laboratories
- More than a factor of 1.5 lower than the respective means of values from other laboratories
- Lower than the respective lowest values available from other laboratories
- Higher than the respective highest values available from other laboratories.

Any of these results might indicate that there may have been an error in the chemical measurements, which might mean that the results of all tests performed in the WER determination need to be adjusted. It is also possible that the metal is more or less toxic in the laboratory dilution water used in the WER determination. Evaluation of results of any other toxicity tests on the same or a different metal using the same laboratory dilution water might be useful.

The laboratory dilution water used in the WER determination will be considered acceptable if, after taking into account any known effect of hardness on toxicity, the new values for the endpoints are not either higher or lower in comparison to data from other laboratories and if both of the new values are within a factor of 2 of the respective means of the previously available values or are within the ranges of the values. If the comparisons do not indicate that the laboratory dilution water, test method, or other factors are acceptable, the tests probably should be considered unacceptable, unless other toxicity data are available to indicate that they are acceptable.

If the total WER (defined in Oklahoma as the laboratory water LC_{50} divided by the simulated downstream site water LC_{50}) is smaller than 0.2, it should be investigated. If a total recoverable WER was less than 0.2 but the dissolved WER was not, the metal associated with WERs may be affected by TSS and/or TOC. Was there a substantial difference between the total recoverable and dissolved concentrations of the metal in the downstream water? If both the total recoverable and dissolved WERs were less than 0.2, there may be nontoxic dissolved metal in the downstream water.

Finally, the results of the chemical measurements of hardness, alkalinity, pH, TSS, TOC, total recoverable metal, and dissolved metal on the effluent and the receiving water should be examined and compared with previously available values for the effluent and receiving water. This comparison can provide an indication of whether the samples were representative and to get some indication of the variability in the composition, especially as it might affect the toxicity of the metal and the WER. The comparison can also help determine if the WER correlates with one or more of the measurements.

At least four acceptable WERs must be calculated, one of which must be from a test that was conducted on a second species. If the results of a particular test are rejected, you must explain the rationale of why you rejected the test in your final report to the agencies. If the toxicity tests are considered acceptable, the calculations necessary to determine a final WER are provided in the main text of this document.

TABLE D-1. RECOMMENDED TOXICITY TESTS FOR DETERMINING WATER EFFECTS RATIOS

Metal	Possible Acute Tests[a]
Aluminum	DA
Arsenic(III)	DA, GM
Cadmium	DA, SL or FM
Chromium(III)	GM, SL, or DA
Chromium(VI)	DA, GM
Copper	DA, FM, or GM
Lead	DA, GM
Mercury	DA, GM
Nickel	DA, FX
Selenium	[b]
Silver	DA, FM
Zinc	DA, FM

Source: U.S. EPA (1994).

[a] The description of a test specifies not only the test species and the duration of the test but also the life stage of the species and the adverse effect(s) on which the endpoint is to be based, all of which can have a major impact on the sensitivity of the test.

DA - A 48-hour EC_{50} (or LC_{50} if there is no immobilization) from a static test with a species in one of three genera (*Ceriodaphnia*, *Daphnia*, *Simocephalus*) in the family Daphnidae (U.S. EPA 1993; ASTM 1993a).

FM - A 48-hour LC_{50} from a static test at 25°C with fathead minnow (*Pimephales promelas*) larvae that are 1□24 hours old (ASTM 1993a; U.S. EPA 1993). The embryos must be hatched in the laboratory dilution water, except that organisms to be used in the site water may be hatched in the site water. The larvae must not be fed before or during the test, and at least 90 percent must survive in laboratory dilution water for at least 6 days after hatch.

