PNNL-13471

Chromium Toxicity Test for Fall Chinook Salmon (*Oncorhynchus tshawytscha*) Using Hanford Site Groundwater: Onsite Early Life-Stage Toxicity Evaluation

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July 2001

Prepared for the U.S. Department of Energy
and the Hanford Natural Resource Trustee Council
under Contract DE-AC06-76RL01830

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Pacific Northwest National Laboratory
Richland, Washington 99352

Summary

The objective of this study was to evaluate site-specific effects for early life-stage (eyed eggs to free swimming juveniles) fall chinook salmon that might be exposed to hexavalent chromium from Hanford groundwater sources. Our exposure conditions included hexavalent chromium obtained from Hanford groundwater wells near the Columbia River, Columbia River water as the diluent, and locally adapted populations of fall chinook salmon. This report describes both a 96-hr pretest using rainbow trout eggs and an early life-stage test beginning with chinook salmon eggs.

The exposure levels for both tests were a control plus 11, 24, 54, 120, and 266 μg/L (target concentrations) of hexavalent chromium. The control treatment was unfiltered Columbia River water. The test was conducted in a modified Mount and Brungs flow-through diluter system. Temperature was controlled by chilling the exposure water before it entered the diluter and placing the exposure aquaria in a temperature-controlled water bath. The photoperiod for the test organisms was controlled to mimic environmental conditions. Specific endpoints measured during the early life-stage test with fall chinook salmon included survival, development rate, and growth. Chromium tissue burdens of fish were measured to evaluate uptake and elimination rates. Specifications of the exposure conditions were within the limits established by the test protocol (Quality Assurance Project Plan 2000).

This study showed that the survival, development, and growth of early life-stage fall chinook salmon from the eyed-egg stage to swim-up stage were not adversely affected by exposures to hexavalent chromium from 11 to 266 μg/L. Survival was high for all treatment levels and controls, exceeding 98% at termination of the test. In addition, there was no difference among the lengths and weights of fish among all treatment groups at test termination. Whole-body concentrations of chromium in early life-stage fall chinook salmon had a typical dose-response pattern; i.e., those subjected to highest exposure concentrations and longest exposure intervals had higher tissue concentrations.

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The authors wish to acknowledge the substantial contributions from A. J. DeLonay and A. M. Farag (United States Geological Survey, Biological Resources Division) in the development of the experimental protocol for the toxicity evaluation and for technical guidance during the evaluation.

S. H. Poirier and S. F. McGovern (Environmental Consulting and Testing) provided excellent technical support and guidance for use of the Mount Brungs proportional diluter. L. S. Bingler and staff at Battelle's Sequim Marine Laboratory provided accurate and timely chromium analysis for water and tissue samples. R. Schalla (PNNL) provided technical assistance in selecting an appropriate groundwater well to meet the test requirements and in specifying groundwater collection equipment. D. A. Neitzel (PNNL) and K. D. Ham (PNNL) provided valuable comments and insights with their peer review of the report. Rosalind Schrempf (PNNL) and S. Q. Bennett (PNNL) provided editorial review; and J. K. Slavens (PNNL) and L. M. Andor (PNNL) provided text processing.

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1.0 Introduction

During 1943, the Hanford Site (1,450 km^2) was created in south-central Washington State for the production of special nuclear materials for national defense (Figure 1.1). The need for large buffer areas for security and public safety effectively prevented dam construction and other development in the area. Consequently, the approximately 90-km stretch of the Columbia River that flows by the Site (the Hanford Reach) has remained the only non-impounded portion of the Columbia River in the United States above Bonneville Dam. One benefit of restrictions on development is that the Hanford Reach is the only remaining area on the Columbia River where significant mainstem spawning of fall chinook salmon (*Oncorhynchus tshawytscha*) occurs (Dauble and Watson 1997).

