United States
Environmental Protection
Agency

Office of Water
4304

EPA-822-R-01-005
March 2001

Streamlined Water-Effect Ratio Procedure for Discharges of Copper

Streamlined
Water-Effect Ratio Procedure
for Discharges of Copper

March 2001

U.S. Environmental Protection Agency
Office of Water
Office of Science and Technology
Washington, D.C.

Notices

This document provides guidance to states and tribes authorized to establish and implement water quality standards under the Clean Water Act (CWA), to protect aquatic life from acute and chronic effects of copper. Under the CWA, states and tribes are to establish water quality criteria to protect designated uses. The CWA and EPA regulations at 40 CFR Part 131 contain legally binding requirements. The statutory provisions and EPA regulations described in this document contain legally binding requirements. This document does not substitute for the CWA or EPA's regulations; nor is it a regulation itself. Thus, it does not impose legally binding requirements on EPA, states, tribes, or the regulated community, and may not apply to a particular situation based upon the circumstances. State and tribal decision makers retain the discretion to adopt approaches on a case-by-case basis that differ from this guidance when appropriate. Therefore, interested parties are free to raise questions and objections about the substance of this guidance and the appropriateness of the application of this guidance to a particular situation. EPA will, and States should, consider whether or not the recommendations or interpretations in the guidance are appropriate in that situation. While this guidance constitutes EPA's scientific recommendations on procedures for obtaining site-specific values for aquatic life criteria for copper, EPA may change this guidance in the future.

This document has been approved for publication by the Office of Science and Technology, Office of Water, U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Acknowledgment

This document was prepared by Charles Delos, Health and Ecological Criteria Division, Office of Science and Technology. Appendix A was adapted and modified from the 1994 document *Interim Guidance on the Determination and Use of Water-Effect Ratios for Metals*, which had been prepared by Charles Stephan and others. Appendix B is based primarily on a literature review by Gary Chapman of the Great Lakes Environmental Center.

Peer review of this document was performed by Paul Jiapizian of the Maryland Department of Environment, William Dimond of the Michigan Department of Environmental Quality, and Cindy Roberts of U.S. EPA. This is documented in *Response to Peer Review Comments on Streamlined Water-Effect Ratio for Discharges of Copper*, available in portable document format (pdf) from the contact below.

Please submit questions to: Charles Delos, U.S. EPA, Mail Code 4304, Washington, DC 20460 (e-mail: delos.charles@epa.gov).

Contents

Page

Introduction

This guidance presents a Streamlined Procedure for determining site-specific values for a Water-Effect Ratio (WER), a criteria adjustment factor accounting for the effect of site-specific water characteristics on pollutant bioavailability and toxicity to aquatic life. This guidance is intended to complement the 1994 *Interim Guidance on Determination and Use of Water-Effect Ratios for Metals* (EPA-823-B-94-001).

Whereas the 1994 Interim Procedure applies to essentially all situations for most metals, the Streamlined Procedure is recommended only for situations where copper concentrations are elevated primarily by continuous point source effluents. Because this is a relatively common regulatory situation, a great deal of experience is available to guide the development of a more efficient procedure.

The Streamlined Procedure does not supersede the 1994 Interim Procedure, even for the limited situations to which it applies. Rather, it provides an alternative approach. In these situations the entity conducting the study may choose between using the Interim Procedure or using the Streamlined Procedure.

Synopsis of the Streamlined Procedure

The Streamlined Procedure involves the sampling of two events, spaced at least one month apart. Flow during each event should be stable, and water quality unaffected by recent rainfall runoff events. Samples of effluent and upstream water are to be taken. These are mixed at the design low-flow dilution, to create a simulated downstream sample, to be used as the site-water sample in toxicity tests spiked with various concentrations of soluble copper salts.

In manner similar to the Interim Procedure, the side-by-side, laboratory-water and site-water toxicity tests are run to obtain the 48-hour acute EC50 with either *Ceriodaphnia dubia* or *Daphnia magna*. The result may be expressed as either dissolved or total recoverable copper. After adjusting for any hardness differences, the WER for the sample is the lesser of (a) the site-water EC50 divided by the laboratory-water EC50, or (b) the site-water EC50 divided by the documented Species Mean Acute Value (the mean EC50 from a large number of published toxicity tests with laboratory water). The geometric mean of the two (or more) sampling event WERs is the site WER.

The design of the Streamlined Procedure is intended as a more efficient approach for generating the information needed to make a pollution control decision. The intent is to provide a method that is both easier for the performing organization to carry out, and easier for the regulatory agency to review. The Streamlined Procedure omits laboratory or field measurements that experience with the Interim Procedure has shown to be of little practical value. The design is also intended to be inherently less subject to random sampling variability, thereby allowing a reduction in the number of samples while maintaining reliability.

Table 1 compares the provisions of the Streamlined Procedure with those of the 1994 Interim Procedure.

Table 1. Comparison of Streamlined Procedure and 1994 Interim Procedure

Characteristic	1994 Interim Procedure	Streamlined Procedure
Applicability	Universal	Copper from continuous discharges
Minimum number of sampling events	3	2 with recommended restrictions
Minimum number of WER measurements	4	2
Minimum number of WER measurements considered in obtaining final site WER	3	2
Preparation of constructed downstream water	Mix effluent and upstream samples at the dilution ratio occurring at the time of sampling	Mix effluent and upstream samples at the design low-flow dilution ratio
Calculation of sample WER	Site water LC ÷ Lab water LC	Site water LC ÷ The greater of (a) Lab water LC, or (b) SMAV
Calculation of final site WER	Complicated scheme with six “if...then...else” clauses and 12 possible paths	Geometric mean of the two measurements

Discussion of Technical Approach

The key facets of the procedure are presented below, with an explanation of their purpose. The detailed protocol for collecting samples, obtaining measurements, and conducting tests is presented in Appendix A. An analysis, through Monte Carlo simulation, of the protectiveness of the approach is presented in Appendix C.

1. *Purpose of procedure.* The procedure is for deriving a dissolved and/or total recoverable WER for copper from continuous point source effluents. The results may be used to obtain:
   a. A dissolved WER used to obtain the site-specific value of a dissolved copper criterion.
   b. A total recoverable WER used to obtain either (i) the site-specific value for a total recoverable criterion, or (ii) a total recoverable effluent limit from a dissolved criterion, merging the functions of a dissolved WER and a dissolved-to-total permit translator factor.

Commentary: If obtained from dissolved copper EC50s, the site WER multiplied by the national or state's hardness-normalized dissolved criterion becomes the site value of the dissolved copper criterion. Likewise, if obtained from total recoverable measurements, the WER is used to obtain the site value of the total recoverable criterion. A total recoverable WER may be applied to a dissolved criterion by first converting the dissolved criterion to a total recoverable criterion, then multiplying by the WER. In this case it eliminates the permit-specific dissolved-total translator factor by replacing the original dissolved criterion with a site-specific total recoverable criterion.

2. *Recommended applicability of the Streamlined Procedure.* The procedure is designed to apply to regulatory situations where most of the copper is from continuous point source effluents. The procedure is not designed for regulatory situations where the copper originates primarily from wet weather or nonpoint sources.

   Commentary: The Streamlined Procedure is intended to apply to situations where the copper from the regulated discharge is expected to attain its maximum concentrations under low-flow or low-dilution conditions. Continuous point source discharges fit this pattern. The most common such situation involves municipal effluents, which past experience and current knowledge have generally shown to yield low risks for copper toxicity, due to the sequestering of copper by organic matter. Nevertheless, the procedure is just as suitable for non-municipal point source effluents. The Streamlined Procedure should not be applied to situations where elevated copper concentrations are the result of wet weather runoff. In this case the critical copper concentrations may not occur under low-flow conditions, such that the Streamlined Procedure's focus on simulating low-flow conditions would not be appropriate.

3. *Collection of samples.* Sampling for WER measurements involves:
   a. Two sampling events, at least one month apart, and as recommended below,
   b. Samples of upstream and effluent during each event.

   Commentary: The Interim Procedure calls for sampling three events. The Streamlined Procedure, by more focused sampling, can achieve equivalent reliability using samples of two events, as indicated by the Monte Carlo modeling presented in Appendix C.

4. *Plant performance during sampling events should be as follows:*
   a. Average or better operating conditions,
   b. CBOD (carbonaceous biochemical oxygen demand) and suspended solids concentrations within permit limits.

   Commentary: Because the WER is sensitive to the concentration of organic matter being discharged, the plant should be operating normally, as measured by CBOD and suspended solids discharges.

5. *Stream conditions during sampling events should be as follows:*
   a. Stable flow condition, preferably during a drier weather season

(wherever regulatory schedules allow).

b. Water quality conditions should be compatible with those occurring during time periods when nonpoint source inputs of organic matter and suspended solids are relatively low.

Commentary: Sampling of events with elevated runoff is not desirable because of the confounding influences of nonpoint inputs that would not be present under low flow conditions. Because the upstream and effluent samples are to be mixed at a ratio equivalent to the low flow dilution, the upstream water quality should not be greatly dissimilar to the quality expected during low flow conditions. Upstream water quality is of greater concern if there is a substantial fraction of upstream water present during the design low-flow event, and if a total recoverable WER is to be used. Conversely, where little upstream water is to be used in creating the simulated low-flow downstream sample, or if a dissolved WER is to be applied, the quality of the sampled upstream water is less important.

6. *Merging of effluent and upstream samples.* The effluent and upstream samples are to be combined at the dilution corresponding to the design low-flow condition that the permitting authority uses in permit limit calculations.

Commentary: Extensive data from the State of Connecticut (discussed in the Supplement to Appendix C) indicate no relationship between upstream dissolved copper WER values and streamflow. Consequently, for continuous discharges the critical condition, that condition where the copper concentration can be expected to be highest relative to the WER, should be expected to occur at low dilution. The most efficient way (and often the only feasible way) to predict conditions at low flow is to mix the upstream and effluent samples at the low-flow dilution ratio.

By contrast, the 1994 Interim Procedure called for mixing the samples at the dilution occurring during the sampling event. The Interim Procedure was, however, a general purpose procedure, not restricted to the situations to which the Streamlined Procedure applies. The Interim Procedure thus also applies to situations where there is little understanding of what flow conditions could be critical.

By restricting the use to well understood situations, the Streamlined Procedure has been designed to be more efficient and its results more predictable than the Interim Procedure. Monte Carlo simulation indicates that the Streamlined Procedure, under the conditions to which it applies, is less subject to chance variation (sampling error) than the Interim Procedure.

7. *Chemical analyses.*
   a. Dissolved and/or total copper concentration
   b. Hardness, alkalinity, pH, dissolved organic carbon, and total suspended solids

Commentary: The method may be applied to either dissolved or total copper. If applied to dissolved copper, the permit authority will need to derive a translator in order to derive a total recoverable permit limit. If applied to total copper, there is no need for a translator.

Hardness measurements are needed to normalize the laboratory water and site-water EC50s to the same hardness. The remaining measurement parameters provide ancillary information for understanding the chemistry influencing the observed results and for providing a link with the Biotic Ligand Model, which is ultimately intended to replace the WER toxicity test procedures for copper.

8. *Toxicity testing.*
   a. Tested taxon: Either *Ceriodaphnia dubia* or *Daphnia magna*.
   b. Test: 48-hr EC50, spiking with copper sulfate, nitrate, or chloride.
   c. Side-by-side tests in laboratory dilution water and site water.

Commentary: These stipulations are more specific than those of the Interim Procedure. Experience has shown that the daphnids, which are quite sensitive to copper, have been the most useful test organisms for WER studies. Furthermore, for these two species there is a substantial amount of data on the range of EC50s observed in laboratory water.

Other daphnid species, such as *Daphnia pulex*, probably have similar sensitivity, but have not been recommended here because they have fewer data available for estimating the appropriate value of the Species Mean Acute Value. Other test species, either fresh or saltwater, could be substituted, provided that ample data were available to determine the appropriate SMAV. There is no technical reason why the approach could not be extended to saltwater copper tests or to other metals in fresh or saltwater. The current document's focus on freshwater copper stems from the greater demand for a streamlined procedure among the numerous freshwater copper dischargers.

The 1994 Interim Procedure recommendation for a test with a second species has been dropped, because the additional test has not been found to have value.

The simultaneously measured laboratory water EC50, as presented in the detailed protocol (Appendix A), is necessary unless the state substitutes a rigorous reference toxicant program involving copper.

9. *Analysis of data.*
   a. For site-water samples, if there is less than 50 percent mortality at the highest copper treatment concentration, then assume that the EC50 is the highest treatment concentration.
   b. The sample WER is the lesser of (i) the site-water EC50 divided by the lab-water EC50, or (ii) the site-water EC50 divided by the Species Mean Acute Value (from Appendix B).
   c. Final site WER is the geometric mean of the two (or more) sample WERs.
   d. The acute and chronic criteria concentrations for the site are the national criteria concentrations (or comparably derived state values) multiplied by the final site WER.

Commentary: (a) As in the Interim Procedure, it is not necessary to ascertain the precise value of the WER, if the actual WER is greater than the range of values of regulatory interest. (b) The Streamlined Procedure eliminates one source of concern about the Interim Procedure: the variability and apparent non-protective bias of the lab water ordinarily used in the side-by-side tests.

(c) Monte Carlo modeling indicates that use of the geometric mean of the two samples can be expected to yield a criterion as protective as or more protective than intended for chemical-specific criteria. (d) In accord with the 1994 Interim Guidance and with common practice, the WER derived from acute tests is applied to both acute and chronic criteria. Because the involvement of strong binding agents causes the WER to increase as the effect concentration decreases, the WER derived from acute tests is expected to be protective of chronic effects.

Implementation

Implementation policies for the Streamlined Procedure are the same as for the 1994 Interim Procedure. WER-based site-specific criteria provisions are subject to EPA review under Section 303(c) of the Clean Water Act and its implementing regulations at 40 CFR Part 131. This can be structured in two ways.

1. A state may submit each individual determination of a WER-based site-specific criteria value to EPA for review and approval.

2. A state may incorporate WER adjustment provisions into its water quality standards, submitted to EPA for review and approval. Once the provisions are in place, the results of each site-specific application of the procedure would be subject to public participation requirements, but would not be submitted for further Section 303(c) review.

In all cases, it should be noted that the WER derivation is part of the standards setting process. In the absence of an appropriate specification of the site criterion, WERs are not used for adjusting reasonable potential calculations, wasteload allocations, or permit limits.

Appendix A

Sampling and Testing Protocol for the Streamlined WER Procedure

The procedure set forth below is for deriving a WER for copper discharged to fresh water primarily from continuous point source effluents, using Daphnia as the test taxon.

A. Background Information

1. Obtain information on the mean flow and design dilution flow for the water body segment for which the WER is being determined. If the proposed effluent limit was calculated using time-variable modeling without reference to a particular design low flow, then for purposes of applying the Streamlined Procedure, use the design flow that the state customarily applies to steady-state dilution calculations.

2. Estimate the values of the site-specific criterion and WER corresponding to key decision points, for example, the minimum values needed for the determination of "no reasonable potential", upon which depends the need for copper permit limits.

3. Consult the state or tribal pollution control agency in order to assure that the study will respond to all concerns with respect to the peculiarities of the specific site.

B. Acquiring and Acclimating Test Organisms

1. Obtain, culture, hold, acclimate, feed, and handle the test organisms as recommended by U.S. EPA (1993) and/or by ASTM (1999, 2000a, 2000b).

2. Acclimation to the site water is desirable but optional.

C. Collecting and Handling Upstream Water and Effluent

1. Obtain samples during two (or more) sampling events, spaced at least four weeks apart. When regulatory schedules allow, schedule events for a season when low streamflows are more likely to occur.

2. For each sampling event, obtain a representative sample of upstream water, relatively unaffected by recent runoff events that might elevate the total suspended solids and organic matter concentrations.

3. For each sampling event, obtain a representative sample of effluent during a period when the discharger is operating normally, relatively unaffected by short-term perturbations due to rainfall inflow or slug loads. Composite samples are preferred over grab samples.

4. Collect, transport, handle, and store samples as recommended by U.S. EPA (1993). Obtain a sufficient volume so that some can be stored for additional

testing or analyses if unusual results are obtained. Store samples at 0 to 4°C in the dark with no air space in the sample container.

5. During the sampling event, measure effluent parameters that are normally required to be reported in the Discharge Monitoring Report for the discharge under study. These measurements provide information on the representativeness of the effluent samples.

6. For the sampling event, obtain streamflow data at the nearest relevant gaging station, and rainfall data and any other relevant meteorological information for the preceding two weeks.

7. Consider using chain of custody procedures for all samples of site water and effluent.

8. Begin toxicity tests as soon as practical, but always within 96 hours after collecting samples, in accord with EPA (1993) recommendations for using site water for the dilution water. Because these tests are not intended for measurement of whole effluent toxicity (which might attenuate over time), it is not essential to begin tests within 36 hours after the collection of the samples (as would be recommended by EPA (1993) for measuring the toxicity of an effluent). This is a change from the 1994 Interim Procedure recommendation.

9. If the site water might contain predators of the daphnid test organisms, remove them by filtering through a 37-60 μm sieve or screen.

D. Laboratory Dilution Water

1. Use laboratory water that accords with U.S. EPA (1993) or ASTM (1999, 2000a). Use ground water, surface water, reconstituted water, diluted mineral water, or dechlorinated tap water that has been demonstrated to be acceptable to aquatic organisms. If a surface water contains predators, remove them by filtering through a 37-60 μm sieve or screen. Do not use water prepared by such treatments as deionization or reverse osmosis unless salts, or mineral water are added as recommended by U.S. EPA (1993) or ASTM (1999, 2000a).

2. Do not use laboratory water with DOC, TOC, or TSS >5 mg/L.

3. Use laboratory water with hardness between 40 and 220 mg/L. Within these ranges use laboratory water with hardness relatively close to that of the site water.

4. The alkalinity and pH of the laboratory dilution water is to be appropriate for its hardness. Values for alkalinity and pH that are appropriate for some values of hardness are given by U.S. EPA (1993) and ASTM (1999, 2000a); other corresponding values should be determined by interpolation. If necessary, adjust alkalinity using sodium bicarbonate, and pH using aeration, sodium hydroxide, and/or sulfuric acid.

E. Conducting Tests

1. Conduct the tests such that there are no differences between the side-by-side tests other than the composition of the

dilution water, the concentrations of metal tested, and possibly the water in which the test organisms are acclimated just prior to the beginning of the tests.

2. A single laboratory water test may be compared with tests of multiple site water samples conducted side by side.

3. Follow the recommendations of U.S. EPA (1993) and/or ASTM (1999, 2000a, 2000b) regarding setting up facilities for conducting toxicity tests and selecting and cleaning the test chambers.

4. Prepare a stock solution of copper chloride 2-hydrate ($CuCl_2{\cdot}2H_2O$), copper nitrate 2.5-hydrate ($Cu(NO_3)_2{\cdot}2.5H_2O$), or copper sulfate 5-hydrate ($CuSO_4{\cdot}5H_2O$).
    a. Use reagent-grade material.
    b. Only as necessary to get the metal into solution, acidify the stock solution using metal-free nitric acid.
    c. Use the same stock solution for all tests conducted at one time.

5. In the unusual situation where the effluent is dominated by highly stable metallo-organic compounds, such as copper phthalocyanine dyes, then an exception to using the above listed copper salts may be considered. That is, it may be acceptable to include the metallo-organic compounds in the stock solution. In such case, prepare the stock solution such that the stable metallo-organic compounds constitute the same percentage of total copper in the stock solution as in the site water to be WER tested. This exception to the usual procedure requires case-by-case review of its appropriateness, after documenting the usual presence of the compounds in the effluent, the stability of the compounds, and the importance of distinguishing the compounds from other forms of copper.

6. Run the 48-hour test with *Ceriodaphnia dubia* or *Daphnia magna* to obtain an EC50 (U.S. EPA 1993; ASTM 1999, 2000a, 2000b), using a sufficient volume to accommodate the needed chemical measurements. With appropriate modification of the protocol, other test species may be substituted on a case-by-case basis, only if (a) the daphnid SMAV is either significantly below the site criterion obtained by the Recalculation Procedure, or daphnia is not viable at the site water salinity; and (b) there is sufficient data to establish an SMAV with high reliability.

7. Static tests may be used if dissolved oxygen remains sufficient, and if either (a) measured dissolved copper concentrations do not decrease more than 50 percent by the end of a test intending to use the dissolved copper measurements as the basis for the WER, or (b) total recoverable concentrations are to be used as the basis for the WER.

8. Renew the test solutions after 24 hours if either (a) dissolved oxygen would decrease too much, or (b) dissolved copper would decrease more than 50% by the end of a test intended for a dissolved WER, or (c) the analyst favors renewal for any other reason.
    a. If solutions in one test in a pair of side-by-side tests are renewed, renew solutions in the other test as well.
    b. To renew site water solutions, prepare new test solutions from the remaining site water sample(s) stored at 0 to 4°C in the dark.

9. Follow recommendations on temperature, loading, feeding, dissolved oxygen, aeration, disturbance, and controls given by U.S. EPA (1993) and/or ASTM (1999, 2000a, 2000b).

10. Use range-finding tests, of 8 to 48-hour duration where necessary and where they will not unduly delay the beginning of the test.

11. Use a dilution factor of 0.6 or greater for nominal concentrations, balancing for the particular situation the needs for covering the range of possible results, reducing the uncertainty in the calculated EC50, and curbing the cost of the test. The value of the WER needed for a "no reasonable potential" determination or other decision benchmarks should be kept in mind in selecting the dilution series. With regard to the risk that the dilution series will not span the possible range of the EC50, note that in *site water* (a) a "less than value" for the EC50 would be unusable for obtaining a WER>1, and (b) a "greater than" value" for the EC50 would be interpreted as "equal to" in calculating the WER. In contrast, in *lab water* (c) a "greater than value" for the EC50 would be unusable for obtaining a WER>1, (d) a "less than value" for the EC50 would be interpreted as "equal to"; however, (e) the SMAV from Appendix B would be used in place of any lab water EC50 less that SMAV.

12. Use an unspiked dilution water control for each test.

13. Use at least 20 organisms for each treatment concentration (including unspiked controls). It is desirable to use two or more test chambers per treatment. Assign test organisms randomly, or at least impartially to the side-by-side tests, and to all treatment chambers (U.S. EPA 1993; ASTM 1999, 2000a). When assigning impartially, do not place more than 20% of any chamber's total number of organisms at one time. In addition, the test chambers should be assigned to location in a random arrangement or in a randomized block design.

14. Use appropriate glassware for making serial dilutions, such as graduated cylinders, volumetric flasks, and volumetric pipettes.

15. For the test using site water, mix effluent and upstream waters to accord with the design dilution, corresponding to the upstream design flow and effluent flow normally used for steady state modeling calculations. Generally use any one of the following procedures to prepare the test solutions for the test chambers and the "chemistry controls", if any (see section F.1):
    a. 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 to 24 hours (if overnight, keep at 0 to 4°C); add the necessary amount of upstream water to each test chamber; mix thoroughly; let stand for 1 to 3 hours.
    b. Add the necessary amount of metal 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 to 24 hours (if overnight, keep at 0 to 4°C); add the necessary amount of upstream water to each test chamber; mix thoroughly; let stand for 1 to 3 hours.

   c. 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 to 4 hours.
   d. 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 to 4 hours.

   Procedures "a" and "b" allow the metal to equilibrate with the effluent before the solution is diluted with upstream water.

16. 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" (see section F.1):
   a. 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 to 4 hours.
   b. 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 to 4 hours.

17. Add the test organisms, acclimated per section B.1, to the test chambers for the side-by-side tests at the same time.

18. Observe the test organisms and record the effects and symptoms as specified by U.S. EPA (1993) and/or ASTM (1999, 2000a).

F. Chemical and Other Measurements

1. To reduce the possibility of contamination of test solutions before or during tests, do not place thermometers and probes for measuring pH and dissolved oxygen into test chambers. Rather, perform such measurements on "chemistry controls" that contain test organisms or on aliquots that are removed from the test chambers. The other measurements may be performed on the actual test solutions at the beginning and/or end of the test or the renewal.

2. Measure hardness, pH, alkalinity, TSS, and DOC for the tested site water and the laboratory dilution water.

3. Measure dissolved oxygen, pH, and temperature during the test at the times specified by U.S. EPA (1993) and/or ASTM (1999, 2000a, 2000b), using the same schedule for both of the side-by-

side tests. If chemistry controls are used, obtain measurements on both the chemistry controls and actual test solutions at the end of the test.

4. For a dissolved WER calculated from dissolved measurements, measure dissolved copper in the appropriate test solutions. For a total recoverable WER, measure total recoverable copper. It is desirable but not essential for both dissolved and total recoverable copper to be measured. EPA methods (U.S. EPA 1997, 1999) are recommended. In any case use appropriate QA/QC techniques to assure attaining the target level of accuracy.
    a. Rather than measuring the metal in all test solutions, it is often possible to store samples and then analyze only those that are needed to calculate the results of the toxicity tests. Measure (i) all concentrations in which some, but not all, of the test organisms were adversely affected, (ii) the highest concentration that did not adversely affect any test organisms, (iii) the lowest concentration that adversely affected all of the test organisms, and (iv) the controls.
    b. For total recoverable copper, measure once for a static test, or twice for a renewal test (once per day). For measurement of total recoverable copper in the test chamber, mix the whole solution in the chamber before the sample is taken for analysis. Do not acidify the solution in the test chamber before the sample is taken. Rather, acidify after it is placed in the sample container.
    c. Measure dissolved metal at the beginning and end of a static test, or at the beginning and end of the first day in a renewal test (that is, just before renewal). For measurement of dissolved metal in a test chamber, mix the whole solution in the test chamber before removing a sufficient amount for filtration. Do not acidify before filtration. Filter the sample within on hour after it is after it is taken, then acidify the filtrate.

5. Perform QA/QC checks.

G. Calculating and Interpreting the Results

1. Evaluate the acceptability of each toxicity test individually.
    a. Reject tests where deviations from the above presented laboratory practices are substantial, particularly with respect to acclimation, randomization, temperature control, measurement of metal, and/or disease or disease-treatment.
    b. Reject tests where more than 10 percent of the organisms in the controls were adversely affected.

