Appendix 5. Rationale for Applying Corrections to Measured Copper and Zinc Values in Water Samples Collected during the Acute Toxicity Test in Round 6 Performed with 74-days-post-hatch Rainbow Trout (*Oncorhynchus mykiss*)

By William G. Brumbaugh

Some of the water samples collected during acute toxicity test Round 6, performed with 74-days-post-hatch (dph) rainbow trout, were filtered using an incorrect filter type that evidently caused errors in the measured concentrations for copper and zinc. These samples include those collected from copper exposures on test day 0 and from cadmium, copper, and zinc exposures on test day 4 of Round 6. Following collection of samples from the cadmium and zinc exposure waters on test day 0 of Round 6, the supply of filters used to collect samples for metals analyses became depleted. Filter cartridges designated for dissolved organic carbon (DOC) sampling of sediment pore water (for a different project) mistakenly were used. These cartridges contain the same 0.45-micron pore-size polyethersulfone (PES) membrane that had been used to collect previous water samples, but also contain a borosilicate glass fiber pre-filter (GFF). Based on results for control waters, an unfiltered sample that was collected in Round 6 (table 5–1), and analyses from previous tests, it became apparent that the GFF component caused partial adsorption losses of dissolved copper and contamination (presumably leaching from the glass fibers) of zinc. No effects on measured cadmium concentrations were evident (data not shown). Notably, there was no evidence of zinc contamination from this GFF filter based on results of de-ionized water filter blanks (table 5–1). Zinc likely was leached from the glass fibers by way of a cation-exchange process (perhaps with calcium and magnesium ions and by complexation with anions of the test water).

During the final acute test with 95-dph rainbow trout, replicate samples were collected from each of the copper exposure test waters to closely compare results from the GFF/PES filter, the PES filter, and unfiltered samples. These results are summarized in table 5–2 and in figure 5–1 and figure 5–2. Based on these data, measured copper concentrations for acute rainbow trout toxicity test Round 6 that were inadvertently sampled using the GFF/PES filter were corrected by multiplying by 1.6 (in accordance with the regression equation displayed on fig. 5–2). Although the measured zinc concentrations for test day 4 samples clearly were erroneous because of filter contamination, those values cannot reasonably be corrected based on the available data. Instead, the measured zinc concentrations obtained for test day 4 of the round 6 toxicity test performed with rainbow trout were disregarded, and the assumption was made that the test day 0 zinc measurements best reflected the actual exposure concentrations for the duration of that test. Results from previous zinc tests indicated close agreement between test day 0 and 4 samples.

Table 5–1. Measured concentrations of copper and zinc in water samples from rainbow trout (*Oncorhynchus mykiss*) acute toxicity test round 6.

[Shaded cells indicate samples filtered using a filter cartridge containing a glass fiber prefilter. Bold and italic indicate concentrations falling below the method quantitation limit; ID, identification; Cu, copper; ng/mL, nanograms per milliliter; <, less than; UF, unfiltered sample; Zn, zinc]

Sample ID	Collection date	Test day	Cu (ng/mL)	Sample ID	Collection date	Test day	Cu (ng/mL)
Copper acute round 6							
A-Filter Blank	12/27/10	0	< 0.14	A-Filter Blank	12/31/10	4	< 0.04
A-Cu-0ppb Control	12/27/10	0	***0.18***	A-Cu-0ppb Control	12/31/10	4	0.22
				A-Cu-0ppb Control UF	12/31/10	4	0.20
A-Cu-18.75ppb Low	12/27/10	0	7.11	A-Cu-18.75ppb Low	12/31/10	4	11.6
A-Cu-37.5ppb Med-Low	12/27/10	0	14.2	A-Cu-37.5ppb Med-Low	12/31/10	4	23.0
				Cu-37.5ppb Med-Low UF	12/31/10	4	35.7
A-Cu-75ppb Med	12/27/10	0	34.2	Cu-75ppb Med	12/31/10	4	42.4
A-DUP-Cu-75ppb Med	12/27/10	0	33.0	A-DUP-Cu-75ppb Med	12/31/10	4	47.0
A-Cu-150ppb Med-Hi	12/27/10	0	58.5				
A-Cu-300ppb High	12/27/10	0	141				

Sample ID	Collection date	Test day	Zn (ng/mL)	Sample ID	Collection date	Test day	Zn (ng/mL)
Zinc acute round 6							
A-Zn-0ppb Control	12/27/10	0	1.3	A-Zn-0ppb Control	12/31/10	4	175
				A-Zn-0ppb Control UF	12/31/10	4	0.8
A-Zn-62.5ppb Low	12/27/10	0	57.6	A-Zn-62.5ppb Low	12/31/10	4	172
				A-Zn-125ppb Med-Low	12/31/10	4	181
A-Zn-125ppb Med-Low	12/27/10	0	104	A-Zn-125 ppb Med-Low UF	12/31/10	4	108
A-Zn-250ppb Med	12/27/10	0	202	A-Zn-250ppb Med	12/31/10	4	207
A-DUP-Zn-250ppb Med	12/27/10	0	203	A-DUP-Zn-250ppb Med	12/31/10	4	229
A-Zn-500ppb Med-Hi	12/27/10	0	399	A-Zn-500ppb Med-Hi	12/31/10	4	245
A-Zn-1000ppb High	12/27/10	0	839	A-Zn-1000ppb High	12/31/10	4	590

Table 5–2. Comparison of nominal, unfiltered, polyethersulfone (PES) filtered, and glass-fiber prefilter (GFF)/PES filtered concentrations of copper and zinc in water samples from rainbow trout (*Oncorhynchus mykiss*) acute toxicity test round 7.

[Bold and italic indicate values that are greater than the detection limit, but less than the quantitation limit. ID, identification; Cu, copper; ng/mL, nanograms per milliliter; Zn, zinc]

Sample ID	Cu (ng/mL)				Zn (ng/mL)			
	Nominal	Unfiltered	PES filtered	GFF/PES filtered	Nominal	Unfiltered	PES filtered	GFF/PES filtered
A-Cu Control	0	1.14	1.11	0.33	0	2.0	1.7	147
A-Cu Low	25	21.3	20.5	14.2	0	1.7	***1.2***	105
A-Cu Med-Low	50	42.3	41.0	32.2	0	***1.2***	***1.0***	81.3
A-Cu Med	100	88.6	88.1	49.9	0	***1.6***	***0.8***	84.7
A-Cu Med DUPL	100	88.1	87.7	50.3	0	***0.8***	***0.7***	89.4
A-Cu Med-Hi	200	176	175	118	0	***0.7***	***0.8***	59.0
A-Cu High	400	360	357	219	0	***0.6***	***0.7***	68.4