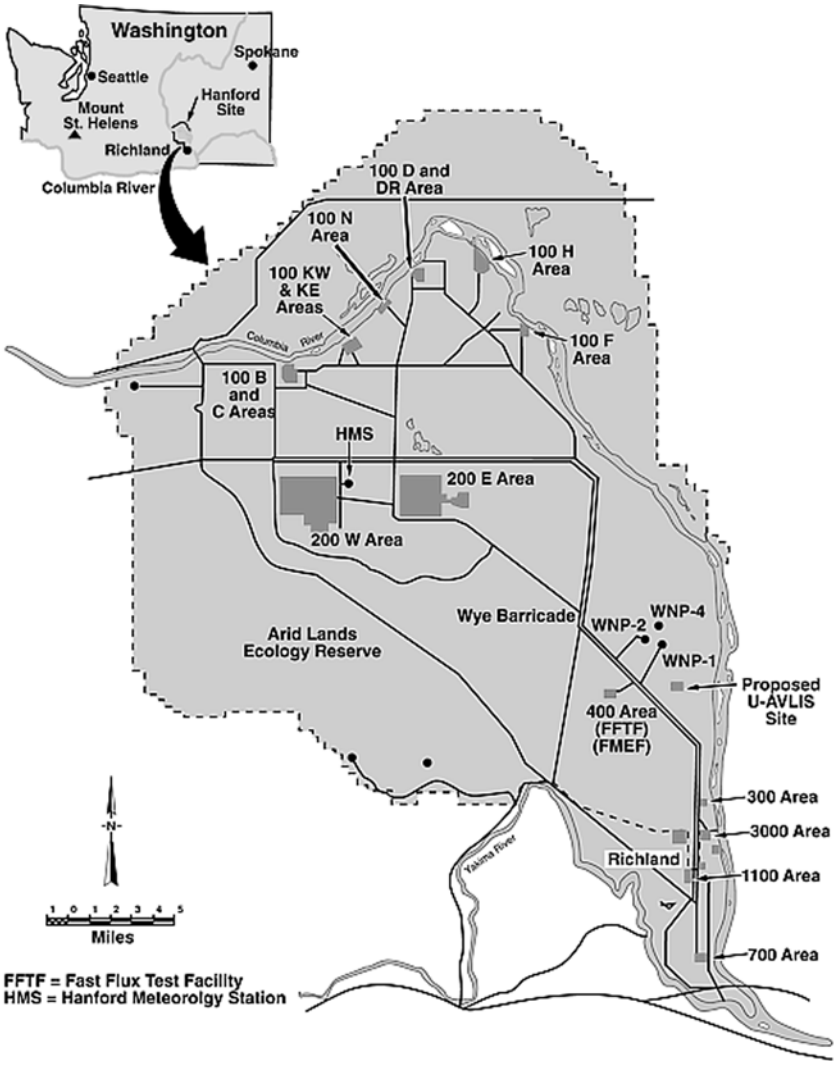

Figure 1.1. Hanford Site Map

Operations at the single-pass reactors discharged substantial quantities of sodium dichromate (hexavalent chromium) to areas adjacent to the Columbia River. Although reactor operations at the single-pass reactors ceased at Hanford during 1971, hexavalent chromium remains a contaminant of concern in the groundwater, particularly in near-shore areas adjacent to the spawning habitat of fall chinook salmon. Recent work has shown that the concentration of hexavalent chromium in groundwater upwelling into the Columbia River was above the ambient water quality criteria of 10 μg/L (Hope and Peterson 1996a; WAC 13-201A-040).