Note: The following 48-hour LC_{50}s were obtained at a hardness of 50 mg/L with fathead minnow larvae that are less than 24 hours old. The metal concentration was measured using the total recoverable procedure (Peltier 1993):

Metal	LC_{50} (μg/L)
Cadmium	13.87
Copper	6.33
Zinc	100.95

FX - A 96-hour LC_{50} from a renewal test (renew at 48 hours) at 25°C with fathead minnow (*Pimephales promelas*) larvae that are 1-24 hours old (ASTM 1993a; U.S. EPA 1993). The embryos must be hatched in the laboratory dilution water, except that organisms to be used in the site water may be hatched in the site water. The larvae mast not be fed before or during the test, and at least

90 percent must survive in laboratory dilution water for at least six days after hatch.

Note: A 96-hour LC_{50} of 188.14 μg/L was obtained at a hardness of 50 mg/L in a test on nickel with fathead minnow larvae that were 1-24 hours old. The metal concentration was measured using the total recoverable procedure (Pettier 1993). A 96-hour LC_{50} is used for nickel because substantial mortality occurred after 48 hours in the test on nickel, but not in the tests on cadmium, copper, and zinc.

GM - A 96-hour EC_{50} (or LC_{50} if there is no immobilization) from a renewal test (renew at 48 hours) with a species in the genus *Gammarus* (ASTM 1993a).

SL - A 96-hour EC_{50} (or LC_{50} if there is no immobilization) from a renewal test (renew at 48 hours) with a species in one of two genera (*Oncorhynchus*, *Salmo*) in the family Salmonidae (ASTM 1993a). In acute tests on cadmium with salmonids, substantial numbers of fish usually die after 72 hours. Also, the fish are sensitive to disturbance, and it is sometimes difficult to determine whether a fish is dead or immobilized.

[b] Because the freshwater criterion for selenium is not based on laboratory data concerning toxicity to aquatic life, they cannot be adjusted using a WER.

TABLE D-2. RECOMMENDED SALTS FOR USE IN DETERMINING WATER EFFECTS RATIOS

Metal	Recommended Salt
Aluminum	Aluminum chloride 6-hydrate: $AlCl_3 \cdot 6H_2O$
	Aluminum sulfate 18-hydrate: $Al_2(SO_4)_3 \cdot 18H_2O$
	Aluminum potassium sulfate 12-hydrate: $AlK(SO_4)_2 \cdot 12H_2O$
Arsenic(III)	Sodium arsenite: $NaAsO_2$
Arsenic(V)	Sodium arsenate 7-hydrate, dibasic: $Na_2HAsO_4 \cdot 7H_2O$
Cadmium	Cadmium chloride 2.5-hydrate: $CdCl_2 \cdot 2.5H_2O$
	Cadmium sulfate hydrate: $3CdSO_4 \cdot 8H_2O$
Chromium(III)	Chromic chloride 6-hydrate (Chromium chloride): $CrCl_3 \cdot 6H_2O$
	Chromic nitrate 9-hydrate (Chromium nitrate): $Cr(NO_3)_3 \cdot 9H_2O$
	Chromium potassium sulfate 12-hydrate: $CrK(SO_4)_2 \cdot 12H_2O$
Chromium(VI)	Potassium chromate: K_2CrO_4
	Potassium dichromate: K_2Cr_2O
	Sodium chromate 4-hydrate: $Na_2CrO_4 \cdot 4H_2O$
	Sodium dichromate 2-hydrate: $Na_2Cr_2O_7 \cdot 2H_2O$
Copper	Cupric chloride 2-hydrate (copper chloride): $CuCl_2 \cdot 2H_2O$
	Cupric nitrate 2.5-hydrate (copper nitrate): $Cu(NO_3)_2 \cdot 2.5H_2O$
	Cupric sulfate 5-hydrate (copper sulfate): $CuSO_4 \cdot 5H_2O$
Lead	Lead chloride: $PbCl_2$
	Lead nitrate: $Pb(NO_3)_2$
Mercury	Mercuric chloride: $HgCl_2$
	Mercuric nitrate monohydrate: $Hg(NO_3)_2 \cdot H_2O$
	Mercuric sulfate: $HgSO_4$
Nickel	Nickelous chloride 6-hydrate (nickel chloride): $NiCl_2 \cdot 6H_2O$
	Nickelous nitrate 6-hydrate (nickel nitrate): $Ni(NO_3)_2 \cdot 6H_2O$
	Nickelous sulfate 6-hydrate (nickel sulfate): $NiSO_4 \cdot 6H_2O$
Selenium(IV)	Sodium selenite 5-hydrate: $Na_2SeO_3 \cdot 5H_2O$
Selenium(VI)	Sodium selenate 10-hydrate: $Na_2SeO_4 \cdot 10H_2O$
Silver	Silver nitrate: $AgNO_3$
Zinc	Zinc chloride: $ZnCl_2$
	Zinc nitrate 6-hydrate: $Zn(NO_3)_2 \cdot 6H_2O$
	Zinc sulfate 7-hydrate: $ZnSO_4 \cdot 7H_2O$

