APPENDIX B (Note Appendix B1 was updated after this report was written)

REPORT ON

ASSESSMENT OF TOXICOLOGICAL EFFECTS OF SULPHATE UNDER VARYING WATER HARDNESS CONDITIONS USING THE EARLY LIFE STAGES OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

Draft

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EXECUTIVE SUMMARY

Several studies have suggested that water hardness may play a pivotal role in modifying the toxicity of a number of environmental chemicals. In an early life stage test, the lethal and sublethal effects of sulphate (SO_4^{2-}) under varying water hardness levels were examined in a model fish species, the Rainbow trout. For this test, a range of water hardness values (6, 50, 100 and 250 mg/L as $CaCO_3$) were used.

Water hardness played a significant role in modifying the toxicity of SO_4^{2-}. The modeling of the results from the embryo mortality studies with SO_4^{2-} at various hardness levels was straight-forward. The plots of the data showed that the concentration-response curves for the different hardness levels were well separated with decreasing SO_4^{2-} toxicity at higher water hardness values.

Results from the 30-d exposure of swim up fry were less clear. Mortality was affected by hardness with lower toxicity with higher hardness values, however, there is less confidence in LCxx values than with embyros. Effects on fry mass were minimal and ECxx values could not be determined.

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1.0 INTRODUCTION

The British Columbia (BC) Ministry of Environment (MOE) develops province-wide ambient water quality guidelines for parameters that are important for the surface waters of BC. This work has the following goals: 1) to provide guidelines for the evaluation of data on water, sediment, and biota and 2) to provide guidelines for the establishment of site-specific ambient water quality objectives.

Specifically, a water quality guideline is a maximum and/or a minimum value for a physical, chemical or biological characteristic of water, sediment or biota, which should not be exceeded to prevent specified detrimental effects from occurring to a water use, including aquatic life, under specified environmental conditions.

Guidelines are set after considering the scientific literature, guidelines from other jurisdictions, and general conditions in BC. Toxicity tests are conducted to provide data used to set water quality guidelines. Aquatic animals are important models for research and monitoring the effects of environmental pollution. Comparison of contaminant effects, however, is often difficult due to factors such as intra- and inter-specific variation in species sensitivity, differences between laboratory practices, variances in animal husbandry, and fluctuations in water quality and test chemical concentration.

In this regard, water hardness is most often defined as the sum of calcium (Ca^{2+}) and magnesium (Mg^{2+}) cations in solution. Hardness is expressed frequently as calcium carbonate ($CaCO_3$) equivalents (mg/L). Hardness has been shown to modify sulphate (SO_4^{2-}) toxicity to several aquatic organisms. The majority of the research regarding the influence of hardness on its toxicity has demonstrated an antagonistic effect. From the large body of research, the majority of studies reported a decrease in toxicity with increasing hardness.

The purpose of these studies was to determine the relationship between water hardness and the toxicity of SO_4^{2-} to the early life stages of Rainbow trout (embryos and swim up fry).

2.0 MATERIALS AND METHODS

2.1 Fish

Fertilized Rainbow trout eggs from adults were obtained from the Miracle Springs Trout Hatchery, Abbottsford, BC and transported shortly after water hardening to the aquatic facilities at Simon Fraser University. Eggs were immediately sorted into test chambers and raised in the dark at 14°C until the eyed egg stage when dosing began. Eggs were initially raised to this stage in dechlorinated water with pH 6.9, hardness 6 mg/L as $CaCO_3$, O_2 saturation >95% (9.5 mg/L).

2.2 Chemicals

All chemicals used were obtained from Fisher Scientific and Sigma-Aldrich Chemical Co. The source of SO_4^{2-} was Na_2SO_4. Four water hardness values were used: 6 (ambient), 50, 100, and 250 mg/L as $CaCO_3$. Test water was prepared by the addition of reagent grade salts to obtain the target hardness concentrations. The source of Ca and Mg for obtaining these water hardness values was $CaSO_4$ and $MgSO_4$. In order to determine the appropriate mg/L Ca or Mg concentrations at these ratios to make the various water hardness values the following equation was used:

$$[CaCO_3]=2.5*[Ca^{2+}]+4.1*[Mg^{2+}]$$

Test water of various hardness values were prepared at 14°C in 5000-L fiberglass tanks, and mixed by submersible pumps for a minimum of 72 h. These mixing times were necessary as $CaSO_4$ is relatively non-soluble in water and does not go into solution easily.

2.3 Dilution water conditions

Dilution water temperature was 14 ±1°C, pH 6.9, and oxygen content 9.8 mg/L. All eggs were raised in the dark. Visual observations were made under red light.

2.4 Chemical QA/QC

It was important to determine if chemical concentrations were maintained at desired concentrations within test chambers. Representative water samples were taken at the start and end of an experiment and placed in appropriate sample bottles and delivered to Maxxam Analytics Inc., Burnaby, B.C. for analysis. Water samples were also taken for hardness, pH and dissolved oxygen measurements from representative tanks.

2.5 Bioassays

Acute lethality tests for fish

Early life stage 21-d LC_{50} tests were carried out according to procedures in Environment Canada (1998) and test conditions are shown in Table 1. Embryos were transferred to incubation units (n=30) within plastic tubs (n=3 per treatment) supplied with fresh flowing dechlorinated municipal water. Eggs were incubated in the dark at 14°C until they reached the eyed stage at which time exposure to water of varying hardness and concentrations of SO_4^{2-} began. Six SO_4^{2-} concentrations were tested, each at 4 hardness values. For each combination of SO_4^{2-} concentration and hardness, 3 different tubs containing 5 incubation units were used (randomly placed), each unit containing 30 embyros for a total of 450 embyros tested per concentration.

Each tub containing incubation units was 8 L in volume and was replaced in total every 40 min. This resulted in 36 total replacements per day. Flow rates for SO_4^{2-} stock solutions were controlled by peristaltic pumps and did not vary by more than 1% at any time.

The test apparatus were modified versions described in Environment Canada (1998). This design enables gentle aeration and continuous circulation of test solutions past incubating embryos. All test requirements outlined in Environment Canada (1998) were satisfied.

Following swim up, fry were released into 1-L plastic grow out chambers within replicate tubs containing water of the appropriate hardness and the appropriate concentration of SO_4^{2-}. Water in each tub was flow through. Fish were fed 4% bw/d. Water temperature was kept at a constant 14 ± 1°C. Chambers were examined for fish mortality and recorded for an additional 30 d. Fish were also examined for behaviour and growth. Fry are assessed for dry weight after the 30 d exposure period. Conditions are found in Table 2.

Table 1. Summary of test conditions for the 21-d Early Life Stage Test (embryo mortality test)

Test organism	*Oncorhynchus mykiss*
Test type	Flow through
Endpoints	Mortality
Organism source	Miracle Springs Hatchery
Organism size	Eyed eggs
Feeding	none
Test chamber	8-L plastic tub

Incubation chamber	800-mL tri-pour beaker
Test volume	400 mL
Test temperature	14±1°C
Control water	Municipal dechlorinated water
Number of organisms/replicate	30
Number of replicates	15
Photoperiod	Dark
Aeration	Yes
Test protocol	Environment Canada (1998); EPS 1/RM/28
Test acceptability criterion for controls	>95% survival

Table 2. Summary of test conditions for the 45-d fish survival and growth test

Test organism	*Oncorhynchus mykiss*
Test type	Flow through
Endpoints	Survival and dry weight at 30 d
Organism source	Miracle Springs
Organism age	Swim up fry
Feeding	EWOS trout feed, 4% bw/d, 4 times/d
Test chamber	Plastic nytex chamber
Test volume	1 L
Test temperature	14±1°C
Control water	Municipal dechlorinated water
Number of organisms/replicate	5-30
Number of replicates	15
Photoperiod	16h light/8 h dark
Aeration	Yes
Test protocol	Environment Canada (1998); EPS 1/RM/28
Test acceptability criterion for controls	>70% survival

2.6 Calculations and Statistics

2.6.1 Modeling

Mortality Responses.

Mortality for Rainbow trout embryos was studied by placing 30 organisms (n=30) in an incubation chamber (5 of these) within a replicate tub (3 of these). The test had 15 replicate chambers under each combination of the concentration of SO_4^{2-} and water hardness. The number of non-viable embyros after 21-d at swim up was measured. The same concentration series was used for all hardness levels and the concentration of SO_4^{2-} were chosen to ensure that the observed mortality rates ranged from 0 to 100% according to previously unpublished studies.

Because multiple replicates were conducted under identical experimental conditions, the mean and variance of the observed mortality rates can be compared to that expected under a binomial distribution with the average mortality rate. Plots of the empirical standard deviation *v*. the

expected standard deviation should fall approximately along the line X=Y if the inter-trial/replicated variability follows a binomial distribution. If the majority of points fall above the line X=Y this would indicate over-dispersion and the need to insert additional variation using random effects as was done in Schwarz (2011).

For the mortality responses, Probit models (Bliss, 1934) were used. The basic Probit model assumes that the number of deaths follows a binomial distribution where the probability of mortality is "linked" to a linear function through the normal distribution. For example, consider the Probit model of mortality as a function of dose for a fixed hardness level. The statistical model is:

$$Dead_{ij} \sim Binomial(BatchSize_{ij}, p_i)$$
$$p_i = \Phi\left(\beta_0 + \beta_i \log\left(D_{ij}\right)\right)$$

where $Dead_{ij}$ is the number of dead organisms observed in the j^{th} batch out of the initial $BatchSize_{ij}$ units on tests at concentration (dose) level (SO_4^{2-}) D_i; β_0, β_1 are the intercept and slope in the Probit model; and Φ is the cumulative normal distribution. [The original papers on Probit analysis added 5 to the linear functions to avoid negative numbers in hand computations, but this is no longer required when using computers.] The parameters are estimated using maximum likelihood (e.g. via Proc Probit or Proc Logistic (with probit link) in SAS). Estimates of the LCxx values (i.e. at what concentration will a fraction xx or organism die or be affected) can be found once estimates of the slope and intercept are found by solving the equation:

$$LCxx/100 = \Phi\left(\hat{\beta}_0 + \hat{\beta}_i \log\left(D_{ij}\right)\right)$$

Maximum likelihood estimates are asymptotically the best possible estimates and extract the maximum amount of information from the data. Estimates of precision (i.e. standard errors) can be found automatically for the parameters of the likelihood equations and by the delta method (Taylor series expansion) for the LCxx values.

In Schwarz (2011), similar models were fit, but in some cases additional complexities presented themselves because of natural responses (i.e. mortality that was present even at dose 0). No such complexities were needed for this report. Additionally, in Schwarz (2011), there was evidence of a lack of fit due to over-dispersion in some cases – again, no evidence of over-dispersion was found in these studies and so models incorporation random effects or variance inflation factors were not needed.

The primary goal of this study was to investigate the effect of hardness levels on the concentration-response curve. This was accomplished by fitting models to the combined concentration-chemical response data using the multiple hardness and mixture levels data.

Suppose, for illustration purposes, that there are 4 hardness levels used for a particular chemical for a total of 4 concentration-response curves. At one extreme, denoted as model M*H*D, a separate concentration-response probit curve is fit to each hardness. So, under this model, 4 separate curves are fit and this model will require 8 parameters (an intercept and a slope for each hardness). This model would indicate that the concentration-response curve is different for all combination of hardness so that no data simplification is possible.

At the other extreme, denoted as model D, a single concentration-response Probit model is fit data over all hardness levels. This model has only 2 parameters (a common slope and intercept over all hardness combinations). This model would indicate that the 4 separate concentration-

response curves are essentially the same, and that the same LCxx values could be used regardless of the hardness.

This suite of models was fit for SO_4^{2-} and rainbow trout embryos. For each model, estimates of the LCxx are found for each hardness. Model selection and averaging was based on AICs as outlined in Schwarz (2011).

30-d fry mortality

The survivors from the egg mortality data were then tracked out to 30 days and the number of survivors at 30 days is measured. The mortality of the egg-fry stage was modeled in a similar fashion as the embryo mortality, i.e. with a set of probit models.

Note that for some sulphate levels, there were only few survivors from the egg stage and so that some trials could not be completed (e.g. if the number of surviving eggs was less than 5, the trial was not extended to the 30 days stage. This has implications for the fitting process as the 30-day mortalities for high sulphate levels are based on vary small numbers of fry and so have a very small weight in the overall model fit.

Also, the highest observed mortality was typically 50% or less, so that estimates of LC50 are likely not well determined except by extrapolation outside of the range of the data. The LC50 should be used cautiously.

30-d fry growth

The average weight of the fry at 30 days was measured finding the total weight of the surviving fish and finding the average weight per fish for each replicate of each container. Up to 15 average values are available for each hardness-sulphate dose combination, but fewer may be available because high mortality from the egg stage would remove the replicate from further study.

The 3-parameter log-logistic model[1] was used to model the average 30-day rainbow trout weight. This model has the form:

$$E[Y] = \frac{C}{\left(1+\left(\frac{X}{LD50}\right)^{B}\right)}$$

where C is the baseline mean weight at X(Dose)=0, $LD50$ is the dose corresponding to the IC50, and B is a scaling factor. This model was fit using maximum likelihood assuming normally distributed residuals about the fit. Goodness-of-fit was assessed using residual and other diagnostic plots. The ECxx values were found by solving the equation

$$C(1-\text{Endpoint}) = \frac{C}{\left(1+\left(\frac{X}{LD50}\right)^{B}\right)}$$

[1] This model has an additional parameter, σ, representing the standard deviation of the residuals around the fit. This parameter has been included in the AIC computations.

where the *Endpoint* is .1, .20., .25 or .50. [The above equation can be solved explicitly for *X*.] For example, the EC10 is the dose where the mean weight is 90% of the baseline mean (the value of *C*).

Three models were fit. In the first model, denoted as Model C(.) LD50(H) B(H) a separate dose-response curve is fit for each hardness but with a common average weight at a 0 sulphate dose. This requires one common parameters for *C;* four separate *LD50* and *B* parameters for each hardness, and variance parameter for a total of 10 parameters.

The second model, denoted as Model C(.) LD50(.) B(.), fits a single dose-response curve is fit for all hardnesses and only requires 4 parameters.

The third model, denoted as Model C(H) LD50(H) B(H), fits a separate dose-response curve for each hardness and requires 13 parameters (4 x 3 parameters for the curves + 1 parameters for the variance).

For each model, estimates of the ECxx are found for each hardness. Model selection and averaging was based on AIC as outlined in Schwarz (2011).

3.0 RESULTS

3.1 Chemical QA/QC

Values for pH, dissolved oxygen, and temperature were all within accepted test parameters. Temperature was held at 14±1°C, and oxygen 9.5±0.3 mg/L. The pH varied with hardness and were 6.9±0.1, 7.2±0.1, 7.4±0.2, and 7.5±0.2 for hardness values of 6, 50, 100 and 250 mg/L as $CaCO_3$. Measured water hardness values were always >95% of nominal values. Water samples were analyzed from typical experiments at the start and end of experiments to determine if actual SO_4^{2-} concentrations were close to nominal concentrations. Measured values were always >77% of nominal values and can be seen in Table 3.

Table 3. Representative sulphate concentrations at the start and end of each experiment in each exposure treatment group in the embryo mortality test.

Sulphate concentrations (mg/L)								
Hardness (mg/L)	6		50		100		250	
Sample	**Nominal**	**Measured**	**Nominal**	**Measured**	**Nominal**	**Measured**	**Nominal**	**Measured**
1 Start	0	2.00	41	38.1	82	85.1	267	252
2 End	0	1.83	41	43.6	82	88.7	267	206
3 Start	125	122	125	126	125	133	500	453
4 End	125	123	125	133	125	133	500	445
5 Start	250	222	250	242	250	205	750	714
6 End	250	204	250	194	250	197	750	691
7 Start	500	423	500	461	500	460	1000	963
8 End	500	492	500	466	500	455	1000	938
9 Start	1000	974	1000	957	1000	960	1500	1380
10 End	1000	1000	1000	972	1000	960	1500	1380
11 Start	2000	1870	2000	1720	2000	1730	2000	1720
12 End	2000	1820	2000	1700	2000	1690	2000	1740

3.2 Bioassay results

3.2.1 Mortality of embryos

Control mortalities in all treatment groups were well within acceptable limits for this bioassay and are shown below in Table 4:

Table 4. Control mortalities of rainbow trout embryos in various hardness treatment groups.