The effect of hexavalent chromium on juvenile chinook salmon has been documented (Olson and Foster 1956; Buhl and Hamilton 1991). However, these studies did not address the potential impacts of Hanford groundwater on chinook salmon under exposure scenarios specific to their early life history. The most likely scenario for salmon being exposed to Hanford groundwater would be at the point when the eggs and eleuthroembryos are present in the river bottom substrate or as salmon redds. Chinook salmon could be exposed to chromium during this early life-stage interval (i.e., during egg development, hatching, and through swim up) at specific locations where contaminated groundwater was upwelling into the river. Surface water monitoring has shown the Columbia River rapidly dilutes the groundwater upwelling (Van Verst et al. 1998; Poston et al. 2000); thus, free-swimming juvenile salmon rearing along the shoreline before migrating downstream to the Pacific Ocean are not likely to be exposed to elevated concentrations of chromium.

This report summarizes the results of laboratory studies conducted from October 1999 through March 2000 in support of the Hanford Natural Resources Trustee Council (HNRTC). These studies are one part of an overall effort to evaluate the potential impacts of contaminated groundwater from the Hanford Site on fall chinook salmon populations in the Hanford Reach of the Columbia River. During 1998 to 1999, at the direction of the HNRTC, the United States Geological Survey (USGS), in cooperation with the U.S. Fish and Wildlife Service (USFWS), initiated studies to investigate the health status of salmon exposed to chromium during the early and parr stages (Quality Assurance Project Plan 2000; Farag et al. 2000). They conducted a series of tests to determine the potential for chromium exposures to impact chinook salmon populations, addressing 1) gametes and the fertilization process, 2) early development using simulated Hanford Site groundwater, 3) degree of health impairment in juvenile salmon, and 4) avoidance response. Fertilization tests revealed that fertilization success was not affected by chromium concentrations ranging from 11 to 266 μg/L. There were no observable adverse effects on growth or survival for the early life-stage test at chromium concentrations from 0 to 120 μg/L. However, the health evaluation revealed that extended exposures to chromium concentrations of 54 to 120 μg/L could impact the health of juvenile chinook salmon with changes noted in DNA, histology, lipid peroxidation, and necropsies (Farag et al. 2000). The avoidance test revealed that parr-stage chinook salmon avoided dissolved chromium concentrations ≥54 μg/L in simulated river water but did not avoid similar concentrations of dissolved chromium in simulated Hanford groundwater (DeLonay et al. 2000). Because Farag et al. (2000) conducted laboratory tests with hexavalent chromium using water reconstituted to simulate conditions present in the Columbia River and fall chinook salmon from a hatchery stock outside of the Columbia River Basin, there was interest in validating the toxicological evaluation of early life history stages using conditions similar to those expected to occur in the Hanford Reach.

The objective of this study was to evaluate site-specific effects for early life-stage fall chinook salmon that might be exposed to hexavalent chromium from Hanford groundwater sources. Our exposure conditions included hexavalent chromium obtained from Hanford groundwater wells, Columbia River water as the diluent, and locally adapted populations of fall chinook salmon. Section 2.0 of this report describes the methods for groundwater collection, a 96-hr pretest, an early life-stage test, and the statistical techniques used. The results for both the 96-hr pretest and the early life-stage test are presented in Section 3.0. A discussion of the results relative to the Hanford Site environment and the scientific literature is provided in Section 4.0. Cited references can be found in Section 5.0, and appendixes are provided for more detailed analytical and statistical results.

2.0 Methods

2.1 Hanford Site Groundwater Collection

The hexavalent chromium source for all testing was obtained from Hanford Site Environmental Monitoring Well 199-D5-43 (called D5-43 in Figure 2.1) in the 100-D Reactor Area (Hartman et al. 2000). Unfiltered groundwater was collected into 10-L carboys or 10-L cubetainers using a dedicated submersible pump. One-third of each container was filled until all containers were full to homogenize the groundwater in each batch. Water samples were collected for chromium analyses for each collection period with samples taken after approximately 5 to 10 L of water had been collected (initial concentration), approximately halfway (midpoint), and at the end of the collection (endpoint). The groundwater was stored in Pacific Northwest National Laboratory's (PNNL's) Aquatic Laboratory at approximately 22°C until needed (~2 to 16 days). The toxicity test groundwater was placed in a 45-L carboy from which a metering pump dispensed the groundwater into the modified Mount and Brungs diluter.