Source: U.S. EPA (1994).

APPENDIX E
CALCULATION WORKSHEETS

This appendix provides worksheets to assist in calculating values that will be required to develop a site-specific criterion. The appendix is organized as follows:

- **Worksheet E1. Determining the Mixing Ratio for the Simulated Downstream Sample-**This worksheet provides the steps for calculating the ratio at which the effluent and receiving water samples are combined to achieve an appropriate simulated downstream water sample.
- **Worksheet E2. Determining the Required Volume for Sample Collection-**After the mixing ratio is known, this worksheet can be used to calculate the sample volume necessary for each sampling event.
- **Worksheet E3. Determining the Final Site-Specific Criterion-**After the required information has been generated for the study, this worksheet provides an example of how the final site-specific criterion is derived. Figure E-1 provides a graphical illustration of how the different components fit together for all three options, resulting in varied site-specific criterion values.

WORKSHEET E1. DETERMINING THE MIXING RATIO FOR THE SIMULATED DOWNSTREAM SAMPLE

To support development of a site-specific chronic criterion, tests must be performed on simulated downstream samples, which are a mixture of upstream water and effluent. The final outcome of the study is likely to be highly dependent on this ratio because the amount of effluent controls the amount of metal in the mixed sample. This worksheet provides step-by-step instructions to determine this mixture ratio, which will remain constant for every sampling event.

Step 1. Determine the maximum effluent flow (Q_E), defined as the highest averaged discharge flow, if sufficient data are available. Are sufficient data available? If yes, then use the average value. If no, then use the maximum design flow as the value for Q_E.

Q_E =

Step 2. Determine the minimum receiving water flow (Q_R), defined as the 7Q2 for the receiving water body, if available. Is a 7Q2 value available (check your existing permit or USGS)? If yes and the 7Q2 is larger than 1.0 cfs, use the 7Q2 value. If no, then use 1.0 cfs as the value for Q_R.

Q_R =

Step 3. Calculate the dilution capacity (Q^*), defined as Q_E/Q_R. Make sure your units are consistent.

Q^* =

Step 4. Calculate the percent of effluent (PE) required in the simulated downstream sample. In Oklahoma, the following formulas are used to determine the PE (Hutcheson 1992):

PE =

If $Q^* < 0.1823$, then $PE = 193.6\,Q^* / (1 + Q^*)$
If $0.1823 < Q^* < 0.3333$, then $PE = 100 / (6.17 - 15.51\,Q^*)$
If $Q^* > 0.3333$, then $PE = 100$

The PE will be used in Worksheet E2 to determine the amount of effluent and receiving water that must be collected to satisfy all of the testing requirements under each option. In this context:

$$PE = 100\,V_E / (V_R + V_E)$$

where:

V_E = volume of effluent
V_R = volume of receiving water.

Each of these steps is shown in the following example.

EXAMPLE

Step 1	$Q_E = 4$ mgd	Value taken from discharge permit calculations.
Step 2	$Q_R = 27$ mgd	Value derived from USGS water gauge records (recorded in ft/second and converted to the same units as Q_E).
Step 3	$Q^* = Q_E/Q_R$ $= 4/27$ $= 0.1482$	Dilution capacity calculated
Step 4	$PE = 193.6Q^*/(1+Q^*)$ $= 25.03$ = 25 percent	Because $Q^* \leq 0.1823$, the first formula is used to determine PE.

WORKSHEET E2. DETERMINING THE REQUIRED VOLUME FOR SAMPLE COLLECTION

To support development of a site-specific criterion, analytical and toxicity tests must be performed on simulated downstream samples, which are a mixture of upstream water and effluent. Chemical analyses are also performed on upstream samples, effluent samples, and simulated downstream samples. This worksheet provides step-by-step instructions to ensure that you collect an adequate volume of both receiving water and effluent during each sampling event. The volume requirements may vary between sampling events, based on different tests that may be performed. The general steps are listed below:

- Determine the value of percent effluent (PE) that will be used in the study (use Worksheet E1). This value is used for each sampling event.
- Determine the known volume requirements for all analyses scheduled for the event (including both toxicity tests and chemical analyses), as appropriate based on the option you pursue. The analytical laboratories should provide you with this information.
- Calculate the amount of effluent and receiving water that must be collected to create the simulated downstream sample. **Add at least 10 percent extra volume in case of unexpected sampling errors or laboratory accidents**.
- Sum the total volume requirements for each event.

Each of these steps is shown in the following example.

EXAMPLE

Step 1

The percent effluent (PE) is 25 percent (from example in Worksheet E1).

Step 2

The known volume requirements are summarized below, where:

V_{EC} - volume of effluent used for chemical analyses
V_{ET} - volume of effluent used for toxicity tests

V_{RC} - volume of receiving water used for chemical analyses
V_{RT} - volume of receiving water used for toxicity tests
-- - no volume required

	Volume Requirements (L)		
	Receiving Water	Effluent	Simulated Downstream
Option One			
WER toxicity tests	V_{RT}	V_{ET}	20
WER chemical analyses[a]	V_{RC}	V_{EC}	1
Other chemical analyses[b]	1	1	--
Totals:	$(V_{RT}+V_{RC}) + 1$	$(V_{ET}+V_{EC}) + 1$	21
Option Two			
WER toxicity tests	--	--	--
f chemical analyses[c]	V_{RC}	V_{EC}	1
Other chemical analyses[d]	1	1	--
Totals:	$V_{RC} + 1$	$V_{EC} + 1$	1
Option Three			
WER toxicity tests	V_{RT}	V_{ET}	20
WER chemical analyses[a]	V_{RC}	V_{EC}	1
f chemical analyses[b]	V_{RC}	V_{EC}	1
Other chemical analyses[b,d]	1	1	--
Totals:	$V_{RT} + (2V_{RC}) + 1$	$V_{ET} + (2V_{EC}) + 1$	22

note: f = dissolved translator

[a] These chemical analyses are conducted to support WER tests (e.g. total and dissolved metals).

[b] These chemical analyses are conducted on the receiving water and effluent samples independent of the WER tests (e.g. total metal concentrations for background)

[c] These chemical analyses are conducted on simulated downstream water only (e.g. total and dissolved metals, total organic carbon)

[d] These chemical analyses are conducted on effluent and downstream receiving water only (e.g. total and dissolved metals, total organic carbon)

Step 3

The volume requirements for effluent and receiving water are calculated as follows:
For toxicity tests:

If PE = 25, and PE = 100 x [V_{ET} / (V_{RT} + V_{ET})],
then V_{ET} = (25/100) x (V_{RT} + V_{ET}) = (25/100) x (20 L) = 5 L
and V_{RT} = (V_{RT} + V_{ET}) - (V_{ET}) = 20 L - 5 L = 15 L

For chemical analyses:

If PE = 25, and PE = 100 x [V_{EC} / (V_{RC} + V_{EC})],
then V_{EC} = (25/100) x (V_{RC} + V_{EC}) = (25/100) x (1.0 L) = 0.25 L
and V_{RC}= (V_{RC} + V_{EC}) - (V_{EC}) = 1.0 L - 0.25 L = 0.75 L