Percent control mortality of eggs (%)					
Tub	Repl.	Hardness: 6 mg/L	50 mg/L	100 mg/L	250 mg/L
1	1	0.0	3.3	0.0	0.0
	2	3.3	0.0	0.0	0.0
	3	0.0	0.0	0.0	0.0
	4	0.0	0.0	0.0	3.3
	5	0.0	0.0	3.3	3.3
2	1	0.0	3.3	0.0	0.0
	2	0.0	0.0	0.0	0.0
	3	0.0	3.3	6.7	0.0
	4	0.0	0.0	3.3	0.0
	5	0.0	0.0	3.3	0.0
3	1	0.0	0.0	0.0	0.0
	2	3.3	0.0	0.0	0.0
	3	0.0	3.3	0.0	0.0
	4	0.0	0.0	0.0	0.0
	5	0.0	0.0	3.3	6.7

The results for the analysis of the mortality data for rainbow trout embryos to SO_4^{2-} exposure is voluminous and rather than placing it in this report, it has been attached as an Appendix.

The following file represents the analysis performed:

- Appendix B-1

Appendix B-1 (rb-mort.pdf) has the relevant information within it and is described here. On page 1 there is a partial list of raw data. On page 2 there is a summary of replicates and tubs used in the study. On page 3 there is a summary of total mortality.

With such large sample sizes, you can directly read LCxx values without having to do any modeling; it is quite obvious that hardness has a protective effect. On page 6 one can see the check for over dispersion which is not a problem, if anything there is some under dispersion which usually isn't a problem. On page 7 a plot of the raw data is shown. No evidence of a natural response as the mortality rates go to 0 except for a few eggs that died at the 0 concentration in the first experiment (about 1/2 of 1%).

On page 8 there is the usual analysis where multiple models are fit and ranked using AIC. Model H*D indicates a separate curve is needed for each hardness; Model H+D indicates a separate curve is needed for each hardness but the curves are simply shifted left/right (i.e. parallel). Model D indicates that a single curve over all hardness levels can be fit. All of the model weight, not surprisingly, is on model H*D. With the very large sample sizes, this is not surprising.

Appendix B-1 page 9 are shown plots of the model fit over the actual data. It is good, but there is evidence of some lack of fit. For example, the curve at hardness 6 is pulled

to the left by the very low dose and so will estimate LC10 a bit too small compared to the actual curve. The general problem is the curve is a bit too steep for a probit model to fit exactly. There isn't any point in fitting a more complex model as the estimates won't change very much.

Appendix B-1 Page 12-27 gives the estimated LCxx values in both the log/anti-log scale which are also given below in Table 2. On page 28/29 the LCxx values on log and anti-log scales are graphed with hardness and are tabulated on page 30. Because of the slight "lack-of-fit" see on age 9, the model averaged SE shouldn't be taken too literally. The same general methods as outlined in the other reports for sulpate are used, i.e. maximum likelihood, probit model but this time no random effects was needed nor natural response.

For a summarized table of LC10, LC20, LC25, and LC50 values at each hardness and for each mixture, see Table 5 below:

Table 5. Estimated LCxx values, Standard Errors (SE) and upper (UCL) and lower confidence limits (LCL) for rainbow trout embyros at 4 hardness values. Values are estimated LCxx values. LCL and UCL are 95% confidence intervals of each value. HD = hardness in mg/L as $CaCO_3$.

O. mykiss	LCxx values (upper and lower confidence limits): endpoint mortality															
	LC10				LC20				LC25				LC50			
	Est	SE	LCL	UCL	Est	SE	LCL	UCL	Est	SE	LCL	UCL	Est	SE	LCL	UCL
HD																
6	175.4	7.8	160.8	191.4	252.0	9.0	235.1	270.2	289.5	9.5	271.6	308.7	507.0	13.6	481.1	534.3
50	299.5	11.9	277.1	323.8	418.9	13.4	393.5	446.0	475.4	14.1	448.5	503.8	792.3	20.6	752.9	833.8
100	419.2	17.4	386.4	454.7	597.1	19.4	560.2	636.4	683.8	20.5	644.9	725.2	1182.1	35.8	1114.0	1254.5
250	673.7	19.6	636.4	713.2	891.7	19.1	854.9	930.0	993.6	20.3	954.7	1034.2	1522.1	33.4	1457.9	1589.1

3.2.2 Mortality of fry

Control mortalities in all treatment groups were well within acceptable limits for this bioassay and are shown below in Table 6:

Table 6. Control mortalities of rainbow trout fry in various hardness treatment groups.