2.2 Exposure Equipment and Chemical Tests

Unfiltered Columbia River water from the 300-Area water intake (Columbia River km 550) was used to dilute the chromium-contaminated groundwater to desired test concentrations. The six treatments were 0, 11, 24, 54, 120, and 266 µg/L (target concentrations) of hexavalent chromium. Assuming a nominal groundwater chromium concentration of 2,400 µg/L (Table A.1), the approximate percentage of groundwater in each chromium exposure concentration were 0%, 0.46%, 1.0%, 2.2%, 5.0%, and 11% for the respective six treatments. The control treatment was unfiltered Columbia River water. The test was conducted in a modified Mount and Brungs (1967) flow-through diluter system (Environmental Consulting and Testing, Superior, Wisconsin). Temperature was controlled by chilling the exposure water before it entered the diluter and by placing the exposure aquaria in a temperature-controlled water bath. The test apparatus was covered with black plastic or blankets to protect the eggs from light before the swim-up stage. The egg cups were suspended into the exposure aquaria from motorized rocker arms to provide a gentle circulation of exposure water past the eggs. Egg mortality was monitored and recorded daily. For the early life-stage test, the hatchlings were released to the exposure aquarium, and the egg cups were removed.

Total hardness (as $CaCO_3$) measurements were made weekly using a HACH test kit (Method 8213, ethylenediaminetetraacetic acid (EDTA) titration, HACH Company, Loveland, Colorado). Total alkalinity (as $CaCO_3$) determinations were made weekly using a LaMotte test kit (Model DR-A, LaMotte Company, Chestertown, Maryland). Conductivity and pH measurements were made daily using an Ultrameter 6P (Myron L Company, Carlsbad, California). Temperature was measured daily using a digital thermocouple verified daily by comparison with a reference thermometer. Dissolved oxygen was measured daily using a YSI Model 52 dissolved oxygen meter equipped with a YSI model 05511-42 self-stirring probe (YSI, Yellow Springs, Ohio).