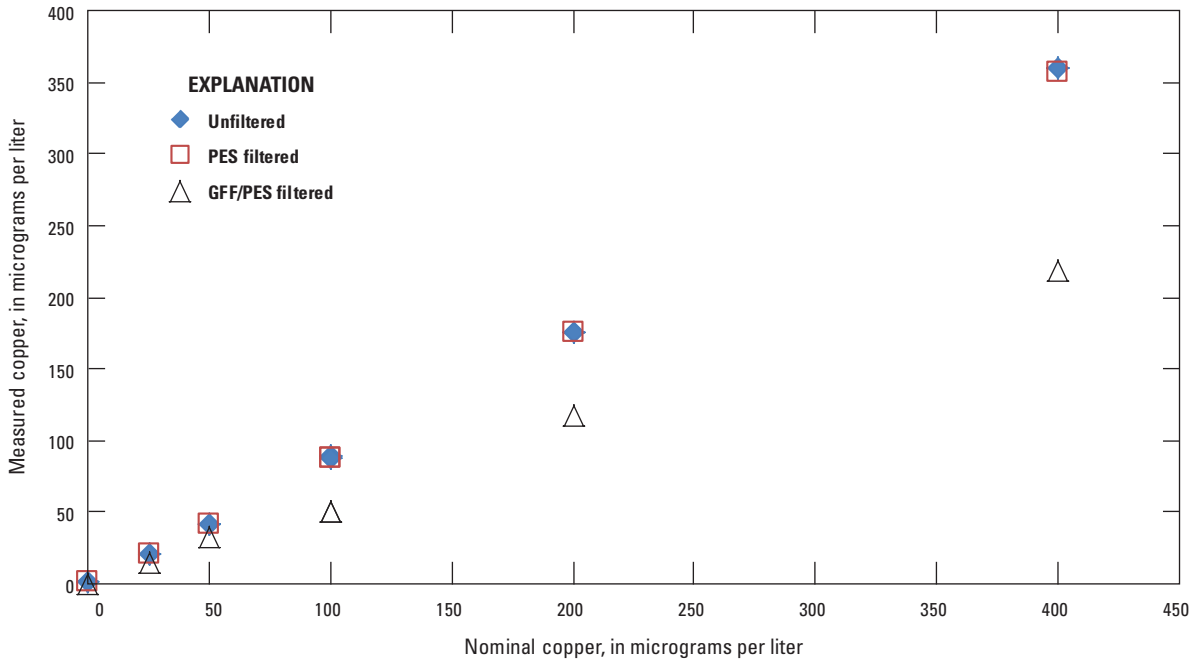

Figure 5–1. Comparison of measured copper concentrations in samples collected from acute test round 7 performed with rainbow trout (*Oncorhynchus mykiss*) using three different collection methods.

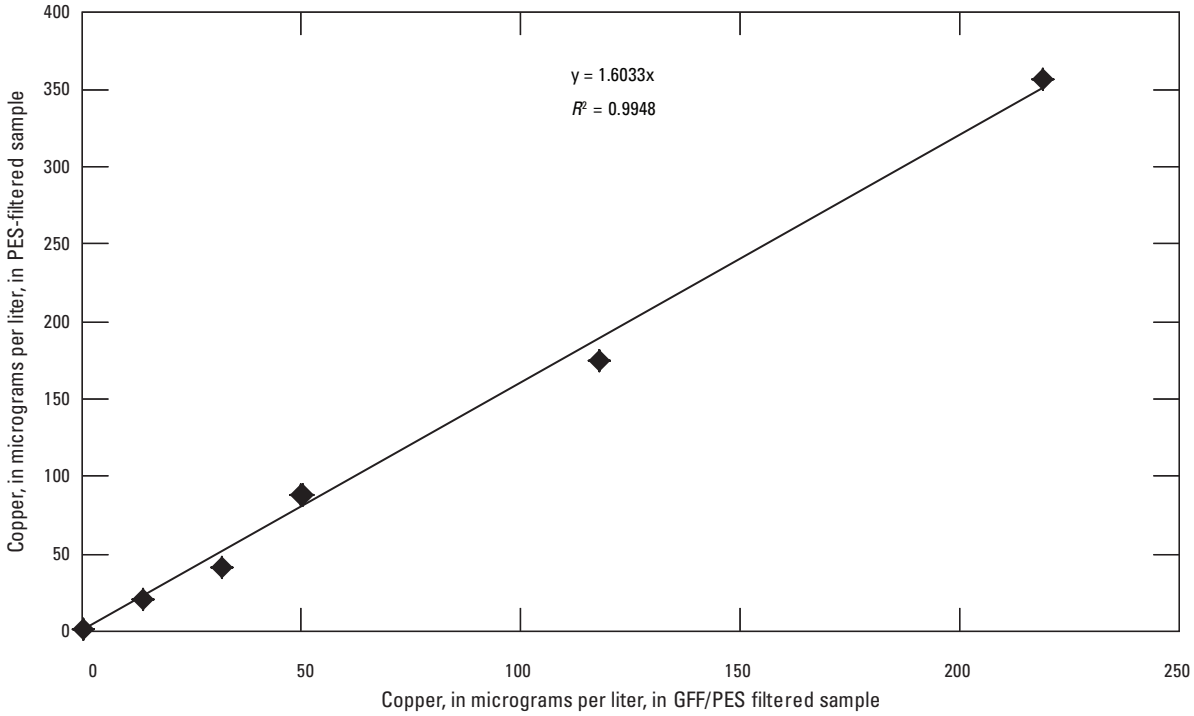

Figure 5–2. Relations between measured copper concentrations for samples from acute test round 7 performed with rainbow trout (*Oncorhynchus mykiss*) that were filtered with a polyethersulfone (PES) cartridge as compared to samples filtered with a glass fiber prefilter (GFF)/PES cartridge.

Appendix 6. Dissolved Organic Carbon Measurement Variability, Bias, and Implications for Biotic Ligand Model Normalization for Toxicity Data Summarized in Chapter A and Chapter B

By William G. Brumbaugh and Christopher A. Mebane

Dissolved organic carbon (DOC) is well established as an important modifier of copper toxicity in freshwaters (e.g., Di Toro and others, 2001; U.S. Environmental Protection Agency, 2007). Yet, what is not well recognized is that the low range of DOC concentrations commonly encountered in ambient and laboratory waters often approach the quantitation limits of routine analyses, and therefore, such DOC measurements are subject to considerable uncertainty and error (Hedges and others, 1993; Yoro and others, 1999). Further, at low DOC concentrations, small differences in DOC that are within the range of analytical variability can result in appreciable differences in modeled and actual toxicity of copper in freshwater (Welsh and others, 2008; Wang and others, 2009). Because of the importance of DOC and potential difficulties in measuring low concentrations involved with these studies, this appendix evaluates the bias and accuracy of DOC measurements in test waters through analyses of quality control blanks, cross-checks, and selected samples analyzed by another laboratory that is experienced in low-level DOC measurements, and DOC measurements performed for other studies performed at the U.S. Geologic Survey Columbia Environmental Research Center (CERC) that used the same test waters; and considers the effect of uncertainties in DOC measurements on biotic ligand modeling (BLM) of copper toxicity for selected results.

Variability in Low-Level DOC Measurements

A summary of all DOC results is presented graphically in figure 6–1. Among all toxicity tests, a total of 233 water samples and 60 method blanks were collected for analysis for DOC. Because concentrations were anticipated to be low enough to cause measurement difficulties, multiple blanks were sampled with each sample collection set for the purpose of applying blank corrections as a means to improve accuracy of sample results. Method blanks associated with sample collections for the first white sturgeon (*Acipenser transmontanus*) acute toxicity test (chapter A) and for the first three sets of sample collections (test days 0, 3, and 9) for the sturgeon chronic toxicity tests (chapter B) were too high (about 1 mg/L) to allow any confidence in measured sample results. Consequently, those sample results were rejected and are not included in fig. 6–1*A*. Contamination for those sets of samples was determined to be because of leaching from the bottles (or caps) that were purported to be suitably clean for DOC analysis without further treatment. Subsequent samples were collected in bottles that had been rinsed and stored in high-purity water before use. With the exception of one sampling set, all blanks obtained beginning on test day 14 of sturgeon test water sampling were considered acceptable (overall mean ±standard deviation (SD) = 0.090 ± 0.090 milligrams per liter (mg/L).

As indicated in figure 6–1, relative variability within and across collection dates was considerable. Such variability was not altogether unexpected because most samples had DOC levels near the CERC method detection limits (about 0.1 to 0.2 mg/L) where measurement uncertainty typically is ± 100 percent. Notably, most of these sample results would actually be reported as less than values or as estimated values under routine analytical testing and reporting procedures (U.S. Environmental Protection Agency, 1999). Despite the expectation that measuring these low concentrations would present difficulties, it was anticipated that collection of multiple samples periodically during each test would allow for determining if any changes in DOC concentrations occurred during the tests. For example, it was hypothesized that DOC in waters might increase as the fish grew larger, and in the case of the chronic tests, as food rations were increased; or that differences between control, medium, and high metal treatments might occur as a result of differences in growth or stress levels among fish having increased metal exposure.

The only trend suggested among the test water samples was that DOC concentrations measured in the chronic rainbow trout (*Oncorhynchus mykiss*) toxicity tests were unusually low during the first 4 weeks of testing, but then seemed to increase during week 5 through 7 (fig. 6–1*B*); however, because most concentrations were near method detection limits, and no such trend was apparent for the chronic tests with sturgeon, it is unlikely that DOC concentrations actually changed much during the chronic trout tests. Instead, it is more likely that (blank corrected) concentrations measured for the first 4 weeks were artificially low, or that during those analyses some type of systematic low bias was present. Collection blanks were greater during those first 4 weeks (mean=0.13 mg/L) as compared with week 5–7 (mean=0.04 mg/L). Therefore, blank subtraction performed with the week 1–4 samples might have resulted in some overcorrection and contributed to lower final results.

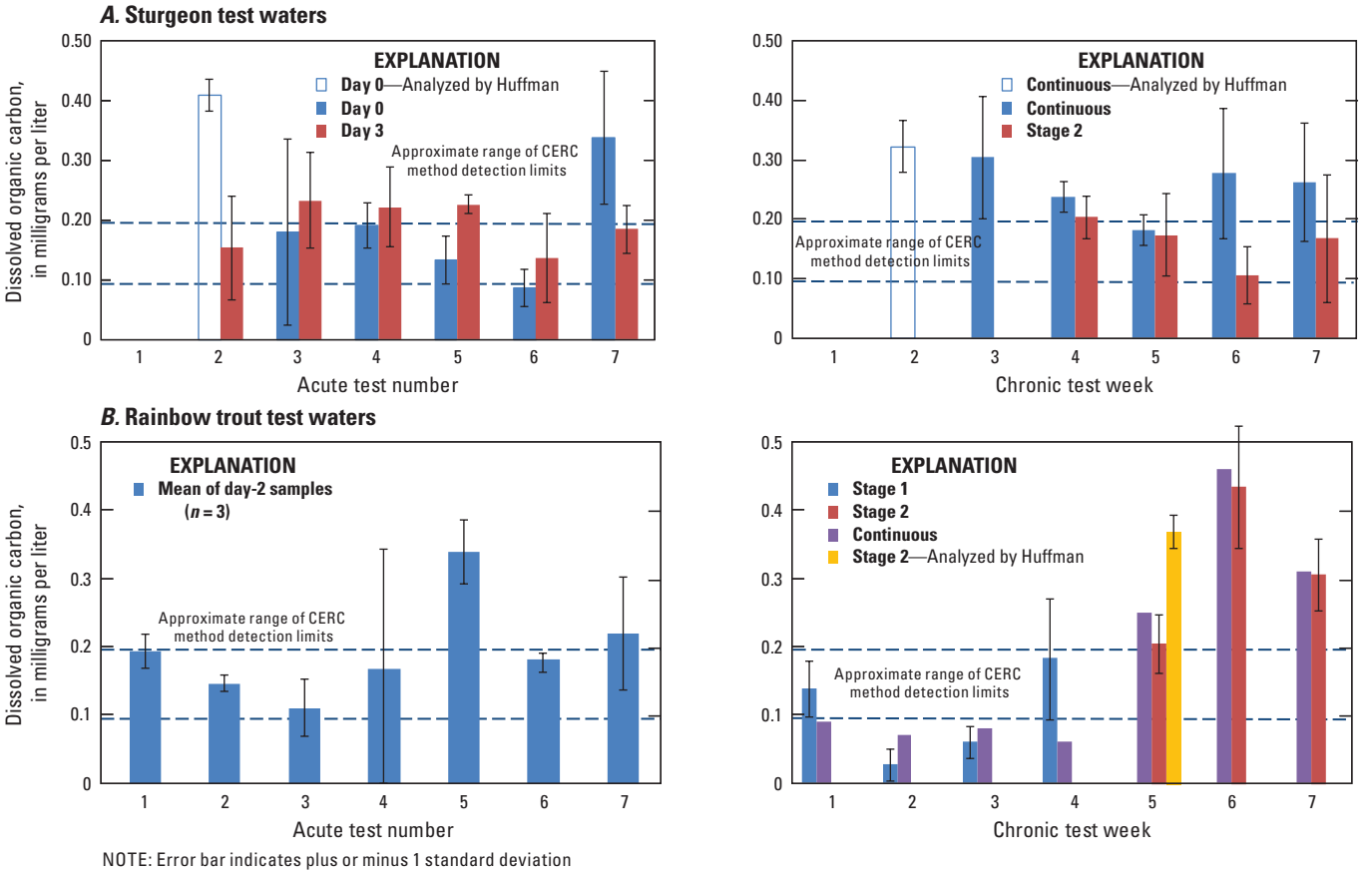

Figure 6–1. Dissolved organic carbon (DOC) of test water sampled during 2010 acute and chronic toxicity testing with *A*, sturgeon and *B*, trout. Values represented by bar graphs are means (n=3 to 12); error bars are ± 1 standard deviation. Dotted blue lines indicate approximate range of Columbia Environmental Research Center method detection limits. For the acute tests, n=3 (controls for each metal tested were sampled). For chronic sturgeon tests, n=12 (control, medium, and high treatments sampled from each of the four metals tested) and for chronic trout tests, n=6 (controls sampled for each metal tested, plus medium and high treatments sampled from copper test). Lighter blue-shaded bars represent samples analyzed by Huffman Laboratory.

Furthermore, some degree of bias (sometimes low, sometimes high) is unavoidable within each instrumental analysis run. Bias can be caused by daily differences in instrumental response, baseline drift, or calibration zero offset, and will be most important for measurements near detection limits where professional judgment might be needed to report the best estimates of actual concentrations of blanks or low-level samples (U.S. Environmental Protection Agency, 1999). Samples collected during weeks 1–4 of the chronic tests with trout were analyzed in two analytical batches and lower values obtained for those samples could have resulted from calibration bias (low in this instance) during those analyses.

Relative variability was large among individual DOC measurements and across collections dates, yet the mean values for each test species and test type remarkably were similar. For example, the mean measured DOC concentration was 0.19 mg/L across all acute sturgeon tests, 0.18 mg/L for all acute rainbow tests, and 0.22 mg/L for chronic tests with sturgeon or trout. Standard deviations about these means were 0.12, 0.11, 0.10, and 0.15, respectively. Although average DOC values across tests were relatively consistent, the measured concentrations determined by CERC on the whole are probably biased low by about 0.2 mg/L. This conclusion is based on multiple lines of evidence. First, mean DOC values obtained from analyses by one or more independent contract laboratories during studies with similarly prepared CERC test waters performed in 2009 (Wang and others, 2011), 2011, and 2012 all were between 0.4 and 0.6 mg/L. For example, during a 2011, 28-d copper exposure to sucker fish species, the average DOC concentration across the control, low, and medium-high treatments was 0.44±0.07 (SD) for samples collected on test day 14 and 0.51±0.02 (SD) for samples collected on test day 28. Undiluted CERC well water analyzed at an oceanographic laboratory with capabilities for analyzing low-level DOC concentrations (University of Miami, Rosenstiel School of Marine and Atmospheric Science) yielded a DOC concentration of 0.54 mg/L (Esbaugh and others, 2011). For a 2012 study with early life sturgeon (test water prepared exactly the same as the 2010 study; appendix 8), the mean DOC result for three separate samplings of control and medium treatments was 0.44±0.03, 0.39±0.02, and 0.47±0.05 mg/L (n=8 for each collection). For the 2012 study, total organic carbon (TOC)-free certified sampling bottles and 100-milliliter

sampling volumes, which helped to minimize procedural blank contamination, were collected. Among the seven blanks collected, four were less than (<) 0.05 mg/L, and the remaining three were 0.05, 0.06, and 0.08 mg/L. Thus, uncertainty caused by procedural contamination was better controlled during that 2012 study as compared to the 2010 studies.