2. Calculate the EC50 using methods described by U.S. EPA (1993) or ASTM (1999, 2000a). If two or more treatments affected between 0 and 100 percent in both tests in a side-by-side pair, use probit analysis to calculate results of both tests, 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, either because fewer than two percentages are between 0 and 100 percent or because

the model does not fit the data, use computational interpolation; do not use graphical interpolation. Use the same computational method for each of the side-by-side tests.

3. For laboratory water:
    a. Calculate or assign the EC50 for the lab water only if the percent of the organisms that were adversely affected is *greater than* 50 percent in at least *one* treatment (although it is preferable if at least 63 percent of organisms were affected). That is, if there is insufficient toxicity at all concentrations in the laboratory water, the side-by-side tests are not usable for obtaining a WER>1.
    b. If no treatment other than the control affected *less than* 50 percent of the test organisms, set the EC50 equal to the *lowest* test concentration (preceded by < sign) . That is, if there is excessive toxicity at all tested concentrations (except the control), the laboratory water EC50 is known only to be less than the lowest treatment concentration.
    c. If the hardness-normalized EC50 in laboratory water is less than the documented SMAV for the species, then use the SMAV in place of the laboratory water EC50 in the dominator of the WER. See Appendix B for the SMAVs for *Ceriodaphnia dubia* and *Daphnia magna*.

4. For site water:
    a. Calculate or assign the EC50 for the site water only if the percent of the organisms that were adversely affected is *less than* 50 percent in at least *one* treatment (although it is preferable if less than 37 percent of organisms were affected). That is, if there is excessive toxicity at all tested concentrations in site water, the sample is not usable for obtaining a WER>1.
    b. If no treatment affected *more than* 50 percent of the test organisms, set the EC50 equal to the *highest* test concentration (preceded by > sign). That is, if there is insufficient toxicity at all tested concentrations, the site water EC50 is known only to be greater than the highest treatment concentration.

5. In reporting results, highlight anything unusual or questionable about the test findings.
    a. Report if dissolved metal decreased by more than 50 percent from the beginning to the end of a 48-hour static test.
    b. Report if there were inversions in the data for more than two concentrations in the range of 20 to 80 percent mortality (or as modified by Abbott's formula).

6. Normalize the (a) laboratory-water EC50, (b) the site-water EC50, and (c) the SMAV EC50 to the same hardness, using the formula:

$$\mathrm{EC50}_{\text{at Std Hdns}} = \mathrm{EC50}_{\text{at Sample Hdns}} \cdot \left(\frac{\text{Std Hdns}}{\text{Sample Hdns}} \right)^{0.9422}$$

Where “Std Hdns” is any particular standard hardness value to which all values will be normalized, and “Sample Hdns” is the hardness of the laboratory water, the site water, or the SMAV. The exponent 0.9422 is the log-log slope for the 1984/1985 and 1995 EPA acute criteria. If different from 0.9422, it is appropriate to use the hardness slope of the state criterion.

7. Calculate the sample WER from values normalized to the same hardness.
   a. If the laboratory-water, hardness-normalized EC50 is greater than the hardness-normalized SMAV, the sample WER equals the site-water EC50 divided by the laboratory-water EC50.
   b. If the laboratory-water, hardness-normalized EC50 is less than the hardness-normalized SMAV, the sample WER equals the site-water EC50 divided by the SMAV. See Appendix B for a value of the SMAV. Other comparably well-documented values for the SMAV may be used.

8. Calculate the site WER as the geometric mean of the two (or more) sample WERs. Compare the result against WER values typically expected for the type of situation under study. Additional chemical or toxicological data may be needed to support unusually high WERs.

9. Calculate the site acute and chronic criteria concentrations as the ordinary hardness-adjusted criteria concentration (that is, the value that would have been applicable for a default assumption that WER=1), multiplied by the final site WER.

H. Reporting the Results

1. Include the following general information in the report submitted to the appropriate regulatory agency:
   a. Identity of the investigators and the laboratory.
   b. Name, location, and description of the discharger; description of the effluent and the receiving water.
   c. Effluent and upstream water flows used to calculate the dilution ratio.
   d. Dilution ratio used in mixing effluent and upstream water to prepare the site water.
   e. Downstream design hardness expected to be used for the permit derivation.
   f. The values of the site-specific criterion and WER estimated to correspond to a determination of no reasonable potential or to other pollution control decision benchmarks relevant to the purpose of the study.
   g. Identification of each sampling station.
   h. Procedures used to obtain, transport, and store the samples of the upstream water and the effluent.
   i. Any pretreatment, such as filtration, of the effluent, site water, and/or laboratory dilution water.
   j. Description of the laboratory dilution water, including source, and preparation.
   k. Results of all chemical and physical measurements on upstream water, effluent, actual and/or simulated downstream water, and laboratory dilution water, including hardness, alkalinity, pH, and concentrations

of total recoverable or dissolved metal, TSS, and DOC.
   - l. Description of the experimental design, test chambers, volume of solution in the chambers, photoperiod, and numbers of organisms and chambers per treatment.
   - m. Source and grade of the copper salt, and how the stock solution was prepared.
   - n. Species and source of the test organisms, age, and holding and acclimation procedures.
   - o. The average and range of the temperature, pH, hardness, alkalinity, and the concentration of dissolved oxygen (mg/L) during acclimation.

2. Include the following information for each sample or toxicity test.
   - a. Date and time of sampling site water and date of toxicity test.
   - b. Effluent flow during the sampling event.
   - c. Upstream flow during and prior to the sampling event, either measured directly or estimated from relevant neighboring gages.
   - d. Prior meteorological conditions affecting flow and sampled water quality.
   - e. Measurements of hardness, alkalinity, pH, and DOC.
   - f. The average and range of the measured concentrations of dissolved oxygen (in mg/L).
   - g. The average and range of the test temperature.
   - h. A summary table of the concentrations of copper in each treatment, including controls, and the number of organisms affected, in sufficient detail to allow independent statistical analysis of the data.
   - i. The EC50 and the method used to calculate it.
   - j. Anything unusual about the test, any deviations from the procedures described above, and any other relevant information.
   - k. 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 site water.

3. Include the following information in a summary table:
   - a. EC50s and hardness for each test in a site-water, laboratory-water comparison, *not* normalized for hardness.
   - b. EC50s for each site-water, laboratory-water (or SMAV) comparison, after normalizing for hardness.
   - c. The calculated sample WERs.

4. Present the calculated site WER and site criterion.

References for Appendix A

ASTM. 1999. ASTM Standards on Biological Effects and Environmental Fate, 2nd Edition. American Society for Testing and Materials, West Conshohocken, PA.

ASTM. 2000a. E729-96 Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians. American Society for Testing and Materials, West Conshohocken, PA.

ASTM. 2000b. E1192-97 Standard Guide for Conducting Acute Toxicity Tests on Aqueous Effluents with Fishes, Macroinvertebrates, and Amphibians. American Society for Testing and Materials, West Conshohocken, PA.

U.S. EPA. 1993. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fourth Edition. http://www.epa.gov/ost/WET/disk2/. EPA/600/4-90/027F. National Service Center for Environmental Publications, Cincinnati, OH.

U.S. EPA. 1997. Trace Metals Guidance (diskette). EPA 821/C-97-002. http://www.epa.gov/OST/pc/analytic.html. EPA Office of Water Resource Center, Washington, DC.

U.S. EPA. 1999. Methods and Guidance for the Analysis of Water, Revision 2 (on CD-ROM). EPA 821/C-99-004. http://www.epa.gov/OST/pc/analytic.html. EPA Office of Water Resource Center, Washington, DC.

Appendix B

Species Mean Acute Values for *Ceriodaphnia dubia* and *Daphnia magna*

Species Mean Acute Values, SMAVs, for *Ceriodaphnia dubia* and *Daphnia magna* were obtained by tabulating available toxicity data, normalizing for hardness differences using the 1985 and 1995 EPA hardness slope for copper, and calculating the geometric mean of all EC50 results for each species.

Normalized to a hardness of 50 and 100 mg/L as $CaCO_3$, the results are as follows:

Species	SMAV EC50, µg/L			
	At Hardness = 50 mg/L		At hardness = 100 mg/L	
	Total Cu	Dissolved Cu	Total Cu	Dissolved Cu
Ceriodaphnia dubia	12.49	11.51	24.00	22.11
Daphnia magna	10.47	10.05	20.12	19.31

The SMAV at any other hardness is obtained as follows:

$$\text{SMAV}_{\text{at Site Hdns}} = \text{SMAV}_{\text{at Hdns}=100} \cdot \left(\frac{\text{Site Hdns}}{100\text{ mg/L}}\right)^{0.9422}$$

The exponent, 0.9422 is the EPA's copper criterion hardness slope. It is slightly higher than found in those Table B-1 studies that investigated the effect of hardness: Belanger et al. 1989, Belanger and Cherry 1990, Chapman et al. manuscript, and Borgmann and Charlton 1984. Other well documented slopes may also be suitable for normalizing the data.

Table B-1: EC50s for 59 reported results with *Ceriodaphnia dubia* and 55 reported results with *Daphnia magna*.