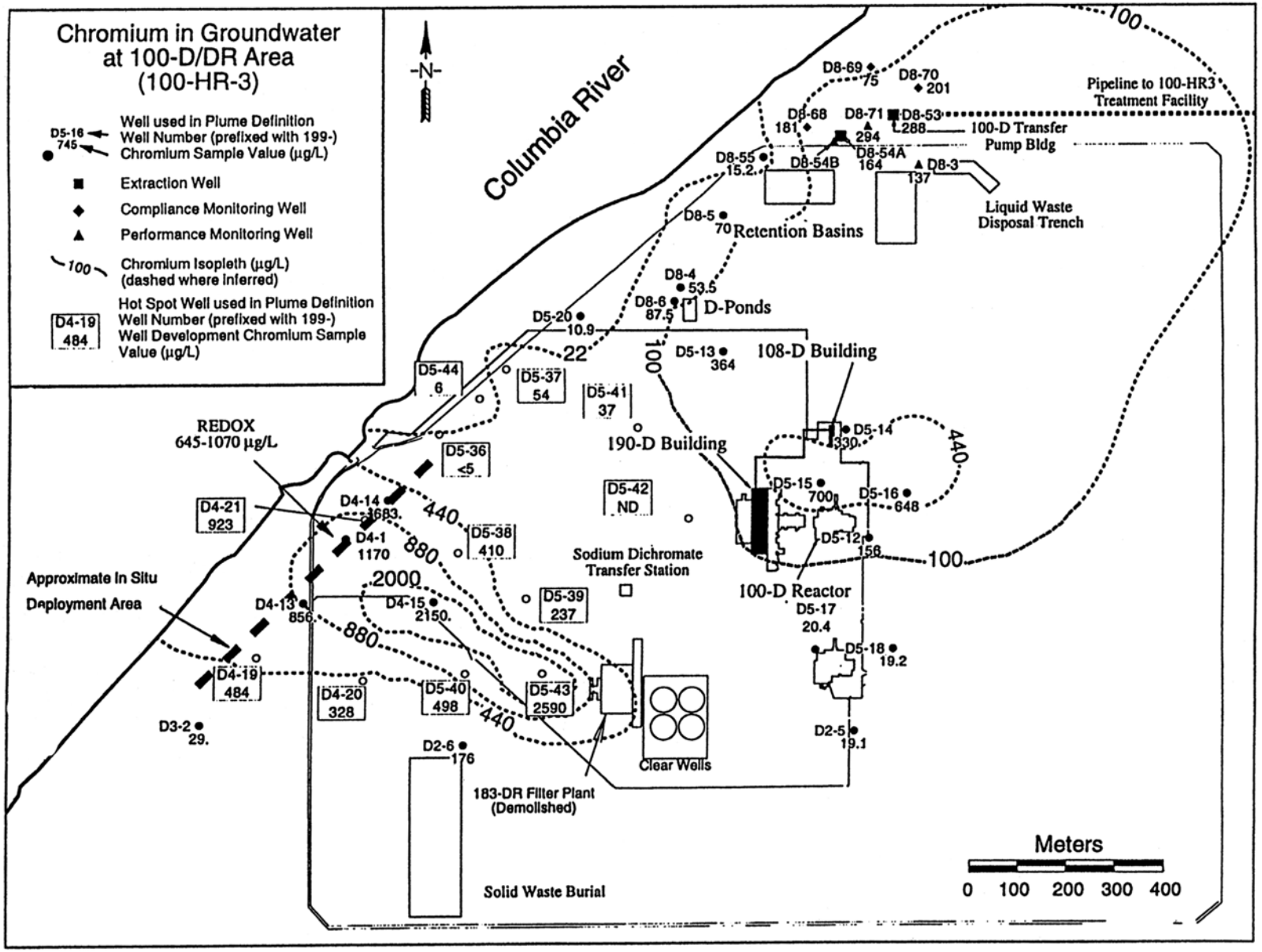

Figure 2.1. 100-D Groundwater Monitoring Wells

Water samples for total and hexavalent chromium were collected from the exposure aquaria using a peristaltic pump to pull water through a Geotech 0.45-µm high-capacity disposable filter (Geotech Environmental Equipment, Inc., Denver, Colorado). The water samples were collected in pre-cleaned plastic bottles. Water samples for hexavalent chromium analysis were preserved at the time of collection with sodium hydroxide (approximately pH 9). Water samples for total chromium analysis were shipped unpreserved to Battelle Marine Sciences Laboratory (Sequim, Washington) where nitric acid preservative was added. Total chromium was analyzed using inductively coupled plasma mass spectrometry (U.S. EPA Method 1638), and the hexavalent form of chromium was analyzed by ion chromatography (U.S. EPA Method 1636).

2.3 96-Hour Pre-Test (rainbow trout)

The 96-hr pre-test started on December 6, 1999 and involved equilibrating the aquarium and dilution equipment with the groundwater/river water solutions for 24 hours. On December 8, we added 2,400 eyed rainbow trout eggs from our research stock to egg cups (100 per cup, one cup per aquaria, six treatments and four replicate aquaria per treatment) and started the exposure.

2.4 Early Life-Stage Test (fall chinook salmon)

Eyed eggs from fall chinook salmon spawned at the Priest Rapids Hatchery were transported to incubators at PNNL's Aquatic Laboratory on December 15, 1999. These adults were spawned on November 15, 1999, and egg development was estimated at 303.3 Celsius temperature units (CTU) on December 15. The eggs were then maintained in an incubator until December 21, 1999 and they incurred an additional 35.8 CTU. CTU is a measure of fish development based on the number of days at a specific temperature; one CTU equals one degree Celsius above freezing for 24 hours.

