Water Effect Ratio (WER) Study Review Checklist

Permittee: _____ **Permit No.:** _____

Date reviewed: _____ **Reviewer:** _____

This checklist is based upon the 1994 Interim Guidance on Determination and Use of Water-Effect Ratios for Metals and applies to Method 1 described therein. The purpose of this checklist is to serve as a useful tool in reviewing Method 1 WER studies. The checklist does not supercede the 1994 Interim Guidance document. In reviewing a WER study, the acceptability of each toxicity test will be evaluated individually based upon the procedures described in the 1994 Interim WER Guidance. Page 57 of the 1994 guidance states that, "If the procedures used deviated from those specified in the guidance, 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." Guidance concerning the calculation of the results of each test and the derivation of the individual test WERs and the FWER is also provided in the 1994 Interim WER Guidance. Review of these results will be in accordance with the guidance document.

General Information

#	**Question** *(If yes, place a "**Y**" in box; if no, place an "**N**" in box. If question cannot be answered in yes/no format, then place answer in "Comments" section.)*	**Workplan**	**Comments**	**1994 Guidance** page #, part
1.	Is the name, location, and description of the discharger provided?	☐		62, J(3)
2.	Is the name of the study investigator provided?	☐		62, J(1)
3.	Is the purpose for conducting the study described?	☐		–
4.	Are requirements that are in the existing permit concerning WET testing, TIE, and/or TRE being met?	☐		9
5.	Is pretreatment, waste minimization, or source reduction an option?	☐		9
6.	Are applicable technology-based limits being met?	☐		9
7.	Is a description of each sampling station provided?	☐		62, J(4)

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Individual Studies

#	Question *(If yes, place a "**Y**" in box; if no, place an "**N**" in box. If question cannot be answered in yes/no format, then place answer in "Comments" section.)*	Study 1	Study 2	Study 3	Comments	1994 Guidance page #, part
Selecting Primary and Secondary Tests						
8.	Species used for primary toxicity test? (Write name.)	_____	_____	_____		45-47, C
9.	Species used for secondary toxicity test? (Write name.)	_____	_____	_____		45-47, C
Acquiring and Acclimating Test Organisms						
10.	Organism culture, hold, acclimation, feed, and handling protocols summarized?	☐	☐	☐		47-48, D(1-2)
11.	Were the organisms acclimated to site water prior to initiating the test?	☐	☐	☐		47, D(1)
12.	Were randomization procedures utilized?	☐	☐	☐		47, D(1) 53, G(9)
Collecting and Handling Upstream Water and Effluent						
13.	Was rainfall data or stream flow data included and was upstream water unaffected by recent runoff events? Rainfall data should be included.	☐	☐	☐		48, E(1)
14.	Is the effluent sample representative of normal operations?	☐	☐	☐		48, E(2)
15.	Was the plant operating at "normal levels"? Flow data should be included.	☐	☐	☐		49, E(5)
16.	Were samples stored at 0-4ºC?	☐	☐	☐		48, E(4)
17.	Are chains-of-custody for samples included, accurate, and filled out completely?	☐	☐	☐		49, E(6)
18.	Were toxicity tests initiated w/in a maximum of 36 hours from the time of sample collection? Test initiation and termination times should be included.	☐	☐	☐		49, E(7) 62, J(1)
19.	If predators in the site water are a concern, was the site water filtered through a 37-60 µm sieve or screen?	☐	☐	☐		49, E(8)

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Laboratory Dilution Water						
20.	Did the lab water have TOC and TSS <5 mg/L as required?	☐	☐	☐		50, F(2)
21.*	Was the hardness of the lab water between the required 40 and 220 mg/L?	☐	☐	☐		50, F(3)
22.*	Was the lab water hardness (w/in the above range) close to the site water? *From 1997 Guidance, page 3, next to last paragraph*	☐	☐	☐		<<1997 Guidance
23.	Are the lab water pH and alkalinity appropriate for the hardness used?	☐	☐	☐		50, F(4)
Conducting Tests						
24.	Was the spiking stock solution made from an appropriate reagent?	☐	☐	☐		50-51, G(4)(a-b)
25.	Was the same stock solution used for lab water and site water tests?	☐	☐	☐		51, G(4)(c)
26.	Was a static test run?	☐	☐	☐		51, G(5)
27.	If the test ran longer than 48 h, was it a renewal test?	☐	☐	☐		51, G(5)
28.	If it was a renewal test, were side-by-side tests renewed at the same time and were proper procedures for renewal followed?	☐	☐	☐		51, G(5)
29.	Was a range finder test conducted?	☐	☐	☐		51, G(7)
30.	Was the dilution factor used in the definitive tests of 0.65 or greater?	☐	☐	☐		53, G(8)
31.	Was an unspiked dilution water control for each test used?	☐	☐	☐		53, G(9)
32.	Were at least 20 test organisms per treatment used?	☐	☐	☐		53, G(9)
33.	Were two or more replicates used per treatment?	☐	☐	☐		53, G(9)