Most importantly, the results of comparisons between pairs of duplicate samples collected during this 2010 study were used for inter laboratory cross-checking purposes. For that evaluation, three sets of test water samples that included spiked samples and blanks were submitted to Huffman Laboratory (Golden, Colorado), henceforth Huffman; an established laboratory with a history of producing high quality DOC measurements at relatively low concentrations. Blanks and spiked samples were prepared using TOC-free water (carbon <0.05 mg/L; EP Scientific, Miami, Oklahoma). Spikes were prepared by serial dilution from a stock solution containing DOC at 1,000 mg/L (Environmental Research Associates, Golden, Colo.). Results of toxicity test water samples analyzed by Huffman (light-blue shaded bars in fig. 6–1) tended to be slightly greater than comparable samples analyzed by CERC. A better comparison is provided in fig. 6–2, where the CERC and Huffman results are plotted together for three test water sample pairs (all blank-corrected values), plus one pair of CERC 100-hard water samples (three samples to each laboratory, uncorrected values; collected June 29, 2010) and two sampling pairs of spiked TOC-free water (three samples to each lab on June 29, 2010, and one sample to each lab on July 26, 2010). Test samples analyzed by each laboratory included water collected from sturgeon chronic copper control, medium and high treatments sampled on July 26, 2010; sturgeon control water from cadmium, copper, and zinc acute test number 2 sampled on July 26 or 29, 2010, and finally the trout chronic tests water treatments C-Cd-0 stage 2, C-Cu-0 continuous, C-Cu-0 stage 2, C-Cu-M stage 2, C-Cu-H stage 2, C-Pb-0 stage 2, and C-Zn-0 stage 2 sampled on November 5, or November 11, 2010. Compared to Huffman results, CERC DOC measurements of the same 13 unspiked test waters (sample pair numbers 1, 2, and 3; fig. 6–2) consistently were lower by an average of 0.19 mg/L. For the spiked samples (sample pair numbers 4 and 6), agreement between laboratories was excellent; however, the CERC value was markedly lower than Huffman for sample pair number 5 (fig. 6–2). A review of all CERC DOC analysis calibrations and QC sample results provided no suggestion that CERC results might be biased; however, the carbon analyzer used at Huffman incorporates a more sensitive (infra-red) detector that produces lower detection limits (about 0.05 mg/L) than the analyzer used by CERC (detection limit about 0.1 to 0.2 mg/L, depending on day-to-day variation in system performance). Presumably, the detector used by Huffman allowed for improved accuracy and precision at the low DOC concentrations in the test waters in the current studies. In addition, it is possible that the system used by Huffman produces a more complete oxidation of organic carbon compared to that used by CERC. Notably, although within-laboratory variability similarly was low for each laboratory for cross-check samples (fig. 6–2 error bars), variability tended to be greater for most other samples sets analyzed by CERC (fig. 6–1).

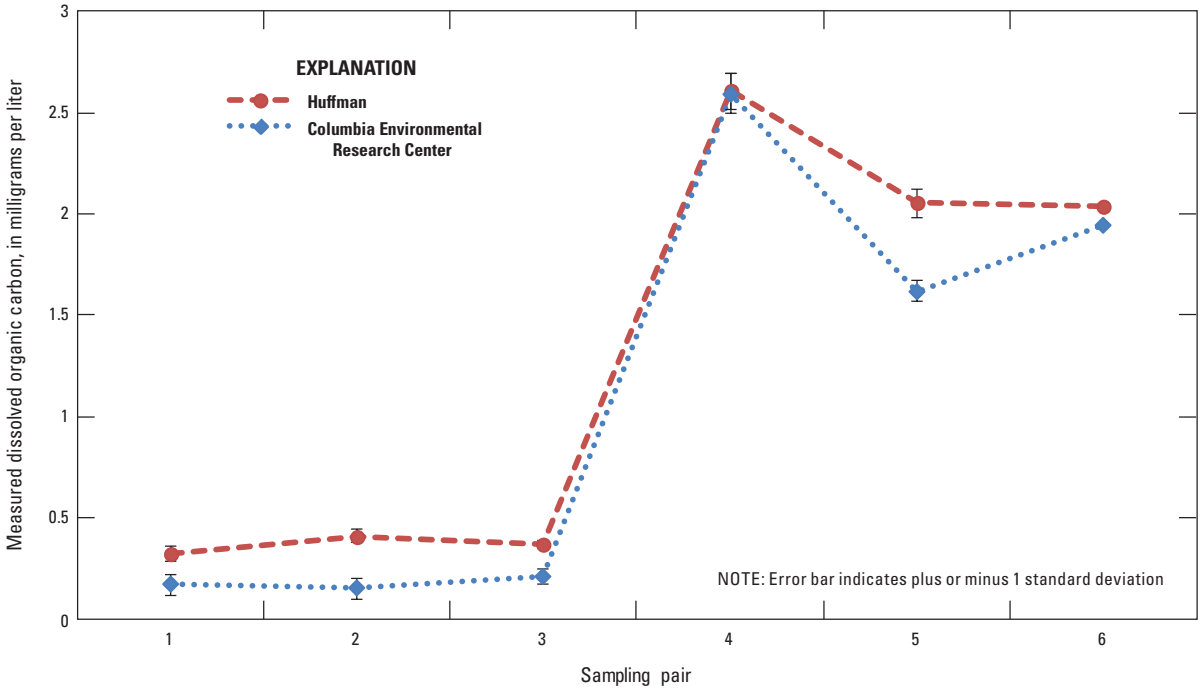

Figure 6–2. Interlaboratory comparison of measured dissolved organic carbon (DOC) for three sets of 2010 test waters (sampling pairs 1, 2, 3) and spiked samples (pairs 4, 5, 6) collected on three different dates. For sampling pairs 1, 2, and 3, n=3, n=3, and n=7, respectively, and CERC mean=0.18 mg/L and Huffman mean=0.37 mg/L. For pairs 4, 5, and 6, n=3, n=3, and n=1, respectively. Error bars represent ±1 standard deviation.

Overall, considering that (1) DOC measurement uncertainty was on the order of ±0.1 to 0.2 mg/L, (2) means between separate sets of toxicity tests were similar and the grand mean for DOC among all tests was 0.21 mg/L, (3) CERC values appear to be biased low by about 0.19 mg/L, and (4) DOC results from other recent CERC studies ranged from about 0.4 to 0.6 mg/L, a DOC value of 0.40 mg/L best approximates actual DOC concentration for all of the CERC test waters in chapter A and in chapter B. Therefore, a DOC value of 0.40 mg/L was used for BLM normalization for all of the toxicity tests described in chapter A, chapter B, and in appendix 8. However, for selected data, BLM modeling also was evaluated with values of 0.3 and 0.5 mg/L so as to perform sensitivity analysis by contrasting the range of potential average DOC concentrations of test waters in the current studies described in the following section.

Implications of Uncertainty in Dissolved Organic Carbon (DOC) Measurements on Estimates of the Sensitivity of Copper to White Sturgeon

As described in the previous section, for the present test waters, the best interpretation of the DOC measurements is that the test water contained close to 0.4 mg/L DOC, with true values ranging between 0.3 and 0.5 mg/L. Similarly, in the University of Saskatchewan testing, reported DOC concentrations ranged from at least 1.9 to 2.6 mg/L (Vardy and others, 2011); however, following comparative cross-check analyses of water at each laboratory, the estimate of the University of Saskatchewan DOC concentrations was about 1.0 mg/L (appendix 7). Thus, these ranges were used to model some of the more copper sensitive results (table 6–1).

The effect of uncertainty in DOC concentrations on BLM results was compared in three ways:

1. First, the empirical acute 50-percent effect concentrations (EC50s) were compared to the BLM-generated final acute value (FAV) used to set acute water-quality criteria. The FAV is the EC50 derived for a hypothetical organism representing the 5th percentile of a rank ordered distribution of mean species sensitivities. In other words, the FAV is a more sensitive EC50 than those for 95 percent of species represented in criteria datasets. The FAV was divided by two to extrapolate from an EC50 for a hypothetical sensitive species to a concentration expected to kill or harm few taxa. Thus, the FAV is the appropriate criteria-related value to compare to acute EC50s.

2. Second, 20-percent effect concentration (EC20) endpoints from chronic tests are compared directly to the copper chronic criterion (CCC, table 6–1) values. The choice to compare 20-percent effect levels to criteria values was arbitrary, but is intended to reflect a less severe effect level than 50-percent mortality.

3. Third, the reported effect concentration values were standardized from their actual test waters to a hypothetical, moderately hard water with 0.5 mg/L DOC. This is the same standard water composition as used by U.S. Environmental Protection Agency (2007) to compare and pool test results that had been performed in various different water compositions. The HydroQual (2007) version of the copper BLM was used for all modeling.

The model results indicate that differences in BLM-predicted copper effects data over an uncertainty band of 0.3 to 0.5 mg/L DOC were within about plus or minus (±)30 percent in the comparisons; that is, from 1.3-fold lower to 1.3-fold higher of the values using 0.4 mg/L (fig. 6–3). For the BLM-based criteria related values (that is, FAVs or CCC), a lower or higher bias to DOC values produces a lower or higher bias respectively to the criteria values; however, what may be less self-evident, with the BLM-standardized EC values, is that the effect of low or high bias in DOC values is reversed. For example, the first three entries in table 6–1 are for a test value with an empirical EC50 estimate of 2.7 µg/L. If the true DOC value in the test was indeed 0.4 mg/L, then the BLM-standardized EC50 would be predicted to be 1.5 µg/L; however, if a lower DOC concentration of 0.3 mg/L was used, the BLM-standardized EC value would be higher, 2.0 µg/L, and if a higher DOC value of 0.5 mg/L was used in the model, a lower standardized EC50 of 1.2 µg/L would be predicted by the BLM.

In the environment and in the modeled simplification, the sensitivity of copper binding to DOC and subsequent differences in toxicity predictions also would be expected to be affected by the copper concentrations in the test; however, for the range of DOC and copper concentrations modeled, the relative differences in BLM-predicted values were similar at high and low copper concentrations (table 6–1).

These modeling scenarios emphasize the critical importance of DOC measurements in toxicity tests with copper that are performed in test waters with low DOC and low copper concentrations. The fact that differences in DOC concentrations within the range of analytical noise (0.1 to 0.6 mg/L) would result in greater than (>)4 fold differences in EC values normalized to standard water conditions through the BLM has important implications for criteria evaluation and updates. In the species-sensitivity rankings of acute and chronic copper data in chapter A and chapter B, factor of four differences could lead to profound differences in relative species rankings, and in turn different conclusions of effect concentrations relative to USEPA national ambient water-quality criteria or Washington State water-quality standards. Because the DOC concentrations that were modeled approach the practical limits of accurate DOC analyses, Wang and others (2009) recommended avoiding the use of reconstituted water recipes with low background DOC in favor of using natural well waters or other waters that have been amended with low levels of DOC (that is, about 1 mg/L).

Table 6–1. Selected examples of biotic-ligand model (BLM) modeled effects of uncertainty in dissolved organic carbon (DOC) measurements on potential criteria values and standardized effect concentrations (LC or EC).

EC, effect concentration; LC, lethal concentration; mg/L, milligrams per liter; BLM, biotic ligand model; µg/L, micrograms per liter; Cu, Copper; FAV, final acute value; CCC, copper chronic criterion; ASTM, American Society for Testing and Materials; USEPA, U.S. Environmental Protection Agency; CERC, Columbia Environmental Research Center; dph, days-post-hatch; EC50, 50-percent effect concentration; Mort, mortality; LOE, loss of equilibrium; CC, continuous chronic; d, day; LH, lack of hiding; EC20, 20-percent effect concentration; h, hour; LC50, 50-percent lethal concentration; UofS, University of Saskatchewan; LC20, 20-percent lethal concentration

Species	Study	Test or scenario	EC or LC	Endpoint definition	DOC (mg/L) used in BLM modeling	Empirical EC in original test waters (µg/L Cu)	BLM-based FAV (acute endpoints) or CCC (chronic endpoints) (µg/L Cu)	Modeled EC for ASTM/USEPA mod-hard water (µg/L Cu)
White sturgeon	CERC, acute	A1, age 2 dph (low DOC estimate)	EC50	Mort+LOE+ lethargy	0.3	2.7	6.2	2.0
White sturgeon	CERC, acute	A1, age 2 dph (best DOC estimate)	EC50	Mort+LOE+ lethargy	0.4	2.7	7.9	1.5
White sturgeon	CERC, acute	A1, age 2 dph (high DOC estimate)	EC50	Mort+LOE+ lethargy	0.5	2.7	9.7	1.2
White sturgeon	CERC, chronic	CC 0-4 d (acute effects during chronic exp.) (low DOC estimate)	EC50	Acute Mort+LOE+ LH	0.3	4.5	6.0	3.5
White sturgeon	CERC, chronic	CC 0-4 d (acute effects during chronic exp.) (best DOC estimate)	EC50	Acute Mort+LOE+ LH	0.4	4.5	7.7	2.7
White sturgeon	CERC, chronic	CC 0-4 d (acute effects during chronic exp.) (high DOC estimate)	EC50	Acute Mort+LOE+ LH	0.5	4.5	9.4	2.2
White sturgeon	CERC, chronic	C2 0-28 d (low DOC value)	EC20	Biomass	0.3	2.7	1.5	2.6
White sturgeon	CERC, chronic	C2 0-28 d (best DOC estimate)	EC20	Biomass	0.4	2.7	1.9	2.0
White sturgeon	CERC, chronic	C2 0-28 d (high DOC estimate)	EC20	Biomass	0.5	2.7	2.3	1.7
White sturgeon	CERC, chronic	CC overall (low DOC estimate)	EC20	Biomass	0.3	1.6	1.6	1.5
White sturgeon	CERC, chronic	CC overall (best DOC estimate)	EC20	Biomass	0.4	1.6	2.0	1.2
White sturgeon	CERC, chronic	CC overall (high DOC estimate	EC20	Biomass	0.5	1.6	2.5	1.0
Rainbow trout	CERC, chronic	C2 0-28 d (growth) [low DOC]	EC20	Growth	0.3	30.0	1.4	29.2
Rainbow trout	CERC, chronic	C2 0-28 d (growth) [best DOC estimate]	EC20	Growth	0.4	30.0	1.8	25.3
Rainbow trout	CERC, chronic	C2 0-28 d (growth) [high DOC estimate]	EC20	Growth	0.5	30.0	2.2	21.9
White sturgeon	UofS, acute	2009 96h, 8 dph (reported DOC)	LC50	Mort	1.9	17.2	15.5	5.2
White sturgeon	UofS, acute	2009 96h, 8 dph (using 1.0 mg/L DOC, blank corrected)	LC50	Mort	1.0	17.2	8.2	10.1
White sturgeon	UofS, acute	2009 96h, 40 dph (reported DOC)	LC50	Mort	2.2	11.7	16.9	3.2
White sturgeon	UofS, acute	2009 96h, 40 dph (using 1.0 mg/L DOC, blank corrected)	LC50	Mort	1.0	11.7	7.7	7.2
White sturgeon	UofS, chronic	CC 59 d UofS (using 2.6 mg/L DOC, w/o blank correction)	LC20	Mort	2.6	10.1	10.0	1.4
White sturgeon	UofS, chronic	CC 59 d UofS (using 1.0 mg/L DOC, blank corrected)	LC20	Mort	1.0	10.1	3.9	3.7

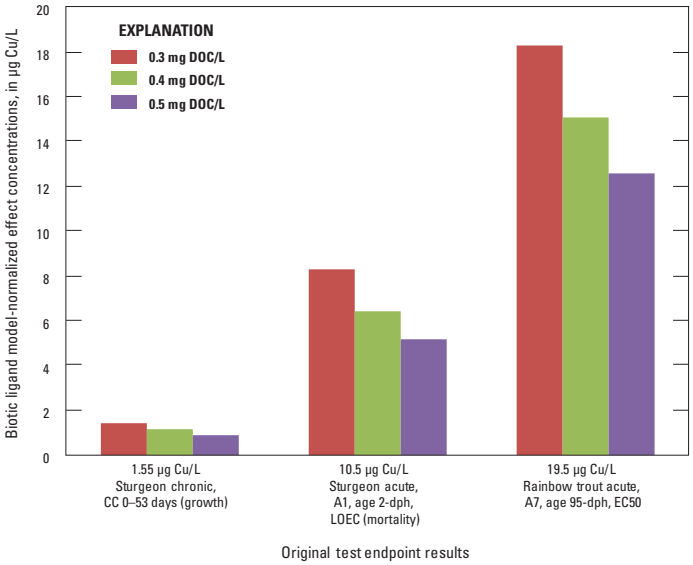

Figure 6–3. Sensitivity of biotic ligand model normalization to uncertainties in dissolved organic carbon (DOC) measurements, across a DOC range of 0.3 to 0.5 milligrams per liter (mg/L), in comparison to the best estimate of the overall DOC concentration of 0.4 mg/L. Comparisons are presented for selected test endpoints that ranged in copper concentrations from about 2 to 20 micrograms copper per liter (µg Cu/L). [dph, days-post-hatch; LOEC, lowest observed effect concentration; EC50, 50-percent effect concentration]

Because differences in DOC concentrations of only ±0.2 mg/L DOC can affect toxicity modeling, the accuracy and uncertainties of measurements in low-DOC laboratory water sources is important. The actual toxicity of copper can differ across that range of DOC concentrations, although apparently less so than predicted by the copper BLM version used in Welsh and others (2008). Further refinements to the copper BLM to dampen the effect of small changes in DOC at low DOC concentrations would seem appropriate, as has been previously recommended [for example, Welsh and others (2008), Wang and others (2009)].

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Appendix 7. Results from U.S. Geological Survey Columbia Environmental Research Center and University of Saskatchewan Interlaboratory Comparison of Analyses for Dissolved Organic Carbon in Water Samples

By William G. Brumbaugh

Because of the importance that dissolved organic carbon (DOC) has on mitigating aqueous copper toxicity to organisms, an interlaboratory study was performed to evaluate comparability of measured DOC results during white sturgeon (*Acipenser transmontanus*) or rainbow trout (*Oncorhynchus mykiss*) toxicity tests performed by the U.S. Geological Survey Columbia Environmental Research Center (CERC) and by the University of Saskatchewan (UofS). Duplicate sets of test water samples and blanks were collected by personnel at the UofS and the CERC on November 15, 2010 (fig. 7–1). Each laboratory then submitted one set of samples for analysis by Columbia Analytical Services (CAS) and the second set by CERC. For the first sample set, all sampling procedures (including collection containers and filters) followed the UofS/CAS protocol whereas the second set followed CERC protocol. The goal of the interlaboratory study was to determine if there were differences between UofS and CERC methods, but without a systematic attempt to identify what factor(s) contributed to differences (if any).

Procedures

1. One week before sampling, CERC shipped eight 120-mL amber glass bottles with tetra-fluoroethylene (TFE) lined lids (presoaked in high-purity water) to the UofS, and CAS sent eight 200-mL high density polyethylene bottles to the UofS and the CERC (each with appropriate amount of 9 normal sulfuric acid (H_2SO_4) preservative in each bottle). Meanwhile, UofS shipped a set of filter cartridges to the CERC.

2. All samples were collected on November 15, 2010. Samples analyzed by the UofS/CAS protocol were collected using a polypropylene syringe and immediately were filtered through a plastic filter cartridge housing a 25-millimter (mm) diameter, 0.45-micrometer (µm) pore-size polycarbonate filter. Samples to be analyzed by the CERC protocol were collected by glass pipette and, after 2 days of storage at 4 degree Celsius (°C), were filtered through a 47-mm diameter, 0.45-µm pore-size nylon membrane mounted on an all-glass filter support.

3. UofS placed their water samples in coolers with extra ice and sent them to CAS and to CERC by way of FedEx on November 15, 2010. Samples were received at CERC on Wednesday, November 17, 2010. The CERC held all CERC test water samples at 4 °C and sent those collected by the UofS protocol/CAS to CAS by way of FedEx overnight on November 17, 2010.

4. The CERC and UofS collected three field blanks, whereas CERC used commercially prepared, certified free (less than 0.05 mg/L) DOC water and UofS used their de-ionized (DI) laboratory water.

5. Samples were analyzed by CAS and by CERC on November 18, 2010. The CAS and CERC included analysis of additional laboratory blanks obtained using DI water at CAS and commercially obtained DOC-free water at CERC.

Results for these analyses are provided in figure 7–2. Based on the information in figure 7–2, the following conclusions were made:

- Sampling procedures used at UofS caused variable DOC contamination (2 to 3 mg/L), presumably caused by leaching from the filter cartridge, but perhaps also from the plastic bottle that was provided by CAS.

- After applying blank correction, mean concentrations of sample pairs were in good agreement, indicating good comparability between CAS and CERC analysis methods

- Test waters sampled during November 2010 at the UofS were estimated to have contained 1.0 mg/L DOC and at CERC were estimated to contain 0.2 mg/L DOC; however, that DOC results of analyses performed by CERC during 2010 studies probably were biased low by about 0.2 mg/L (appendix 6).