Before the early life-stage test began, the aquarium and dilution equipment were equilibrated with the groundwater/river water solutions at 5°C. The exposure was initiated on December 21, 1999 (Day 0), when 2,400 eyed eggs were added to the egg cups (50 eggs per cup, two cups per aquarium, six treatments, four replicate aquaria per treatment). Test treatments were 0, 11, 24, 54, 120, and 266 µg/L (target concentrations) of hexavalent chromium. The control treatment was unfiltered Columbia River water containing background concentrations (<5 µg/L) of hexavalent chromium. Temperature was maintained at 5 ± 2°C

Juvenile fish were released from the egg cups and into the aquaria on Day 70 (median hatch occurred from days 41 to 47). Photoperiod control began on Day 97. On the median swim-up date (Day 98), the chromium exposure was discontinued, and the juvenile fish were held in 100% Columbia River water until the test was terminated on Day 132. Fish were not fed until the median swim-up date occurred.

Egg mortality, hatching, post-hatch deformities (visible spinal curvature), and post-hatch mortality were monitored and recorded daily. Dead organisms were removed from the egg cups or aquaria and discarded. The behavior and development of the juvenile fish was recorded daily to document behavioral differences between exposure groups.

Samples of juvenile fish (n=15) were taken from each of the four replicate exposures at the median hatch date (from Day 41 to 47), halfway between hatch and swim up (Day 70), at median swim up (Day 98), and at test termination (Day 132). These samples were collected and preserved for analysis of whole-body chromium concentrations, lipid peroxidation, and DNA strand breakage. Three fish were collected from each replicate and preserved in 10% neutral buffered formalin for histological analysis at median swim up and at test termination. However; based on the preliminary results from the 1998 early life-stage test (Farag et al. 2000) that showed little difference between indices of physiological function and development, only the whole body chromium concentration assay was conducted. The analysis for chromium in whole body tissues was a destructive test that used all material collected; therefore no whole body tissue is available for additional analysis. The histology samples are currently in storage at PNNL, but were not assayed as part of this study.

At the request of USGS scientists, blood was collected from three fish in each exposure aquarium 3 days before test termination (Day 129). The USGS had some success with analysis of DNA strand breakage for their parr heath studies and were hopeful that the method could be extended to the early-life stage exposures. To obtain blood samples, we excised the tail using a scalpel and collected a drop of blood by holding the fish over a centrifuge tube containing 100 μL of a citrate freezing medium. The blood samples were frozen with liquid nitrogen and stored in a -70°C freezer. The blood samples were stored at PNNL for 11 months during which an equipment malfunction caused the freezer to shut down. The thawing event compromised the viability of the samples for DNA analysis, so they were destroyed. Determination of whether lipid peroxidation values were elevated and/or individual tissues were damaged by chromium exposure was not feasible for early life stage tests because there was insufficient tissue mass for analysis.

At test termination, all surviving fish were euthanized using MS-222 (tricaine methanesulfonate) and measured (fork length, mm), blotted dry, and weighed to the nearest mg using a top-loading Satorius (Model L0620S) balance. Fish were not fed for 24 hours before all tissue sampling events.

Whole-body tissue samples for chromium analysis were digested using a nitric acid total digestion method based on U.S. EPA Method 200.2. The digested tissue samples were then analyzed for chromium using U.S. EPA Method 1638.

2.5 Statistical Methods

Statistical analyses and graphics were performed using the SAS software system, version 8 (SAS Institute, Lary, North Carolina) and Splus software version 4.0 (Mathsoft Inc., Seattle, Washington).

Lengths and weights of the juvenile chinook salmon at study termination were analyzed for differences among the six chromium concentration exposure groups using standard analysis of variance (ANOVA) techniques. Similar ANOVA modeling was done on the results for concentration of chromium in whole-body tissue for each tissue sampling event (hatch, midway between hatch and swim up, at swim up, and at study termination).