Species	Method[a]	Hrdns (mg/L as $CaCO_3$)	EC50 Total (µg/L)	EC50 Diss. (µg/L)	EC50 Total, Adjusted to Hrdns=100 (µg/L)[b]	EC50 Diss., Adjusted to Hrdns=100 (µg/L)[b]	Reference
Ceriodaphnia dubia (<24 h)	S, M	45		25.0		53.05	Belanger et al. 1989
				17.0		36.07	
				30.0		63.66	
				24.0		50.93	
				28.0		59.42	
				32.0		67.90	
				23.0		48.81	
				20.0		42.44	
				19.0		40.32	
Ceriodaphnia dubia (<24 h)	S, M	94.1		26.0		27.53	Belanger et al. 1989
				21.0		22.24	
				27.0		28.59	
				37.0		39.18	
				34.0		36.00	
				66.0		69.89	
				63.0		66.72	
				71.0		75.19	
Ceriodaphnia dubia (<24 h)	S, M	179		67.0		38.71	Belanger et al. 1989
				38.0		21.96	
				78.0		45.07	
				81.0		46.80	
Ceriodaphnia dubia (<24 h)	S, M	97.6	14		14.32		Belanger and Cherry 1990
			28		28.65		
			31		31.72		
Ceriodaphnia dubia (<24 h)	S, M	113.6	52		46.11		Belanger and Cherry 1990
			76		67.40		
			91		80.70		
Ceriodaphnia dubia (<24 h)	S, M	182.0	56		31.85		Belanger and Cherry 1990
			84		47.78		
			93		52.90		
Ceriodaphnia dubia (<12 h)	S, M	90	13.4		14.80		Oris et al. 1991
Ceriodaphnia dubia	S, M	87.5	11.25		12.76		Neserke 1994
		80.8	13.17		16.10		
		80.8	25.25		30.87		
		60	11.25		18.20		
		30	4.5		13.99		
Ceriodaphnia dubia	S, U	188	36.6		20.19		Bright 1995
		204	19.1		9.76		
		428	36.4		9.25		
		410	11.7		3.10		
		494	12.3		2.73		
		440	12.0		2.97		
Ceriodaphnia dubia (<24 h)	S, M	80	6.98		8.61		Diamond et al. 1997b

Species	Method[a]	Hrdns (mg/L as $CaCO_3$)	EC50 Total (µg/L)	EC50 Diss. (µg/L)	EC50 Total, Adjusted to Hrdns=100 (µg/L)[b]	EC50 Diss., Adjusted to Hrdns=100 (µg/L)[b]	Reference
Ceriodaphnia dubia	S, M	99	10.1	10.65	10.20	10.75	Tetra Tech 1998
		70	14.65	13.34	20.50	18.67	
		74	6.72	6.53	8.92	8.67	
		72	6.59	4.55	8.98	6.20	
		148	23.3	17.74	16.10	12.26	
		148	24.99	15.34	17.27	10.60	
		142	18.91	14.7	13.59	10.56	
		144	73.5	49	52.13	34.75	
		148	18.48	8.24	12.77	5.70	
		200	31.77	20.99	16.53	10.92	
		193	58.82	32.39	31.66	17.43	
		198	31.53	21.14	16.57	11.11	
		194	39.38	18.25	21.09	9.77	
Ceriodaphnia dubia	S, M	78	13.1		16.56		Dimond 2000
		90	8.88		9.81		
		90	10.3		11.37		
Daphnia magna (<24 h)	S, U	45.3	9.8		20.67		Biesinger & Christensen 1972
Daphnia magna (1 d)	S, U	99	85		85.81		Adema & Degroot-van Zijl 1972
			50		50.48		
Daphnia magna	S, U	45	10		21.22		Cairns et al. 1978
Daphnia magna	S, M	100	31.8		31.80		Borgmann & Ralph 1983
Daphnia magna (<24 h)	S, U	45	54		114.59		Mount & Norberg 1984
Daphnia magna (<24 h)	S, U	240	41		17.97		Elnabarawy et al. 1986
Daphnia magna (<4 h)	S, M	54	7		12.51		Nebeker et al. 1986
Daphnia magna	S, M	80					Nebeker et al. 1986
(1 d)			10		12.34		
(2 d)			6		7.40		
(3 d)			14		17.28		
(4 d)			7		8.64		
(5 d)			10		12.34		
(6 d)			18		22.21		
Daphnia magna	S, U	240	93		40.76		Khangarot and Ray 1989
Daphnia magna	S, U	10	21.5		188.21		Hickey and Vickers 1992

Species	Method[a]	Hrdns (mg/L as $CaCO_3$)	EC50 Total (µg/L)	EC50 Diss. (µg/L)	EC50 Total, Adjusted to Hrdns=100 (µg/L)[b]	EC50 Diss., Adjusted to Hrdns=100 (µg/L)[b]	Reference
Daphnia magna	S, U	33.8	11.5		31.96		Koivisto et al. 1992
Daphnia magna	S, U	50	7		13.45		Oikari et al. 1992
Daphnia magna	S, M	52	26		48.15		Chapman et al. Manuscript
		105	30		28.65		
		106	38		35.97		
		207	69		34.76		
Daphnia magna	S, M	170	41.2		24.99		Baird et al. 1991
			10.5		6.37		
			20.6		12.50		
			17.3		10.49		
			70.7		42.88		
			31.3		18.99		
Daphnia magna (<24 h)	S, M	100	7.1		7.10		Meador 1991
			18.7		18.70		
			18.9		18.90		
Daphnia magna (<24 h)	S, M	170	31		18.80		Lazorchak and Waller 1993
			38		23.05		
			35		21.23		
			58		35.18		
			37		22.44		
			51		30.93		
			39		23.66		
			50		30.33		
			52		31.54		
			31		18.80		
			30		18.20		
			46		27.90		
			63		38.21		
Daphnia magna (<24 h)	S, M	72	4.8		6.54		Dimond 2000
		76	6.24		8.08		
		80	6.17		7.61		
		72	6.62		9.02		
		84	10.2		12.02		
		84	10.8		12.73		
		44	4.6		9.97		
		44	7.8		16.91		
		36	3		7.86		
		76	10.4		13.47		
		88	16.9		19.06		

[a] Methods: S = Static, M = Measured, U = Unmeasured

[b] Adjusted EC50 at hardness 100 mg/L is obtained from the test measured dissolved or total EC50 as follows:

$$\text{Adjusted EC50} = \text{Test EC50} \cdot \left(\frac{100\ \text{mg/L}}{\text{Test Hdns}}\right)^{0.9422}$$

Where only the test total EC50 is known, the test dissolved EC50 is estimated from $EC50_D = EC50_T \cdot 0.96$.
Where only the test dissolved EC50 is known, the test total EC50 is estimated from $EC50_T = EC50_D/0.96$.

References for Appendix B

Adema, D.M.M. and A.M. Degroot-van Zijl. 1972. The influence of copper on the water flea *Daphnia magna*. TNO Nieuws 27:474.

Baird, D.J., I. Barber, M. Bradley, A.M.V.M. Soares and P. Calow. 1991. A comparative study of genotype sensitivity to acute toxic stress using clones of *Daphnia magna* straus. Ecotoxicol. Environ. Saf. 21(3):257-265.

Belanger, S.E., J.L. Farris and D.S. Cherry. 1989. Effects of diet, water hardness, and population source on acute and chronic copper toxicity to *Ceriodaphnia dubia*. Arch. Environ. Contam. Toxicol. 18(4):601-11.

Belanger, S.E. and D.S. Cherry. 1990. Interacting effects of pH acclimation, pH, and heavy metals of acute and chronic toxicity to *Ceriodaphnia dubia* (Cladoceran). J. Crustacean Biol. 10(2):225-235.

Biesinger, K.E. and G.M. Christensen. 1972. Effects of various metals on survival, growth, reproduction, and metabolism of *Daphnia magna*. Jour. Fish Res. Board Can. 29:1691.

Borgmann, U. and K.M. Ralph. 1983. Complexation and toxicity of copper and the free metal bioassay technique. Water Res. 17:1697.

Bright, G.R. 1995. Variability of the “water effect ratio” for copper toxicity - a case study. Proc. Toxic. Subst. Water Environ. 5/23-5/30. Water Environment Federation, Alexandria, VA.

Cairns, J., Jr. et al. 1978. Effects of temperature on aquatic organism sensitivity to selected chemicals. Bulletin 106. Virginia Water Resources Research Center, Blacksburg, VA.

Chapman, G.A. et al. Manuscript. Effects of water hardness on the toxicity of metals to *Daphnia magna*. U.S. EPA, Corvallis, OR.

Diamond, J.M., D.E. Koplish, J. McMahon, III and R. Rost. 1997b. Evaluation of the water-effect ratio procedure for metals in a riverine system. Environ. Toxicol. Chem. 16(3):509-520.

Dimond, W.F. 2000. Letter to Charles Delos: peer review of Streamlined Water-Effect Ratio Procedure. July 21, 2000. State of Michigan, Department of Environmental Quality. Lansing, MI.