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34.	Were the laboratory hard water and the site water prepared in accordance with appropriate guidelines?					53, G(10) 54, G(11)
35.	Were the test organisms (already acclimated), added to the test chambers for the side-by-side tests at the same time?					54, G(12)
Chemical and Other Measurements						
36.	Were hardness (or salinity for marine water), pH, alkalinity, TSS, and TOC measured at test initiation for both site water and lab water?					55, H(2)
37.	If "yes" to the above question, did the dissolved oxygen level remain acceptable throughout the entire test?					55, H(3)
38.	Were dissolved oxygen, pH, and temperature measured for each treatment at the appropriate times during the test?					55, H(3)
39.	Were both total recoverable and dissolved metal measured for all samples?					54, H(4)
40.	Were the metal concentrations measured at the appropriate frequency?					54, H(4)(d)
41.	Were QA/QC requirements summarized?					57, H(4)(d)(5)
Calculating and Interpreting the Results						
42.	To prevent roundoff error in subsequent calculations, were at least four significant digits retained in all endpoints and WERs?					57, I(1)
43.	Were greater than 10% of control organisms adversely affected (for acute tests)?					57, I(2)(b)
44.	The percent of organisms that were adversely affected must have been less than 50%, and should have been less than 37%, in at least one treatment other than the control. Did this occur?					57, I(2)(c)(1)

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45.	For lab water, at least one treatment showed at least 50% of the organisms to be adversely affected?	☐	☐	☐		57, I(2)(c)(2)
46.	For site water, at least one treatment showed at least 63% of the organisms to be adversely affected?	☐	☐	☐		57, I(2)(c)(2)
47.	Did a lower concentration kill a higher % of organisms than a higher concentration?	☐	☐	☐		57, I(2)(c)(3)
48.	If so, did this occur for more than two concentrations affecting between 20-80% of the organisms?	☐	☐	☐		57, I(2)(c)(3)
49.	If a static test was run, did the dissolved metal concentration at the end of 48 hours decrease by more than 50% from test initiation?	☐	☐	☐		58, I(2)(e)
50.	Did it increase by more than 10% from test initiation?	☐	☐	☐		–
51.	Did each individual test meet all acceptability requirements, as specified in #43-49 above?	☐	☐	☐		57, I(2)
52.	Were the LC50 (or EC50) values calculated appropriately and with similar statistics?	☐	☐	☐		58, I(4 & 6)
53.*	Was the hardness of the laboratory dilution water normalized (to obtain an adjusted LC50) according to the guidance document?	☐	☐	☐		39-43
54.	Do the results from the laboratory dilution water compare with results that were obtained using a comparable laboratory dilution water in one or more other laboratories?	☐	☐	☐		59, I(5)
55.	Is the WER larger than 5? If so, investigate results further as specified in the 1994 Interim Guidance on page 61.	☐	☐	☐		61, I(7)(c)(3)
56.	Were summary tables provided containing metal concentrations and organism response for each concentration?	☐	☐	☐		64, J(3)

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Final Report

#	Question *(If yes, place a "Y" in box; if no, place an "N" in box. If question cannot be answered in yes/no format, then place answer in "Comments" section.)*	Final Report	Comments	1994 Guidance page #, part
57.	Were toxicity tests conducted at least three weeks apart?	☐		48, E(3)
58.	Three WERs for primary test developed?	☐		45, C(1)
59.	At least one WER for secondary test developed?	☐		45, C(1)
60.	Are the WERs obtained with the primary and secondary tests w/in a factor of 3? If yes, then results are further confirmed.	☐		61, I(7)(b)(1)
61.	Does the test with the higher endpoint give the higher WER? If yes, then results are further confirmed.	☐		61, I(7)(b)(2)
62.	Were both total recoverable and dissolved WERs calculated?	☐		60, I(6)
63.	Was the final WER calculated as the geometric mean of the three individual test WERs? Provide the final WER in the comments section.	☐		37-38
64.	Were acute and chronic criteria calculated? If yes, provide the results in the comments section.	☐		–
65.	Were any individual studies eliminated from consideration in the final WER calculation? If yes, provide an explanation.	☐		–
66.	Was an explanation of "unusual" observations and/or any procedural deviations provided if necessary?	☐		–

* As an alternative to conducting testing with laboratory water with a hardness between 40 and 220 mg/l total hardness and then mathematically adjusting the LC_{50} of the laboratory water to the segment regulatory hardness, testing can be conducted using laboratory water with the total hardness chemically adjusted to be the same as the segment regulatory hardness. If the laboratory water is adjusted, then no mathematical adjustment should be necessary.

Additional Comments: