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SUBJECT: Spokane River Metals
Water Effects Ratio Study

INTRODUCTION

The Spokane River is known to have elevated concentrations of metals due in large part to leaching of tailings from historic mining operations in the area. Concern over these elevated levels has resulted in a number of studies over the years to develop an understanding of the effects they may have on biota in the river system.

The Water Effects Ratio procedure, developed by U.S. Environmental Protection Agency Duluth Laboratory, gives an indication of the impact a receiving water may have on the toxicity of a specific pollutant in that system. To evaluate differences in bioavailability and effective toxicity of a toxicant, the LC50 in site water is compared to the LC50 in Laboratory reconstituted water.

For the Spokane River Study, the two sites selected as receiving water collection sites had previously been identified as having relatively different chemical characteristics. Five metals, cadmium, copper, lead, mercury, and zinc, were selected for testing. *Daphnia magna* and Rainbow Trout (*Oncorhynchus mykiss*) were used to evaluate the relative toxicity of each metal in the two river water samples and in laboratory reconstituted water.

SAMPLE IDENTIFICATION

The samples were collected September 24, 1992, and held on ice until delivered to Manchester Laboratory September 25. Sample identification was as follows:

Sample No. 39-8250: "Metals in River Water; 24-Sep-92; Bioassay; Station: SPKN96"
A clear liquid

Sample No. 39-8254: "Metals in River Water; 24-Sep-92; Bioassay; Station: SPKN65"
A clear liquid

Collection of samples was scheduled to minimize the time lapse between the probable latest low flow period on the Spokane River, and the earliest fall hatching of Rainbow Trout for testing.

METHODS

Testing was conducted following the methods defined in EPA (1992) *Interim Guidance on Interpretation and Implementation of Aquatic Life Criteria for Metals*, following the guidance for evaluation of the Water-Effect Ratio. A dilution series was prepared for each metal using each of the receiving waters and the laboratory water as dilution water. The range of dilutions was determined by the hardness of each of the three dilution waters. Toxicity data from U.S. EPA Ambient Water Quality documents for each of the metals was used as a guide to estimate a probable LC50 for that metal in each water. Based on a recommendation from Charles Stefan (EPA Duluth Lab), a dilution factor of 0.6 was used to establish the appropriate dilution series range for each test. Because of the uniqueness of each of the river water samples, it was not possible to ensure that the chosen dilution series would bracket the LC50 for a given metal. For situations in which the LC50 was not initially bracketed, testing was reinitiated based on information gained from the first test, or on data from screening tests. Prior to testing, screening tests on both samples were done with the two test organisms to ensure the river waters were not inherently toxic to either species; no mortality was noted.

Capacity of temperature controlled testing facilities dictated the testing routine. Two metals were tested on the three waters, using both test organisms, each week of the study. Cadmium and copper were tested the week of October 6; lead and zinc were tested the week of October 13; and, mercury was tested the week of October 10. Retesting was as soon as possible, as capacity allowed.

Five dilutions were made from a laboratory stock solution of each metal for each of the three waters. The stock solutions for cadmium and copper were prepared from EPA reference toxicants; cadmium chloride, lot 786, and copper sulfate, lot 188 (EPA/EMSL, Cincinnati). The remainder of the stock solutions were prepared from Baker Analyzed Reagents; lead nitrate, mercuric chloride, and zinc chloride.

Preparation of most test solutions was in 20 liter Pyrex tanks. If it was determined that the two test organisms would require different dilution series, test solutions for *Daphnia magna* were prepared in one liter Pyrex beakers. After each dilution was prepared and mixed thoroughly, a sample was collected for metals analysis, and an appropriate volume was placed in each of the test chambers. A control was also tested for each of the three waters concurrently with metals testing for each organism.

Original testing with the two organisms was done concurrently. The trout tests were initiated on Monday and terminated on Friday; *Daphnia magna* tests were initiated on Tuesday and terminated on Thursday. This routine was selected in consideration of the conditioning and handling requirements of the test organisms. Specific test methods for each of the organisms are described below.

Metals analyses were done by the Manchester Laboratory Inorganics/Metals Unit. Samples were prepared for total recoverable metals analysis by US EPA Method 200.2. Lead, copper, and zinc were analyzed by Inductively Coupled Plasma; cadmium was analyzed using graphite furnace atomic absorption spectroscopy. Mercury was determined by CLP method 245.1, cold vapor atomic absorption spectroscopy.

Survival data were used to calculate an LC50 for each metal in each of the three waters. EPA software for the Trimmed Spearmann-Karber Method or Probit was used for LC50 calculations. Results were verified by graphical LC50 estimates. Water-Effect Ratios for each metal were calculated by dividing the river water LC50 by the Laboratory water LC50 for each river water sample. Nominal concentrations were used for mercury calculations; analyzed concentrations were used for the remainder of the tests.

SALMONID

Testing was conducted following the method described in U.S. EPA (1991) *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*. Test organisms were hatched at Manchester Laboratory from eggs obtained from Washington State Department of Wildlife. Eyed eggs were received at Manchester on August 27; animals were two weeks post swim-up at initiation of testing. Hatching, and holding of organisms was in flow-through tanks using dechlorinated Manchester City Water. Fish were fed three times daily during holding; feeding was withheld 48 hours prior to and during testing.

Each test replicate was prepared with four liters of test solution in a two and one-half gallon glass aquarium. Two replicates were prepared for each test concentration. Ten trout were added randomly, two at a time, to each replicate. Testing was in a temperature controlled chamber at 12 ± 1°C, with a 16:8 light:dark photoperiod.

Dissolved oxygen, pH, and temperature were measured daily during testing, and mortalities were enumerated and removed from test chambers. Samples were removed at 0 and 96-hours to analyze for hardness, alkalinity, and conductivity. After 96-hours, the number of survivors was recorded, and the control organisms were weighed and measured for loading calculations.

Daphnia magna

The *Daphnia magna* test of survival and reproduction was conducted following the method described in U.S. EPA (1991) *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*. Test organisms were from cultures maintained at Manchester Laboratory. Animals had been conditioned for two months in preparation for testing.

Test vessels were 30 ml Pyrex beakers containing 25 ml of test solution. Four replicate beakers were prepared for each test concentration. Five less-than-24-hours-old neonates were placed in each replicate beaker at test initiation. Replicates were placed in a $25 \pm 1^{\circ}C$ incubator, with a 16:8 light:dark photoperiod. Water quality parameters (dissolved oxygen, pH, and temperature) were measured daily throughout testing. Samples were taken for hardness, alkalinity, and conductivity on initiation and termination. After 48-hours, testing was terminated, and the number of survivors was enumerated and recorded.

RESULTS

Test results are summarized in Tables 1 and 2. Complete tabulation of data from these tests are presented in Appendix I for Salmonid tests, and in Appendix II for *D. magna*. Printouts from statistical analysis are included in each Appendix. Copies of bench sheets from metals analyses for test solutions are in Appendix III.

Table 1. A summary of LC50 estimates and resulting Water Effects Ratios for five metals in 96-hour Salmonid tests conducted using Laboratory Reconstituted Water and water from two sites on the Spokane River as dilution water.

Metal	Dilution Water	LC50 (ug/L)	Water Effects Ratio
Cadmium	Lab Water	3.90	
	39-8250	1.10	0.28
	39-8254	5.53	1.42
Copper	Lab Water	30.9	
	39-8250	27.5	0.89
	39-8254	67.0	2.17
Lead	Lab Water	371	
	39-8250	76	0.20
	39-8254	549	1.48
Mercury	Lab Water	197	
	39-8250	219	1.11
	39-8254	309	1.57
Zinc	Lab Water	423	
	39-8250	229	0.54
	39-8254	594	1.40

Table 2. A summary of LC50 estimates and resulting Water Effects Ratios for five metals in 96-hour *Daphnia magna* tests conducted using Laboratory Reconstituted Water and water from two sites on the Spokane River as dilution water.

Metal	Dilution Water	LC50 (ug/L)	Water Effects Ratio
Cadmium	Lab Water 10/14	9.82	
	39-8250	35.3	3.60
	Lab Water 10/6	3.01	
	39-8254	17.1	5.68
Copper	Lab Water	25.7	
	39-8250	6.22	0.24
	39-8254	85.2	3.31
Lead	Lab Water	692	
	39-8250	83	0.12
	39-8254	286	0.41
Mercury	Lab Water	2.23	
	39-8250	3.00	1.34
	39-8254	7.38	3.30
Zinc	Lab Water	484	
	39-8250	150	0.31
	39-8254	799	1.65

CADMIUM

The initial testing the week of October 5, bracketed the Trout LC50s for all three waters, and for two of the waters for *D. magna*. Sample 39-8250, however, was more protective of the test organisms against cadmium than was expected, based on its hardness. The test was repeated the following week for that sample, using a higher range of dilutions, and for the Lab Water; and the LC50 was bracketed in the retest. The LC50 for the Lab Water was 3.01 ug/L for the first set of tests and 9.82 ug/L for the second set; these values are consistent with the range of values this Laboratory normally expects using cadmium chloride as a reference toxicant. The Water Effects Ratio for each set of data was calculated using the Lab Water LC50 for that particular test period.

The Water Effects Ratios for cadmium are summarized below. Sample 39-8250 affected a greater sensitivity in the Salmonid test to cadmium; sample 39-8254 made them somewhat less sensitive than the reconstituted Lab Water. The Daphnids were markedly less sensitive to cadmium in both of the Spokane River samples than in the Laboratory Water.

Water Effects Ratios

Sample Number	Salmonid	*Daphnia magna*
39-8250	0.28	3.60
39-8254	1.42	5.68

COPPER

Copper was also tested during the week of October 5. All estimated testing ranges effectively bracketed the LC50's for both test organisms.

The Water Effects Ratios for copper are summarized below. Sample 39-8250 rendered both test organisms more sensitive to copper relative to their response in Laboratory Reconstituted Water. The ratios indicate Sample 39-8254 was protective of both test organisms relative to the Lab Water.

Water Effects Ratios

Sample Number	Salmonid	*Daphnia magna*
39-8250	0.89	0.24
39-8254	2.17	3.31

LEAD

Initial lead tests yielded unusual results. In all cases either the estimated range failed to bracket the LC50, or dose-response curves were atypical. The atypical response occurred in Lab Water tests for both organisms, and Sample 39-8254 for *D. magna*; survival initially decreased with concentration, then increased at higher concentrations.

The phenomenon was discussed with Charles Stefan, EPA Duluth Laboratory, an author of the Water Effects Ratio method. He said other researchers had observed a similar effect in silver tests using marine organisms, however the phenomenon had not been documented in the literature to this point. His explanation was that the solubility of lead was probably exceeded mid-range in these tests. That, combined with the complexing effects of several components of harder waters, could produce two, three, or even more LC50s for lead.

An attempt was made to further bracket higher concentration LC50s to test this hypothesis. Four 1-liter beakers were prepared with lead concentrations of 9,646 ug/L, 16,075 ug/L, 26,792 ug/L, and 44,653 ug/L; five trout were added to each. After 96 hours, mortality was observed only in the two highest concentrations; 100% at 26,792 ug/L, and one mortality at 44,653 ug/L. This suggests that the results obtained in the initial testing were not anomalous.

A second set of tests were done, with a goal of bracketing the lowest LC50, since that is the level most likely to be observed in a receiving water. For tests that initially did not bracket the LC50, a lower range of concentrations were prepared based on information gained from the earlier tests. The multiple peak response curve was once again seen in trout tests for Lab Water and for Sample 39-8250. The testing range for 39-8250 in the second Salmonid test was still high, however the supply of sample was depleted by that time; an LC50 was therefore estimated using the graphical method.

The Water Effects Ratios for Lead are summarized below. Only the data from the second set of tests are included in this summary. Complete data from both tests are tabulated in Appendix A and B, however. Sample 39-8250 affected a greater sensitivity in both organisms than did the Lab Water. *D. magna* was more sensitive to the lead in Sample 39-8254, however the Salmonid test showed less sensitivity than in the Lab Water.

Water Effects Ratios

Sample Number	Salmonid	*Daphnia magna*
39-8250	0.20	0.12
39-8254	1.48	0.41

MERCURY

A pretest was done to establish appropriate test ranges for mercury, to minimize waste volumes, considering the special problems of disposing of mercury wastes. Testing was the week of October 23. All tests effectively bracketed the LC50s for both organisms. Analyzed concentrations for mercury were consistently slightly less than nominal concentrations. The analysts suggested mercury concentrations may have decreased slightly during holding, since analysis was near the end of the 28-day holding period. Therefore, statistics have been using nominal rather than analyzed concentrations for mercury.

The Water Effects Ratios for mercury are summarized below. Sample 39-8250 provided only slightly greater protection for the test organisms than did the Lab Water. Sample 39-8254 affected somewhat more protection for Salmonids; *D. magna* was markedly less sensitive to mercury using this dilution water.

Water Effects Ratios

Sample Number	Salmonid	*Daphnia magna*
39-8250	1.11	1.34
39-8254	1.57	3.30

ZINC

Zinc was tested the week of October 12. Estimated testing ranges effectively bracketed all LC50's for both test organisms.

The Water Effects Ratios for zinc are summarized below. Ratios were similar for both organisms. Sample 39-8250 rendered both test organisms more sensitive to copper relative to their response in Laboratory Reconstituted Water. The organisms were somewhat less sensitive when sample 39-8254 was used as dilution water relative to Lab Water responses.

Water Effects Ratios

Sample Number	Salmonid	*Daphnia magna*
39-8250	0.54	0.31
39-8254	1.40	1.65

REFERENCES

U.S. EPA. 1989. *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms.* EPA/600/4-89/001.

EPA (1992) *Interim Guidance on Interpretation and Implementation of Aquatic Life Criteria for Metals.* Health and Ecological Criteria Division, Office of Science and Technology, Washington, DC 20460.