Elnabarawy, M.T., A.N. Welter and R.R. Robideau. 1986. Relative sensitivity of three daphnid species to selected organic and inorganic chemicals. Environ. Toxicol. Chem. 5(4):393-8.

Hickey, C.W. and M.L. Vickers. 1992. Comparison of the sensitivity to heavy metals and pentachlorophenol of the mayflies *Deleatidium* spp. and the cladoceran *Daphnia magna*. N.Z.J. Mar. Freshwater Res. 26(1):87-93.

Khangarot, B.S. and P.K. Ray. 1989. Investigation of correlation between physicochemical properties of metals and their toxicity to the water flea *Daphnia* *magna* Straus. Ecotoxicol. Environ. Saf. 18(2):109-20.

Koivisto, S., M. Ketola and M. Walls. 1992. Comparison of five cladoceran species in short- and long-term copper exposure. Hydrobiol. 248(2):125-36.

Lazorchak, J.M. and W.T. Waller. 1993. The relationship of total copper 48-H LC50s to *Daphnia magna* dry weight. Environ. Toxicol. Chem. 12(5):903-11.

Meador, J.P. 1991. The interaction of pH, dissolved organic carbon, and total copper in the determination of ionic copper and toxicity. Aquat. Toxicol. 19(1):13-31.

Mount, D.I. and T.J. Norberg. 1984. A seven-day life-cycle cladoceran toxicity test. Environ. Toxicol. Chem. 3:425.

Nebeker, A.V., M.A. Cairns, S.T. Onjukka and R.H. Titus. 1986. Effect of age on sensitivity of *Daphnia magna* to cadmium, copper and cyanazine. Environ. Toxicol. Chem. 5(6):527-30.

Neserke, G. 1994. Written testimony to Colorado Department of Health Water Quality Control Commission on behalf of the Coors Brewing Company.

Oikari, A., J. Kukkonen and V. Virtanen. 1992. Acute toxicity of chemicals to *Daphnia magna* in humic waters. Sci. Total Environ. 117-188:367-77.

Oris, J.T., R.W. Winner and M.V. Moore. 1991. A four-day survival and reproduction toxicity test for *Ceriodaphnia dubia*. Environ. Toxicol. Chem. 10(2):217-24.

Tetra Tech. 1998. Determination of Copper LC50s for Calculation of Water Effect Ratios and Effluent Limits for POTWs in Southeastern Pennsylvania. Tetra Tech, Owings Mills, MD.

Appendix C

Assessment of the Streamlined Water-Effect Ratio Procedure

Abstract

The protectiveness of the Streamlined Procedure for obtaining a copper water-effect ratio (WER) for streams affected by point source discharges has been assessed using Monte Carlo probabilistic modeling.

The Streamlined Procedure uses two WER samples to set the site WER. The probabilistic modeling considered that the two WER samples were collected from a situation where the flow, toxicant concentration, and WER vary over time.

This analysis evaluated the suitability of calculating each site's WER as the geometric mean of the two WER measurements for the site, when the effluent and upstream samples are mixed at the design low-flow dilution. Comparison was made against the predicted unbiased value for the WER: that is, the value that the WER would have if the site-specific criterion were to have the same level of protection as intended for the national criterion.

Overall the results of this work indicated that the Streamlined Procedure tends to yield a site WER slightly more restrictive than the unbiased site WER. In 50 percent of the Monte Carlo trials, the calculated WER was less than 0.84 times the unbiased site WER. Within the range of conditions investigated, the design downstream dilution had no significant effect on the level of protection provided.

In addition, in the Supplement to this Appendix, an estimate was made of the effect, relative to the 1994 Interim Procedure, of having the Streamlined Procedure restrict the lab water EC50 to a value not less than the EPA SMAV. Sixteen lab water EC50s from three WER studies were evaluated. By including the difference in the way the two procedures set the lab water EC50, the Streamlined Procedure and the Interim Procedure could be appropriately compared through the Monte Carlo simulation. Results indicated that the two procedures yielded similar results.

Introduction

The purpose of this assessment is to determine, through modeling, whether the Streamlined Procedure provides the degree of protection intended for aquatic life criteria. The Supplement to this Appendix also deals with the following issues: (a) the purpose of streamlining the copper WER procedure, (b) the reason for preparing samples by mixing at design dilution, (c) the need for the simultaneous laboratory water test, (d) the potential for reducing the number of samples, and (e) a comparison with the 1994 Interim Procedure.

Assessment Strategy

The water-effect ratio (WER) reflects the effect that local site water constituents have on increasing or reducing the pollutant bioavailability and toxicity. The concentrations of the site water constituents that control the WER can be expected to vary over time, as do all water quality parameters. A site's WER would thus also vary over time. Consequently, the number of samples in any reasonably feasible sampling scheme cannot fully characterize the site.

The question investigated here is whether it is appropriate to calculate the site WER as the geometric mean of the two samples, mixed at the low-flow dilution. This issue can be addressed by asking: How does the level of protection provided by such a WER derivation compare with the level of protection intended for aquatic life criteria?

If one continually knew what the actual WER was during each increment of time, then one could continually reset the site criterion such that it would always correctly reflect the actual conditions. Thus, if one made continual WER measurements and continual adjustments to the site criterion, then there would be no "final WER" and no issue about its method of calculation. Assuming that the WER measurements were correct, the continual adjustments of the site criterion would yield a level of protection exactly equal to what was intended for the criterion.

It is of course infeasible to continually measure the WER over time. However, since we know something about the statistical properties of water quality parameters, it is feasible to set up a mathematical modeling experiment to mimic continual measurements, using realistic-looking hypothetical distributions of WER values and other site parameters. In this way it is possible to examine the behavior of various sampling and calculation alternatives. That is, given an assumed distribution of time-variable WERs, one can compare (a) the level of protection provided by any alternative for obtaining the final WER (and site criterion), to (b) the neutral or unbiased level of protection provided by continually resetting the site criterion using the WER specific to each event.

The basic strategy for performing this analysis (modified from Delos 1998, which in turn evolved from Delos 1994) is to set up one or more scenarios of hypothetical site applications by specifying the statistical properties of the facility WERs, flows, and toxicant concentrations. Reflecting the time variability of a site, a very large number of such WERs, with accompanying flow and toxicant concentration values, can then be randomly generated. One may then compare the behavior of (a) the Streamlined Procedure for deriving a site WER, with (b) the unbiased technique of continually resetting the WER and site criterion.

For a situation where the WER is varying over time, and the site criterion is continually being re-set in accordance with the WER, the time variations in the toxicant concentration will yield some frequency of criteria violations. Assuming that this frequency of criteria violations is acceptable, one can find, by trial and error, a single, fixed (time-invariant) value for the WER, and likewise a single, fixed site criterion, that yields exactly the same frequency of violations as the time-variable WER and time-variable site criterion. This single, fixed value for the WER, yielding the same violation frequency as the time-variable WER, may be termed the *unbiased final WER*.

This fixed unbiased WER represents the “correct” value for site’s final WER. However, its value could only be ascertained if one had a long record of measured WERs and toxicant concentrations for the site, something that can be obtained for a model simulation site but not a real-world site. In a real-world site only a small sampling is obtained for the values that the WER may take over time. The purpose of the Monte Carlo modeling is to compare the final WER values derived from small samples of the of the site's distribution of WERs to the correct value represented by the unbiased final WER.

The Monte Carlo analysis considers that a site has time-variable values for streamflow, toxicant concentration, and WER. It also considers that the sites to which the Streamlined Procedure applies differ somewhat in their design flow dilution ratio.

Modeling Procedure

It is *not* necessary to read this section in order to understand and use the modeling results presented later. Readers not interested in the procedural details may skip to **Results**, the next section.

The Monte Carlo model is set up as follows. For any particular discharge situation, the following sources of variability are taken into account:

- Time variable dilution ratio of upstream flow to effluent flow
- Upstream time-variable toxicant concentration
- Upstream time-variable WER
- Effluent time-variable toxicant concentration
- Effluent time-variable WER

That is, the modeling work assumes that the dilution flow, toxicant concentration, and WER vary over time. In addition to time variability within a site, the analysis recognizes that between various sites, discharges differ somewhat in their design-flow dilution.

The steps for setting up and evaluating a site scenario are set forth below. The computations were set up as a Quattro Pro spreadsheet.

1. Consider an effluent discharged to a stream. The effluent and upstream waters are each characterized by three parameters: WER, toxicant concentration, and flow. The downstream toxicant concentration and WER are calculated by dilution. Such a calculation for the WER assumes that the WER is controlled by and directly related to the underlying concentrations of water quality parameters (such as dissolved organic carbon) that are appropriately calculated by the dilution formula.

2. Assume that effluent and upstream WERs, toxicant concentrations, and flows are log normally distributed, each with particular values for its log mean and log standard deviation. Time variable effluent and upstream flows were made to fit one of three categories: (a) design IWC of 91%, (b) design IWC of 50%, and (c) design IWC of 33%. Flow parameters were selected such that downstream flows less than the design low flow occurred 3% of the time. Because the copper discharged by sewage treatment facilities (by far the most common copper discharge) is physico-chemically associated with the discharged organic matter, effluent toxicant concentration and effluent WER were modeled to be 40-50% correlated with each other. Such degree of correlation is directly supported by State of Connecticut data (Dunbar 1997c), by wastewater treatment

process modeling, and indirectly by Pennsylvania municipal discharger data (Hall and Associates 1998).

3. Using random number generators that produce log normal values having the proper mean and standard deviation, generate random values for five parameters: effluent and upstream WERs and toxicant concentrations, and upstream flow. This constitutes one event.

4. Using the dilution formula, calculate the downstream WER and toxicant concentration for the Step 3 event. Because the streamlined approach mixes effluent and upstream water at the design IWC, irrespective of the actual IWC occurring during the sampling event, this modeling analysis must keep track of two separate downstream WERs for the event: (a) the downstream WER calculated to occur for the actual event IWC, and (b) the downstream WER that would be measured if effluent and upstream waters were mixed at the design IWC.

5. In like manner, generate a large number of events. For this work, 1000 events were used for each of the three dilution categories (33%, 50%, and 91% design IWC). Each event is characterized by the upstream and effluent values for WERs, concentrations, and flows, and by the calculated downstream values for these parameters.

6. Specify an appropriate relative value for the national criterion for the toxicant. Then for each of the 1000 events calculate a site criterion as the product of the national criterion times the event's downstream WER (at the actual event dilution). For each event determine whether the toxicant concentration exceeded the event-specific site criterion. Count the number of excursions of the event-specific criterion in all 1000 events.

Toxicant concentration and WER mean values and relative national criterion values in Steps 2 and 6 were selected such that using reasonable values for log variances, the site criterion was violated 1% of the time overall.

7. Arbitrarily pick some value for the overall final WER, to remain a fixed constant across all events. Calculate a fixed value for the site criterion, as the product of the national criterion times the fixed final WER. For each event determine whether the toxicant concentration exceeded the fixed value for the site criterion. Count the number of excursions of the event-specific criterion for all 1000 events. Compare this excursion frequency with that obtained in Step 6.

8. Repeat Step 7, adjusting the value by trial and error, until the fixed final WER yields the same excursion frequency as did the variable criterion from Step 6. The value so obtained may be called the *unbiased final WER*. It is the value the final WER should have if it were to produce the level of protection intended for the criterion.

The remaining steps consider the possible outcomes if (a) sample WERs were obtained for two events, in accord with the Streamlined Procedure, and (b) the final WER is set equal to the geometric mean the two sample WERs.

9. For each of 999 pairs of WER measurements in each of the three

dilution scenarios (33%, 50%, and 91% IWC), calculate a site WER equal to the geometric mean of each pair of measurements. (Form pairs from the first and second events, second and third events, and so on down the list, thereby yielding 999 pairs.)

10. For each of the three dilution scenarios, examine the distribution of 999 site WERs obtained by Step 9. Determine the WERs corresponding to various percentiles in the distribution (such as 50th, 85th, and 95th percentiles). Compare these with the unbiased WER obtained from Step 8, in order to ascertain how often the site WER would be under-protective, and how often it would be over-protective.

Values for key parameters are shown in Table C-1. The log standard deviations are based on past experience with selected water quality measurements from well conducted studies.

Table C-1. Values for key input parameters and selected intermediate outputs.

0.5	Standard deviation of log upstream toxicant concentration
0.5	Standard deviation of log upstream WER
1.0	Standard deviation of log upstream flow
0.5	Standard deviation of log effluent concentration
0.5	Standard deviation of log effluent WER
0.0	Standard deviation of log effluent flow
0.5	Correlation (r^2) between log effluent toxicant concentration and log effluent WER
0.43	Correlation (r^2) between effluent toxicant concentration and effluent WER
4.0	Ratio, effluent mean WER : upstream mean WER
33%	Design IWC for higher dilution facility in scenario
51%	Design IWC for medium dilution facility in scenario
91%	Design IWC for low dilution facility in scenario
8%	Overall geometric mean IWC for higher dilution facilities in group
14%	Overall geometric mean IWC for medium dilution facilities in group
63%	Overall geometric mean IWC for low dilution facilities in group
3%	Frequency of flows below design flow
1%	Frequency of site-specific criteria violations

Results

The results were used to address the question: How much protection is provided if the site WER is set equal to the geometric mean of two sample WERs?

The WER established as such a geometric mean will be here termed the *procedure WER*. Table C-2 shows the ratio of the procedure WER to the unbiased WER for the typical or 50th percentile situation and worst case 95th percentile situation among the 999 final WERs obtained from the Monte Carlo analysis of each of the three dilution scenarios.

The level of protection provided by the Streamlined Procedure does not vary significantly among the dilution scenarios, relative to other uncertainties and random influences. Irrespective of design IWC, the Table C-2 Monte Carlo results indicate that the substantial majority of cases, a streamlined Procedure WER will be below the site's unbiased WER. The probability of obtaining a procedure WER greater than the site's unbiased WER averaged 29% among sites.

Data from Dunbar (1997a, 1997b, 1997c) indicate that over time, the measured WERs at a site are less variable than assumed in this Monte Carlo analysis. Consequently, these results probably represent a conservative worst case portrayal of the performance of the Streamlined Procedure for the type of scenarios considered.

The possibility that a site could be assigned a criterion concentration somewhat greater than ideal is an inherent risk associated with both national and site-specific criteria. If most dischargers are to be assigned a WER not too far below what they deserve, the luck of the draw during sample collection will yield some site WERs somewhat higher. However, for the criterion in question, there is no reason to expect aquatic communities to be sensitive to minor errors or uncertainties in criteria setting. Application of any criterion will always involve some potential for inaccuracy, whether adjusted using the Streamlined Procedure, the 1994 Interim Procedure, the Biotic Ligand Model, an empirical hardness relationship, or whether not adjusted at all for site water quality.

The performance of the Streamlined Procedure was also compared with the 1994 Interim Procedure. This comparison is discussed in the Supplement to Appendix C.

Table C-2. Monte Carlo prediction of relationship between the procedure WER and the unbiased WER.

Design IWC	Ratio of Procedure WER : Unbiased WER		Probability of exceeding the Unbiased WER
	50th Percentile	95th Percentile	
91% Design IWC	0.82	1.49	30%
50% Design IWC	0.81	1.38	25%
33% Design IWC	0.90	1.42	33%
Mean of scenarios	.84	1.43	29%

Conclusion

Analysis of the behavior of the Streamlined Procedure using Monte Carlo modeling techniques has indicated that the procedure provides a level of protection close to that intended for the criteria.

References for Appendix C

Biological Monitoring, Inc. 1992. Site-Specific Study for Computing Metal Standards for the Lehigh River and City of Allentown, Pennsylvania. BMI, Blacksburg, VA.

Delos, C. 1994. Probabilistic analysis of the level of protection provided by the interim guidance on determining water-effect ratios. Draft. Office of Water, U.S. EPA, Washington, DC.

Delos, C. 1998. Assessment of WER Study for Aggregated Southeast Pennsylvania Facilities. Health and Ecological Criteria Division, U.S. EPA, Washington, DC 20460.

Dunbar, L.E. 1997a. Effect of streamflow on the ability of ambient waters to assimilate acute copper toxicity. Connecticut Department of Environmental Protection.

Dunbar, L.E. 1997b. Derivation of a site-specific dissolved copper criteria for selected freshwater streams in Connecticut. Connecticut Department of Environmental Protection.

Dunbar, L.E. 1997c. Lotus spreadsheet (data).

Hall & Associates and Environmental Engineering & Management Associates. 1998. Evaluation of copper toxicity and water effect ratio of treated municipal wastewater. Prepared for Pennsylvania Copper Group.

MacKnight, E.S. 1997. September 17 letter to James Newbold. U.S. EPA, Region 3.

Neserke, G. 1994. Written testimony of George Neserke on behalf of the Coors Brewing Company. Colorado Department of Health, Water Quality Control Commission.

U.S. EPA. 1994. Interim guidance on determination and use of water-effect ratios for metals. EPA-823-B-94-001.

Supplement to Appendix C

Discussion of Issues Pertaining to the Streamlined Procedure

This supplement discusses several issues associated with the Streamlined Procedure.

Why was a streamlined WER procedure developed?

The national recommended criterion for copper has now taken on the role of a screening tool. Violation of that criterion does not usually trigger immediate pollution control, but rather triggers WER studies to derive a site-specific value for the criterion.

It is common for sewage treatment facilities to exceed the national recommended copper criterion. However, when evaluated by TIEs, actual toxicity due to copper from such facilities is rare, apparently because of the excess of complexing organics.