Percent control mortality of fry					
Tub	Repl.	Hardness: 6 mg/L	50 mg/L	100 mg/L	250 mg/L
1	1	3.3	0.0	3.3	0.0
	2	0.0	0.0	0.0	0.0
	3	0.0	0.0	3.3	0.0
	4	0.0	0.0	0.0	3.3
	5	0.0	3.3	0.0	0.0
2	1	3.3	0.0	0.0	0.0
	2	0.0	0.0	3.3	3.3
	3	0.0	6.9	6.7	0.0
	4	3.3	3.3	3.3	3.3
	5	3.3	3.3	0.0	3.3
3	1	0.0	0.0	3.3	0.0
	2	0.0	0.0	0.0	3.3
	3	3.3	0.0	0.0	6.7
	4	0.0	0.0	3.3	3.3
	5	3.3	3.3	0.0	7.1

The results for the continued (30-d) SO_4^{2-} toxicity test with swim up fry is voluminous and rather than placing it in this report, it has been attached as an Appendix.

The following file represents the analysis performed:

- Appendix B-2

Appendix B-2 Page 1 shows part of the raw data and Pages 2/3 are a summary of the number of replicates. Note that for higher sulphate doses, the number of available replicates is small because only trials that had sufficient survival of the eggs are allowed to proceed to the 30 day mortality trial.

Pages 4/5 show the same on an egg basis. The last two columns show the remainder after the initial mortality that are allowed to continue to the end. Notice the very small number of eggs at the very high doses (e.g. 37 eggs left for a dose of 1000). This implies that the right end of the dose-response curve is not well estimated because of the very small sample size and so values such as the LC50 are likely not reliable at these higher doses.

Page 9 is a check for overdispersion; none was seen, in fact, there existed some underdispersion).

Page 10 is a plot of raw mortality curves. The "protective" effect of hardness (curves shifted to the right, and the curves seem to be properly "nested" within each other) can be seen. It is difficult to estimate the 50% mortality for higher hardnesses as the data

doesn't cover the LC50 point.

The plot also shows no natural response, so a probit model was fit without a natural response.

Page 22 is the Model ranking table. Not unexpectedly, a separate curve is required for each hardness (the H*D) model. The parallel curve (i.e. simple shift to the right as hardness increases) was also not supported.

Page 23 is the fit of the curves. It is not unusual that the curves may not appear to fit the data well at the higher sulphate levels; this is not due to the higher doses having very small sample sizes. The dose-response curves for the two higher hardness values cross, which is an artifact of the data. The LC50 estimates may not be reliable, however, the LC10/20/25 should be reasonably reliable.

Pages 26-29 show the individual estimates of the LCxx at each hardness on the log-scale. Pages 30-33 show the same, but on the anti-log scale. Pages 34-41 are the model averaged LCxx values. Because of the large weight given to the H*D model, the estimates are essentially those from that model.

Page 42 are plots of the LCxx by hardness on the anti-log scale. Note that the LC50 curve bends down, but these may not be actual LC50 values because the 50% mortality is often well outside the range of the observed data. Page 43 is the same plot on the log-scale.

Page 44/45 is the model averaged estimates of the LCxx values. These are the two tables in the *-short.rtf file.

Definite evidence of protective effects of hardness are seen in this data set. Because of the small number of eggs available at the higher sulphate levels and because the raw data rarely reaches the 50% mortality point, the LC50 values may not be useful. The LC10/20/25 seem to be well fit from the data and are shown in the Table 7 below:

Table 7. Estimated LCxx values, Standard Errors (SE) and upper (UCL) and lower confidence limits (LCL) for rainbow trout swim up fry through a 30-d continued exposure to sulphate (mg/L) at 4 hardness values. HD = hardness in mg/L as $CaCO_3$.

O. mykiss	LCxx values (upper and lower confidence limits): endpoint mortality															
	LC10				LC20				LC25				LC50			
	Est	SE	LCL	UCL	Est	SE	LCL	UCL	Est	SE	LCL	UCL	Est	SE	LCL	UCL
HD																
6	363.2	9.3	345.4	382.0	534.4	8.9	517.2	552.1	619.3	9.6	600.7	638.5	1122.8	31.6	1062.5	1186.5
50	367.9	19.9	330.9	409.1	707.7	20.1	669.4	748.2	907.6	24.5	860.8	956.8	2477.5	170.1	2165.5	2834.4
100	771.7	31.2	712.9	835.3	1559.0	68.5	1430.4	1699.2	2036.2	114.0	1824.6	2272.4	3670.0	0.0	3670.0	3670.0
250	1224.7	15.9	1193.8	1256.3	1626.4	14.9	1597.4	1655.9	1813.7	17.4	1780.0	1848.1	2797.8	60.2	2682.2	2918.4

3.2.3 Growth

The results for the continued (30-d) SO_4^{2-} toxicity test with swim up fry for growth is voluminous and rather than placing it in this report, it has been attached as an Appendix.

The following file represents the analysis performed:

- Appendix B-3

Page 1 is part of the raw data.

Pages 2/3 shows the number of trails. As in the 30-d mortality data, fewer trials are seen at higher doses.

Pages 4/5 is the summary of number of fish. Notice the smaller numbers at the higher doses. Here the sample size is the number of "averages" available as each rep gives a single average final body mass.

Page 10 shows the graph of mean weight with dose of chemical and hardness. The average weight is about 850 mg per fish. The LC10 point would be a reduction to 850*.9=760. None of the experiments even comes close to reaching the LC10 point. So none of the LC10/20/25/50 will have any reliability except based on extrapolations from the models. As well, the final "points' where the curve bends are based on very small sample sizes (the higher doses) and so most models give little weight to these points.

Page 11 shows the same plot, except on anti-log scale.

On Page 12 the data from a log-logistic model which starts with an asymptote and then tries to curve down as the dose gets larger is shown. The model at first appears sensible - the model with a common intercept c (.) but different curves for each hardness (ld50(h) b(h)) is the "best" fit. However, on page 13 which is a graph of this data, the curve for the lowest hardness is good, but the curves for the other hardness are essentially flat. The 'dip' at the end is not picked up because of the small sample size at the higher doses. The model tries to concentrate most of its "fit" where the data are richer and gives little credence to a dip based on a small number of data points. A number of different models for the data were attempted with the same results. It is virtually impossible to capture the dip at the end based on a small number of data points. As noted earlier, none of the raw data even comes close to an LC10 effect, nor the LC20/25/50, which lends to these results being somewhat uniformative.

The rest of the printout does not appear useful. It estimates LCxx values on the log-scale in the thousands which corresponds to exp(thousand) on the anti-log scale (e.g. 14 on the anti-log scale is about a million) So, a successful experiment, but not very helpful in determining an LCxx value based on growth.

4.0 SUMMARY AND DISCUSSION

In this report, the toxicity of SO_4^{2-} to rainbow trout embyros and fry was examined where nonviability and growth were the measured endpoints.

Among the various water quality parameters that could potentially influence the uptake and toxicity of other chemicals or elements, hardness is often reported to be a major

factor influencing both. Moreover, given that hardness usually co-varies with pH and alkalinity, the normalization of bioavailability or toxicity values for water hardness would incorporate these factors as well. Most studies used to derive log-linear hardness equations (USEPA 1999) used in determining regulatory criteria have been derived from studies conducted in waters with hardness values between 25 to 400 mg/L (as $CaCO_3$), as there is a paucity of studies using very hard waters (>400 mg/L), although they do occur naturally in many jurisdictions. In this study, a range of 6 to 250 mg/L was used.

There were two parts to this early life stage test; 1) acute mortality of embryos, and 2) further mortality and growth measures for a 30-d period following swim up. The results of the second part of the experiment were not as clear as the first part. With swim up fry, definite evidence of a protective effect of hardness was seen in this data set. Because of the small number of eggs available at the higher sulphate levels and because the raw data rarely reaches the 50% mortality point, the LC50 values may not be useful. The LC10/20/25 seem to be well fit from the data. For the growth data, none of the raw data comes close to an LC10 effect, nor LC20/25/50, which lends to these results being somewhat uniformative.

The modeling of the results from the acute egg mortality study was straight forward. The experimental data were fit by relatively simple probit models with no evidence of natural mortality or over-dispersion. The plots of the data showed that the dose-response curves for the different hardness levels were well separated and that hardness provided a protective effect to embryos from sulphate toxicity.

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6.0 REFERENCES

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