Step 4

The total volume requirements are summed below:

	Volume Requirements (L)		
	Receiving Water	Effluent	Simulated Downstream
Option One			
WER toxicity tests	15	5	20
WER chemical analyses	0.75	0.25	1
Other chemical analyses	1	1	--
Totals:	16.75	6.25	21
Option Two			
WER toxicity tests	--	--	--
f chemical analyses	0.75	0.25	1
Other chemical analyses	1	1	--
Totals:	1.75	1.25	1
Option Three			
WER toxicity tests	15	5	20
WER chemical analyses	0.75	0.25	1
f chemical analyses	0.75	0.25	1
Other chemical analyses	1	1	--
Totals:	18.5	7.5	22

WORKSHEET E3. DETERMINING THE FINAL SITE-SPECIFIC CRITERION

Various calculations must be performed to derive the final site-specific criterion. This worksheet provides an example to help you fit the pieces of the puzzle together based on the option that you decide to pursue.

The steps of the calculations are as follows:

- Step 1. Calculate the total and dissolved water effect ratios (WERs) for at least four acceptable toxicity tests by dividing the laboratory LC_{50} by the site LC_{50} (see page 19 of main text for formula).
- Step 2. Calculate the total and dissolved final WERs (FWERs) by taking the geometric mean of the four respective WER values (see page 21 of main text for formula).
- Step 3. Calculate the dissolved translator by taking the geometric mean of the ratio of dissolved to total metal concentrations in at least 10 simulated downstream samples.
- Step 4. Calculate the new site-specific criterion by applying the values derived in Steps 1, 2, or 3 to the statewide criterion.
- Step 5. Calculate the background concentration (if required) of total metal by taking the average total metal concentration in at least 12 upstream samples.

Each of these steps is illustrated in the example data set in the following tables and figure.

Total WER

1	Fathead minnow	102.3	65.60	0.6413
1	Daphnid	123.5	42.12	0.3411
2	--	--	--	--
3	--	--	--	--
4	Fathead minnow	98.60	51.20	0.5193
5	--	--	--	--
6	--	--	--	--
7	--	--	--	--
8	--	--	--	--
9	--	--	--	--
10	Fathead minnow	112.9	54.26	0.4806
11	--	--	--	--
12	--	--	--	--
Geometric Mean:				0.4833

STEP 2	**TOTAL FWER =0.4833**

Dissoved WER

1	Fathead minnow	23.8	13.50	0.5663
1	Daphnid	34.8	23.50	0.6757
2	--	--	--	--
3	--	--	--	--
4	Fathead minnow	14.70	9.20	0.6259
5	--	--	--	--
6	--	--	--	--
7	--	--	--	--
8	--	--	--	--
9	--	--	--	--
10	Fathead minnow	29.6	18.60	0.6292
11	--	--	--	--
12	--	--	--	--
Geometric Mean:				0.6231

STEP 2	**DISSOLVED FWER =0.6231**

Note: Site water is a simulated downstream sample.

The WER is calculated by dividing the laboratory LC_{50} by the site LC_{50}.