Screening tools can be judged by their sensitivity and efficiency. EPA's approach for copper appeared to have few problems with sensitivity, but its efficiency (actual problems divided by the sum of false positives and actual problems) appeared to be very low, and when applied to sewage treatment facilities, possibly approaching zero. Because the cost and complication of the needless WER studies divert federal, state, and local government resources away from more productive efforts, the criteria program has been subjected to criticism both from within EPA and from outside EPA.

Recognizing the need for improving the efficiency of the copper WER approach, EPA developed a more streamlined approach, simpler to perform, simpler to review, but fully protective.

What is involved in recommendations about the mixing of effluent and upstream waters in preparation for the toxicity tests?

The Streamlined Procedure mixes effluent and upstream waters at the design low-flow dilution ratio (the design IWC). The 1994 Interim Procedure mixes effluent and upstream waters at the dilution actually occurring during the sampling event.

The thinking behind the 1994 Interim Procedure recommendation, which was designed primarily for application to total recoverable metals, was that the suspended solids concentration tends to increase with increasing flow, such that merging the high-flow, high-solids event and the low-dilution, high-organic-matter sample mixture might yield an unjustifiably optimistic scenario for a total recoverable WER. However, for dissolved metals, particulate matter is not relevant, and should have little effect on the dissolved WER. Furthermore, for copper, since total recoverable is generally not much greater than dissolved, and since the method is restricted to copper problems associated with continuous point sources, it is only necessary to assure that the event sampled is not substantially influenced by rainfall runoff.

State of Connecticut (Dunbar 1997a) data support this: *in upstream waters*, there is no apparent relationship between the WER and streamflow. Absent such a relationship in upstream waters, there is no particular reason not to measure the WER using the IWC in which one is most interested: the design flow condition.

The State of Connecticut data comparing the WER with the IWC also suggest that the WER does not increase as rapidly as the IWC. In this case, the IWC of greatest concern would be the design low-flow IWC. At this time the discharged metal would be expected to be at its highest level compared to the WER and the sample-specific criterion.

The modeling analysis in the main part of Appendix C nevertheless recognizes and accounts for the potential difference between the a sampling-event WER measured for the actual event IWC, and the sampling-event WER measured when the sample is prepared at the low-flow design IWC. Consequently, the assessed protectiveness of the Streamlined Procedure is *not* an artifact of any assumption that the low flow condition is the critical condition with respect to criteria excursions. The modeling analysis considered all criteria excursions no matter when they occurred.

Mixing upstream and effluent samples at a specified dilution substantially reduces the unpredictability of the results, and allows the needed sampling work to proceed in a timely manner (an essential feature for an efficient approach), the only stipulation being that rainfall runoff impacted events be avoided.

How does the protectiveness of the Streamlined Procedure compare with that of the 1994 Interim Procedure?

The features of the Streamlined Procedure and Interim Procedures were compared in Table 1 in the main body of this document. There are two key differences that affect the relative protectiveness of the two procedures. (1) The Interim Procedure mixes effluent and upstream water at the dilution occurring at the time of sampling; the Streamlined Procedure mixes effluent and upstream at the low-flow dilution. (2) The Interim Procedure uses the laboratory water EC50 in the WER denominator; the Streamlined Procedure uses the SMAV if it is greater than the laboratory water EC50. The influence these differences have on the protectiveness of the procedures appear largely to balance each other. The two procedures are expected to be equally protective.

Mixing effluent and upstream water: Because flow at the time of sampling is such a random parameter, effluent dilution and downstream organic carbon concentration are thus random uncontrolled factors in the Interim Procedure. In contrast, flow occurring at the time of sampling is not relevant in conducting the test under the Streamlined Procedure. In general, it can be expected that organic carbon concentrations in the tested site water will be higher and less variable when tested per the Streamlined Procedure than when tested per the Interim Procedure. This tends to slightly elevate the WER under the Streamlined Procedure.

SMAV versus laboratory water EC50: Experience with the 1994 Interim Procedure has caused some concern about the values of the lab-water EC50 used for the side-by-side comparison. There is a perception among many WER study reviewers that these lab-water EC50s, while within the range of reasonable values, are usually less than the SMAV for the test species, thus yielding a slight bias toward increasing the WER. The comparatively low calcium-magnesium ratio of the laboratory water commonly used in WER tests may account for their relatively low EC50s.

Data for 30 separate WER measurements from studies by four different laboratories indicated that the laboratory water EC50 for

Ceriodaphnia dubia and *Daphnia magna*, when measured for WER purposes, averaged less than 67% of the SMAVs shown in Appendix B. There was relatively little difference among the four laboratories in this regard. This potential for variability and bias has been eliminated from the Streamlined Procedure by stipulating that the denominator of the WER is the greater of the lab water EC50 or the SMAV. This stipulation tends to slightly depress the WER under the Streamlined Procedure.

The net result is that the differences between the two procedures appear to essentially balance each other. As shown in Table C-3, the Streamlined Procedure yields results that are comparable to the 1994 Interim Procedure, analyzed by the Monte Carlo technique in a similar scenario, while accounting for the differences between the two procedures.

Relative to the Streamlined Procedure, the Interim Procedure may be more variable and uncertain than Table C-3 would suggest, because this analysis accounts only for the typical bias of the lab-water EC50, and not for its imprecision or variability. Overall the Streamlined Procedure is expected to yield somewhat more stable results than the Interim Procedure.

Could a single WER measurement ever be used to derive a site WER?

The modeling analysis in the main part of Appendix C easily lends itself to evaluating the suitability of using a single WER measurement. Table C-4, in format similar to the Table C-2, presents the results. The results did not vary significantly with design IWC, so the table shows the combined results for all three dilution scenarios (33%-91% IWC).

Table C-3. Comparison of Protectiveness of Streamlined Procedure and 1994 Interim Procedure.

	Ratio of Procedure WER : Unbiased WER		Probability of exceeding the Unbiased WER
	50th Percentile	95th Percentile	
Streamlined Procedure	0.84	1.43	29%
Interim Procedure	0.83	1.51	30%

Table C-4. Monte Carlo predictions comparing the two-sample Streamlined Procedure with a single-sample approach, both relative to the true unbiased WER.

	Ratio of Procedure WER : Unbiased WER		Probability of exceeding the Unbiased WER
	50th Percentile	95th Percentile	
Two-sample Procedure	0.84	1.43	29%
Single-sample Approach	0.84	1.72	35%

Using a single sample rather than the geometric mean of two samples has no effect on the median result. However, the luck of the draw has more influence on a single sample than the mean of two samples. Of greatest concern is large difference between the median and the 95th percentile in the single-sample approach when compared to the two sample approach.

Nevertheless, it is recognized that in many municipal discharge situations, there is a wide margin of safety between value of the WER needed to conclude that there is no reasonable potential for impairment and the value of the measured values of the site WER. In a tiered testing approach the criterion would be more stringent where less data are available. For a single sample, obtained during stable flow events, not influenced by rainfall runoff, with treatment plant operating well, if the measured WER is more than a factor of two greater than WER needed to conclude that there is no reasonable potential, the above modeling results do not suggest any particular need for an additional sample before concluding the decision making process.

If a single sample is obtained for purposes of reconfirmation of a previously established site-specific criterion, then a single reconfirmation sample should be compared with the original sample results, without reference to the above factor of two. In all cases, occasional reconfirmation testing is desirable, perhaps on concert with the permit issuance cycle, even though site-specific criteria do not generally incorporate any legally recognized expiration date.

Is simultaneous side-by-side laboratory water and site water testing needed? Can the laboratory water EC50 be dispensed with and replaced by the SMAV in conjunction with reference toxicant testing?

Over the years this question has remained with the WER approach. Insistence on simultaneous side-by-side testing implies that the relative positions of the laboratory water and site water EC50s are more reliable than the absolute values. Nevertheless, the Agency clearly puts a substantial amount of trust in the absolute values: they are the basis for the national criterion and the basis for determining compliance with WET limits.

The advantage of having the simultaneous laboratory water test is that it accounts for any variations in the organism culture or test condition that might affect sensitivity to copper, even though such variations toward non-optimal test conditions might often be expected to depress rather than increase the EC50. The disadvantage of the laboratory water test is that it is an additional source of variation and uncertainty in the WER, and may be subject to unjustified manipulation. It also adds somewhat to the study cost.

Nevertheless, without some type of laboratory water EC50 check, there is nothing to discourage use of a daphnid culture that had been bred in water having high concentrations of copper. This might or might not mimic genetic adaptation processes actually occurring at the site. In any case, it is considered undesirable for the national criteria program to depend on such adaptation processes to assure aquatic life protection.

If the simultaneous laboratory water EC50 were to be eliminated, a state or tribe would need to substitute in its place a reference toxicant testing program involving copper. This would be technically necessary to assure that the organism culture used for testing was not insensitive to copper toxicity. The state or tribe would be responsible for assuring the adequacy of such an approach.

References for the Supplement

Dunbar, L.E. 1997a. Effect of streamflow on the ability of ambient waters to assimilate acute copper toxicity. Connecticut Department of Environmental Protection