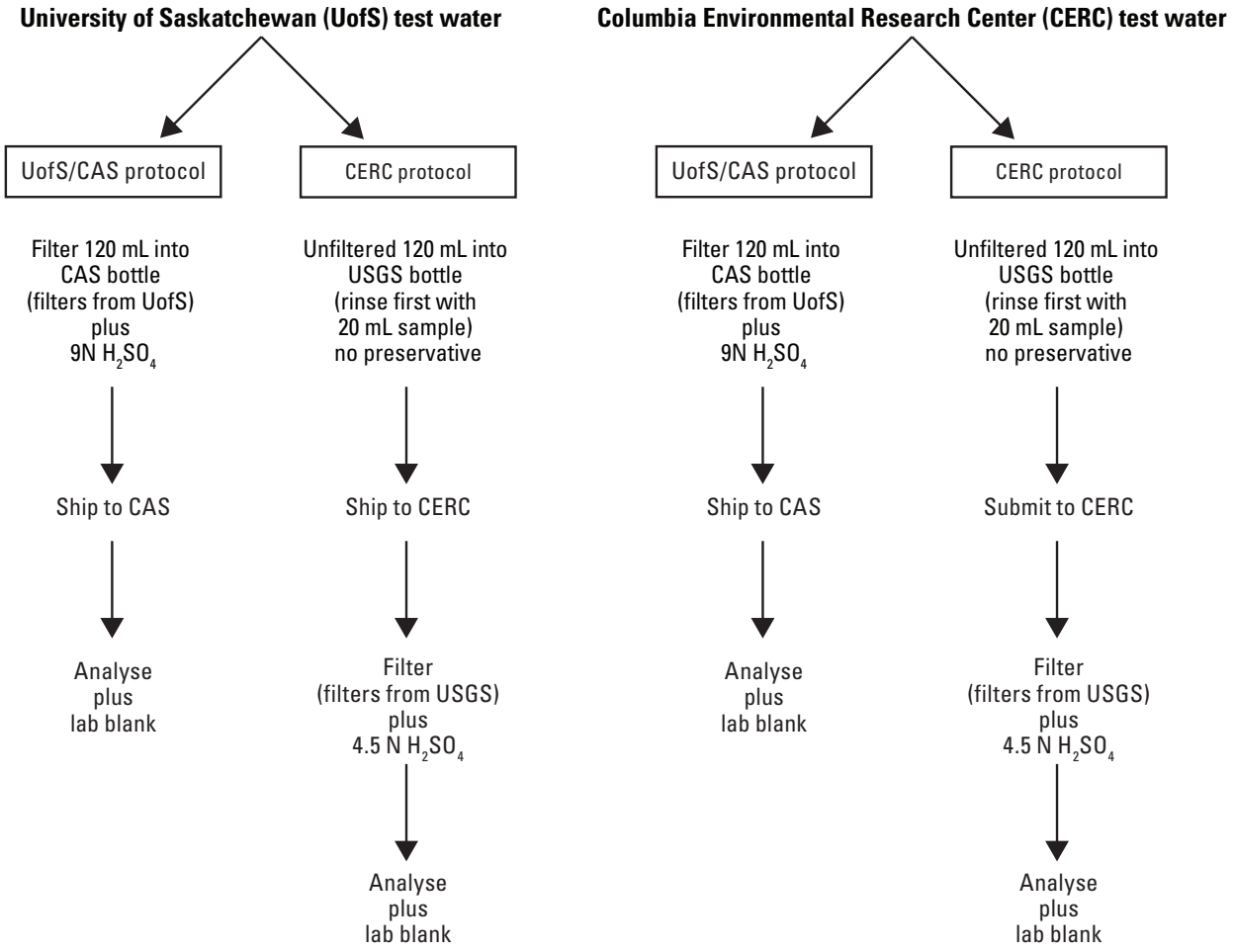

Figure 7–1. Schematic for interlaboratory comparisons of dissolved organic carbon analyses.

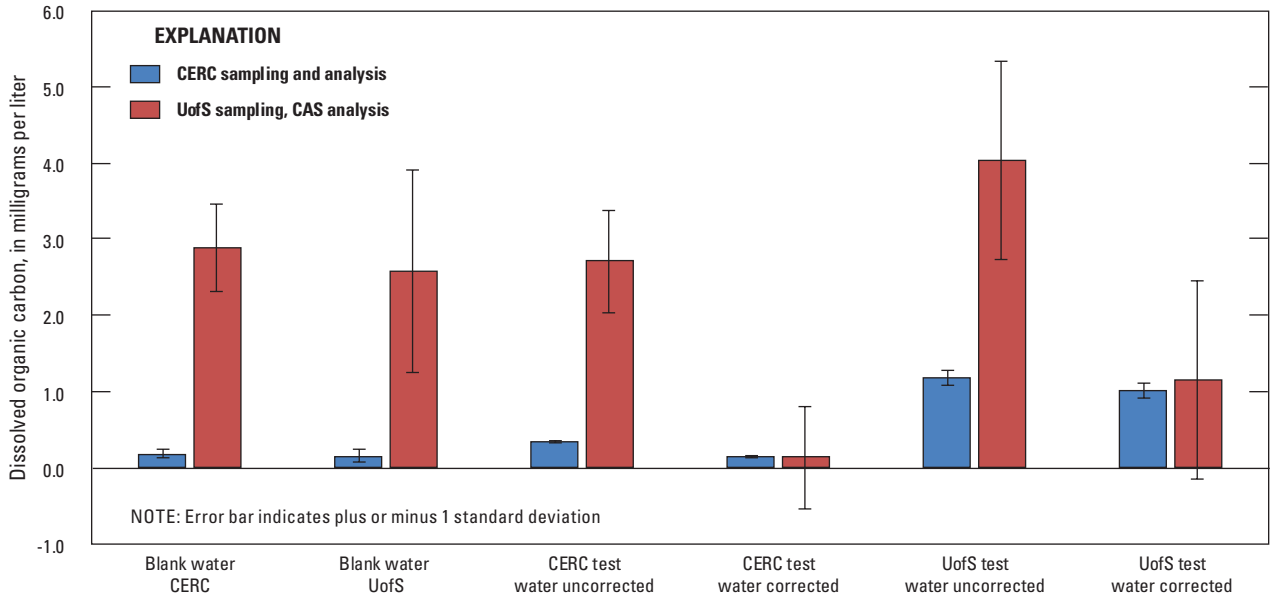

Figure 7–2. Results from U.S. Geological Survey Columbia Environmental Research Center (CERC) and University of Saskatchewan (UofS) November 2010 interlaboratory dissolved organic carbon study. Values represent means (n=3); uncorrected = not blank corrected; corrected = blank subtraction applied.

Appendix 8. 2012 Chronic Copper Toxicity Test and Light Intensity Test with White Sturgeon (*Acipenser transmontanus*)

By Ning Wang, Christopher G. Ingersoll, Rebecca A. Dorman, Brittany King, William G. Brumbaugh, and Christopher A. Mebane

Chronic Copper Toxicity Test

In the 2010 study, control survival in the chronic 25-day (d) life-stage 1 exposures (C1), starting with newly hatched white sturgeon, was low and did not meet chronic test acceptability requirement of greater than or equal to (≥)80-percent control survival (table B–2). A repeated copper exposure with newly hatched sturgeon was performed at the U.S. Geological Survey, Columbia Environmental Research Center in 2012. The 2012 study was performed to determine if improved survival of newly hatched sturgeon could be achieved in a chronic toxicity test. Methods for culturing the sturgeon and for performing the 2012 toxicity tests were consistent with the methods used to culture and perform toxicity tests with sturgeon in 2010 (table B–1). Newly fertilized eggs from two female and one male white sturgeon were provided by Yakima Fish Hatchery (Toppenish, Washington) on June 16, 2012. The sturgeon were caught between May 23 and 25, 2012, in the John Day pool at the McNary Dam tailrace on the Columbia River, near Benton County, Wash., and held individually in 4-meter (m) diameter (1.2-m high) fiberglass circular tank at 14 degrees Celsius (°C). The fish were not fed in the hatchery before spawning on June 14, 2012. The 24-d copper exposures started with 1-day-post-hatch (dph) larval sturgeon under similar test conditions that were used in the 25-d C1 exposure of the 2010 study (table B–1). The exceptions were that (1) the light intensity was reduced to about 50 lux during the first 14 days of the 2012 exposure when the larval sturgeon were in the hiding phase, and (2) the number of fish per replicate was 30. Food was provided starting on test day 9 before fish began to swim up. The fish were fed less than 1-d-old brine shrimp (*Artemia*) nauplii and laboratory-cultured aquatic oligochaetes (*Lumbriculus variegatus*) 2 or 3 times daily in excess (food available for at least 4 hours (h) after each feeding). The oligochaetes initially were cut into pieces (about 5-millimeter, mm, length) to facilitate initial feeding of larval fish. When the fish started feeding on oligochaetes, more oligochaetes were added and the amount of brine shrimp was reduced gradually until the fish were fed only oligochaetes. The light intensity was increased and maintained at about 500 lux on test day 15 when the sturgeon started swimming up and actively feeding.

Water quality was measured weekly on composite water samples collected from the replicates in the control, medium, and high exposure concentrations. Mean dissolved oxygen (plus or minus, ±, standard deviation; n=15) was 9.04±0.47 milligram per liter (mg/L), conductivity 256±2 microsiemens per centimeter (µS/cm), pH 8.10±0.08, alkalinity 95±5 mg/L as calcium carbonate ($CaCO_3$), hardness 102±2 mg/L as $CaCO_3$, and total ammonia nitrogen 0.07±0.05 mg/L. Water samples for the analysis of major cations and major anions, and dissolved organic carbon were collected biweekly in water samples from the control and medium exposure concentrations. Mean calcium (± standard deviation; n=4) was 26±0.7 mg/L, magnesium 8.9±0.2 mg/L, potassium 0.9±0.02 mg/L, sodium 9.0±0.3 mg/L, chloride 9.5±0.1 mg/L, sulfate 18±0.2 mg/L, and dissolved organic carbon 0.41±0.1 mg/L.

Mean control survival was 93 percent by the end of the 24-d copper exposure, and met the test acceptability of ≥80-percent control survival (American Society for Testing and Materials, 2012). The 20-percent lethal concentration (LC20) was 3.01 microgram copper/L (µg Cu/L), and 20-percent effect concentration (EC20) was 1.44 µg Cu/L based on dry weight, and 1.72 µg Cu/L based on biomass (table 8–1). The LC20 or EC20 obtained from the 2012 24-d copper exposure with a ≥90-percent control survival did not substantially differ from the LC20s or EC20s for copper obtained from the 2010 53-d exposure with low control survival (table B–6) and the previous 66-d exposure with low control survival (Vardy and others, 2011).

Light Intensity Test

At the start of the 2010 chronic 53-d toxicity tests, performed starting with newly hatched white sturgeon, the light intensity was inadvertently set high (ranging from about 900 to 1,300 lux, instead of about 200 to 500 lux) for the first 21-d exposures and was close to an upper range of light intensity of 10–20 microeinstein per square meter per second (about 500 to 1,000 lux) recommended by the U.S. Environmental Protection Agency for estimating chronic toxicity of effluents and receiving waters (U.S. Environmental Protection Agency, 2002). To determine whether the high light intensity was stressful to the newly hatched sturgeon, a follow-up study was performed in 2012 for 25 days starting with 3-dph sturgeon under the control conditions with two different light intensity levels (50 and 1,000 lux). Newly fertilized eggs from one female and one male were provided on July 11, 2012, from

Table 8–1. White sturgeon (*Acipenser transmontanus*) chronic responses (mean of four replicates unless noted) and effect concentrations in a 24-day copper exposure conducted in 2012 starting with 1-day-post-hatch larvae.

[Yellow shading indicates significant reduction relative to the control. Due to 100-percent mortality in two replicate at a high exposure treatment, replicate number for weight calculation was 2 at the 8 µg Cu/L treatment. µg Cu/L, microgram copper per liter; %, percent; SD, standard deviation; g, gram; NOEC, no-observed-effect concentration; <, less than; LOEC, lowest-observed-effect concentration; Geomean, geometric mean of the NOEC and LOEC; LC/EC10, 10-percent lethal or effect concentration; CL, 95-percent confidence limits; LC/EC20, 20-percent lethal or effect concentration]

Nominal concentration (µg/L)	Measured concentration (µg/L)	Survival (%)		Dry weight (g)		Biomass (g, dry)	
		Mean	SD	Mean	SD	Mean	SD
0	0.15	93.4	6.1	0.0123	0.0004	0.344	0.020
0.5	0.53	79.2	11.0	0.0109	0.0012	0.258	0.036
1	0.92	87.5	5.0	0.0103	0.0008	0.272	0.030
2	1.90	85.9	1.7	0.0085	0.0003	0.220	0.008
4	3.67	51.7	23.8	0.0030	0.0002	0.048	0.023
8	7.40	4.2	5.0	0.0020	0.0004	0.003	0.003
NOEC		1.90		<0.53		<0.53	
LOEC		3.67		0.53		0.53	
Geomean		2.64		<0.53		<0.53	
LC/EC10 (CL)		2.56 (1.79–3.67)		1.02 (0.73–1.43)		1.39 (1.05–1.85)	
LC/EC20 (CL)		3.01 (2.39–3.79)		1.44 (1.13–1.85)		1.72 (1.39–2.12)	

the Sherman Creek Hatchery, the same hatchery that provided eggs for the 2010 study. The fish were caught 2 days earlier on July 9, 2012, at Five Mile Creek, Northport, Washington, on the Columbia River, the same location where the sturgeon were caught for the 2010 study. Test conditions were similar to those for the C1/CC exposures performed in the 2010 study (table B–1), except that (1) no toxicant was added in test water, (2) light intensity was about 50 lux in a low-light treatment and about 1,000 lux in a high-light treatment, and (3) the number of fish was 10 in each of six replicates. Mortality and behavior were recorded daily at 9:00 a.m. during the 25-d study.

Most of the fish hid under stones and mean percent hiding was similar between the low- and high-light treatments during the first 10 days of the study (fig. 8–1). Less than 50-percent hiding was observed on test day 11 in the high-light treatment, whereas less than 50-percent hiding was observed on test day 14 in the low-light treatment (fig. 8–1). Mean survival

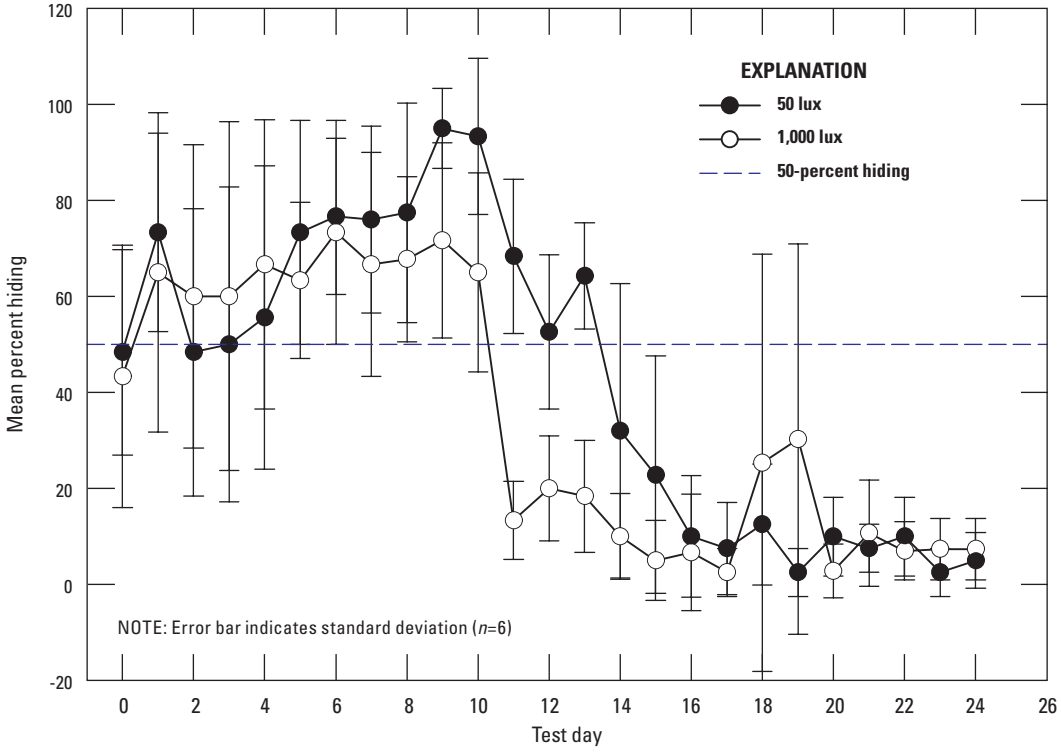

Figure 8–1. Mean percentage of white sturgeon (*Acipenser transmontanus*; n) hiding under stones in the low (50 lux) and high (1,000 lux) light intensity treatments during a 25-day study period.

(87 percent) and mean dry weight (14.2 mg/individual) at the end of the low-light treatment were not significantly different from mean survival (96 percent) and mean dry weight (13.5 mg/individual) at the end of the high-light treatment (*t*-test, $p>0.05$). The results indicate that the high light intensity of 1,000 lux may shorten the hiding phase by about 3 days compared to the low-light intensity of 50 lux at 15 °C; however, the light intensity did not affect the 25-d survival or growth.

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