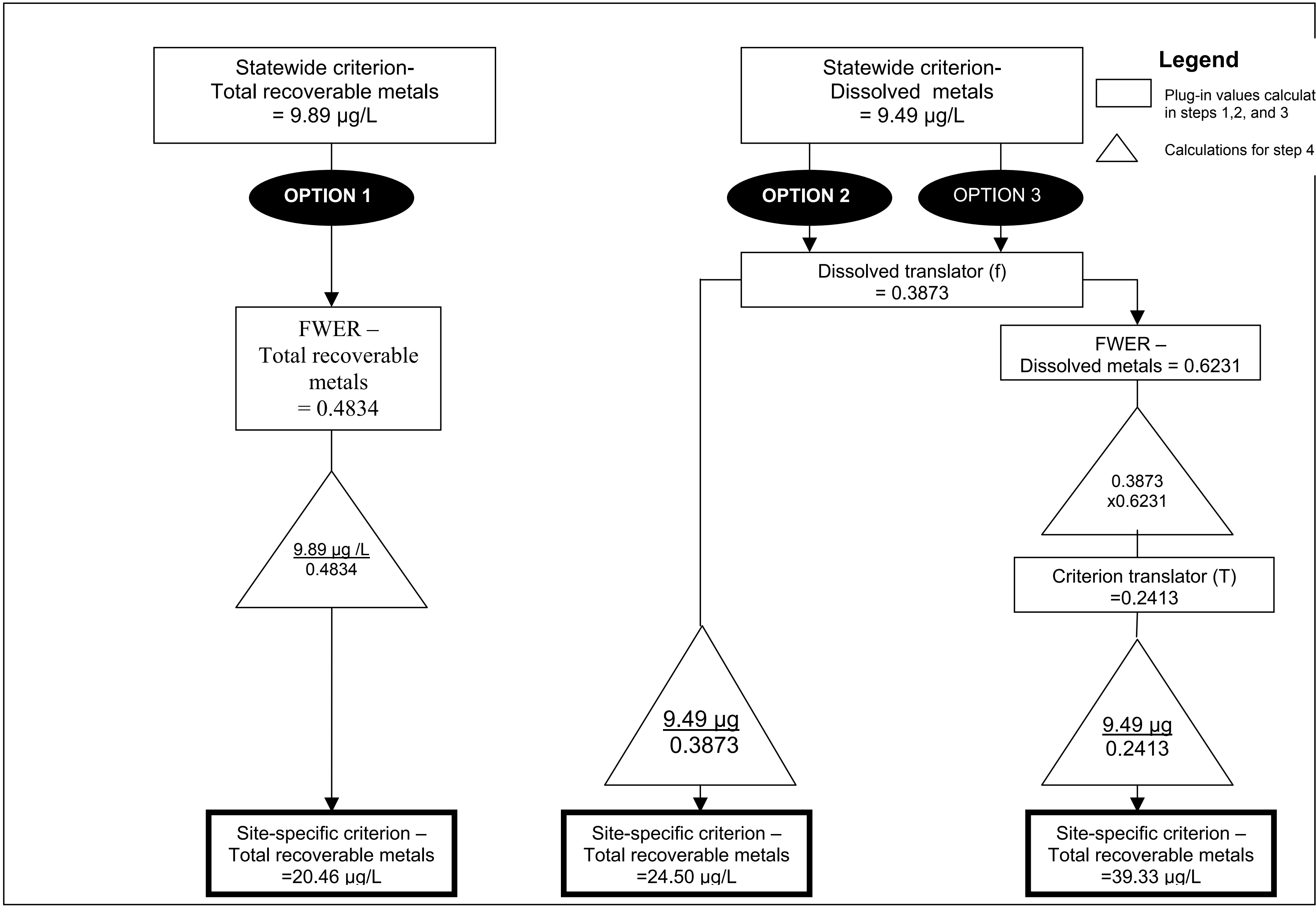

Figure E-1. Calculation of site-specific criterion (step 4).

SUMMARY OF ANALYTICAL RESULTS
(STEPS 3 AND 5)

	Sampling event	Total metals (µg/ L)	Dissolved metals (µg/ L)	Dissoved metals (percent of total)
Receiving water				
	1	2.3	0.5	21.74
	2	5.6	1.2	21.43
	3	7.8	1.5	19.23
	4	8.2	3	36.59
	5	0.5	0.4	80
	6	14	9.3	66.43
	7	10.4	1.8	17.31
	8	1.7	0.5	29.41
	9	6.6	1.3	19.7
	10	4	1.6	40
	11	8.3	1.6	19.28
	12	5.4	1.2	22.22
	Geometric mean	4.715	1.348	28.599
	Average	6.233	1.992	32.778
	Step 5	**background = 6.233**		
Effluent				
	1	25.3	15.6	61.66
	2	8.7	5.2	59.77
	3	15.3	6.5	42.48
	4	10.7	5.8	54.21
	5	9.1	3.4	37.36
	6	16.2	6.3	38.89
	7	12.4	5	40.32
	8	7.9	6.9	87.34
	9	29.6	18.5	62.5
	10	21.6	8	37.22
	11	30.3	8.5	28.05
	12	53.6	22.3	41.6
	Geometric mean	16.894	7.946	47.056
Simulated downstream				
	1	8	5.6	70
	2	6.5	2	30.77
	3	16.3	5.2	31.9
	4	10	2.1	21
	5	2.5	0.8	32
	6	14.3	5.6	39.16
	7	5.6	1.6	28.57
	8	1	0.3	30
	9	15.2	9.6	63.16
	10	5.2	1.3	25
	11	13.5	8.5	62.96
	12	16.9	12.5	73.96
	Geometric mean	7.478	2.897	38.731

Step 4 is shown graphically on Figure E-1.

APPENDIX F
REPORTING REQUIREMENTS

The final report of the study must include all relevant information for the agencies to review. Although the organization of the final report is flexible, the document will need to address the methods and the results of the study. Specifically, the final report should summarize the methods that were used to collect each of the types of samples (e.g., receiving water, effluent). If any deviations from the work plan were encountered, the final report must also summarize these deviations and provide an explanation of why they occurred. The following results must also be submitted as part of the final report:

- Field sampling information
- Water effect ratio (WER) toxicity test data
- Chemical analytical data.

FIELD SAMPLING INFORMATION

The field sampling information that must be reported as part of the final report includes the following:

- The name, location, and description of the discharger, a description of the effluent, the design flows of the effluent, and recorded historic flows for the receiving water
- The name(s) and title(s) of the people who collected the samples
- A description of each sampling station, date, and time, with an explanation of why they were selected, and the recorded flows of the receiving water and the effluent at the time the samples were collected
- The procedures used to obtain, transport, and store the receiving water and effluent samples.

WER TOXICITY TEST DATA

The reporting requirements for WER toxicity testing state that the following elements must be included in the final report:

- Name(s) of the analyst(s), name and location of the laboratory, and dates and times of initiation and termination of the tests.

- A description of the laboratory dilution water, including source, preparation, and any demonstrations that an aquatic species could survive, grow, and reproduce in the water.
- Any pretreatment, such as filtration, of the effluent, simulated downstream site water, and/or laboratory dilution water.
- Results of all chemical and physical measurements on upstream water, effluent, actual and/or simulated downstream water, and laboratory dilution water, including hardness (or salinity); alkalinity; pH; and concentrations of total recoverable metal, dissolved metal, total suspended solids, and total organic carbon.
- Description of the experimental design, test chambers, depth and volume of solution in the chambers, loading and lighting, and numbers of organisms and chambers per treatment.
- Source and grade of the metallic salt and how the stock solution was prepared, including any acids or bases used.
- Source of the test organisms, scientific name and how verified, age, life stage, means and ranges of weights and/or lengths, observed diseases, treatments, holding and acclimation procedures, food, and feeding frequency.
- The average and range of the temperature, pH, hardness (or salinity), and concentration of dissolved oxygen (as percent saturation and as mg/L) during acclimation, and the method used to measure them.
- All differences, other than the dilution water and the concentrations of metal in the test solutions, between the side-by-side tests using laboratory dilution water and simulated downstream site water.
- The following must be presented for each toxicity test:
    - ➢ The average and range of the measured concentrations of dissolved oxygen, as percent saturation and as mg/L
    - ➢ The average and range of the test temperature and the method used to measure it
    - ➢ The schedule for taking samples of test solutions, and the methods used to obtain, prepare, and store them
    - ➢ A summary table of the total recoverable and dissolved concentrations of the metal in each treatment, including all controls
    - ➢ A summary table of the values of the toxicological variable(s) for each treatment, including all controls, in sufficient detail to allow an independent statistical analysis of the data
    - ➢ The endpoint, and the method used to calculate it

    - Comparisons with other data obtained by conducting the same test on the same metal using laboratory dilution water in the same and different laboratories; such data may be from a criteria document or from another source
    - Anything unusual about the test, any deviations from the procedures described above, and any other relevant information.

- Comparison of results obtained with the primary and secondary tests.
- The total and dissolved LC_{50} values and WERs for each test and an explanation of their calculation.

CHEMICAL ANALYTICAL DATA

The reporting requirements for chemical analyses state that the following information must be included in the final report:

- Results of all chemical and physical measurements on upstream water, effluent, simulated downstream water, and laboratory dilution water. These measurements must include hardness, alkalinity, pH, total suspended solids, total organic carbon, and concentrations of total recoverable and dissolved metal.
- Results of any data validation that was performed on the chemistry data.
- A summary of relevant calculations from the data set (e.g., dissolved translator, background concentrations of total metal).

After all of the results have been reported, a summary of calculations could also be included. See Appendix E for examples of how the relevant calculations are performed.

APPENDIX G
WATER QUALITY STANDARDS REVISION PROCESS

Prior to any site-specific criteria being available for use in a discharge permit, it must first become a criterion in the state Water Quality Standards (WQS). Department of Environmental Quality permit writers rely upon the WQS to provide the in-stream limit that are then modeled back to “Long Term Averages”, “waste load allocations” and NPDES permit limits. Although these may be approved through the appropriate state agencies, regional EPA offices must also approve this criterion for use in a discharge permit.

The job of the contractor who actually develops the criterion is not done when the criterion is calculated and the final report submitted. There is still process of getting the criterion into “rule”, which in this case is Oklahoma Administrative Code title 785 Chapter 45 Appendix E. Since this process is, in essence, a request from the affected entity (municipality or industry) for a change to the WQS, it will be their responsibility to justify that change. OWRB staff will provide the necessary technical and logistical assistance to make this presentation as effective as possible but the request and the justification will the responsibility of the presenter.

The contractor and the entity for whom the criterion is being developed should be prepared to present and defend their findings to the public at one of the informal public meetings held to present criteria changes and justification to the public and interested industry representatives. Traditionally, this will involve some sort of multi-media presentation (e.g. handouts, Power Point slides, overhead transparencies, etc) where the summary of the project and results are encapsulated for the general public. At the end of the series of informal meetings, an official comment period is opened for state agencies, general public and others to present their views on any or all of the issues presented for consideration during the revision process.

The contractor and the entity for whom the criterion is being developed are also encouraged to attend the formal hearing on the proposed revisions, held at the end of the official comment period. This gives the affected entity a chance to voice their support for the findings and the promulgation of the results.

One of the last chances for an affected entity to voice their support and request adoption of the site-specific criterion is at the Board meeting(s) when this topic is formally presented to the Oklahoma Water Resources Board members for their consideration and potential adoption. The Board will be presented the packet of rule changes to be considered along with brief explanations of the development process for each. At some point in the meeting, the Board traditionally accepts oral comments from the public, state agencies and affected stakeholders. After official promulgation by the Board, the process continues through the Attorney General, Legislative and Gubernatorial reviews and ultimately to EPA for final approval.

The criterion will be effective as state law upon approval by the Governor but ODEQ will not be able to use it until EPA has approved it. There may be questions or requests for additional information. Be prepared to provide it. It is critical that Appendix E and this guidance document be followed as closely as possible. OWRB staff is better able to defend proposed revisions of the WQS if EPA is assured that the development process has been a rigorously scientific one and the public has had ample opportunity to participate in the adoption process.

APPENDIX H
EPA OPINION LETTER ON STATES AUTHORITY TO DETERMINE ACCEPTABILITY OF WER