Survival data were analyzed for differences between treatment groups using a Kaplan-Meier survival model and non-parametric tests for significance between treatment strata. The Kaplan-Meier model accounts for fish removed from the aquaria for tissue concentration analysis at different times during the study by censoring those observations at the times they were removed. Alternatively, comparative analysis was done on survival by comparing the survival rates of each of the four aquaria at each level of the treatment factor using a non-parametric test on differences in the medians for each group.

Uptake and elimination rates for chromium were estimated from tissue concentration data for each treatment level. The primary objective of this analysis was to determine whether uptake and elimination rates differed across treatments and exposure intervals. Bioaccumulation factors (BCFs) were estimated as the ratio of the uptake to the elimination rate (after Hamelink 1977). These rates were estimated by fitting a non-linear model to the tissue concentration data by least squares and taking the parameter estimates K_1 and K_2 from the equation

$$C_t = \frac{K_1 Cw}{K_2}(1 - e^{-K_2 t})$$

where C_t is the tissue concentration at time t
C_w is the concentration in the water
K_1 is the uptake rate parameter
K_2 is the elimination rate parameter
(Blanchard et al. 1977).

3.0 Results

This section includes results of both water chemistry and toxicity studies. Specific end points measured during the early life-stage test with fall chinook salmon included survival, development rate, and growth. We also measured chromium tissue burdens of fish to evaluate uptake and elimination rates. Specifications of the exposure conditions were within the limits established by the test protocol (Quality Assurance Project Plan 2000).

This study showed that the survival, development, and growth of early life-stage fall chinook salmon from the eyed-egg stage to swim-up stage were not adversely affected by exposure to hexavalent chromium from 11 to 266 μg/L. Survival was high for all treatment levels and controls, exceeding 98% from hatch through swim up. In addition, there was no difference among the lengths and weights of fish among all treatment groups at test termination.

3.1 Groundwater Analysis

Total chromium in the groundwater used in these tests (Well 199 D5-43) was relatively constant and ranged from 2,350 to 2,420 μg/L for the 96-hr test (three samples, one sampling event) to 2,037 to 2,980 μg/L for early life-stage test (27 samples, nine sampling events). Concentrations of total chromium on individual sampling dates were generally within ±5% (Appendix A, Table A.1). Hexavalent chromium in groundwater was only determined for the 96-hr pretest, with these results ranging from 2,320 to 2,420 μg/L. Since the 96-hr test results revealed that the chromium in the groundwater was essentially all hexavalent chromium, only total chromium concentrations were determined for the groundwater used for the early life-stage. These groundwater chromium concentrations were used to adjust the Mount Brungs diluter, both total chromium and hexavalent chromium concentrations were measured in the exposure aquariums to determine the actual exposure concentrations. The groundwater consistently had a light green tint when viewed through a 10-L container, and there was no sediment visible. The pH and conductivity of the groundwater ranged from 7.6 to 8.2 and 467 to 529 μS/cm, respectively. Groundwater from Well 199 D5-43 was collected on February 3, 2000 and analyzed by the Hanford Site Groundwater Monitoring Program for inorganics, anions, and radionuclides. These results are presented in Appendix A, Table A.2.

3.2 96-Hour Pre-Test

Water temperatures during the 96-hr test period ranged from 6.1 to 7.8°C. Day 1 values were slightly higher than the other test days because the water bath temperature equilibrated with room temperature during the egg transfer process. For Day 2 through Day 4, water temperatures ranged from 6.1 to 7.2°C. Dissolved oxygen concentrations were near saturation, ranging from 11.5 to 13.7 mg/L. The highest chromium concentration group consistently showed a slight decrease in dissolved oxygen. The pH ranged from 7.6 to 7.8 with no differences between concentration groups. Alkalinity ranged from 64 to 72 mg/L (as $CaCO_3$), and hardness ranged from 54 to 75 mg/L (as $CaCO_3$). Conductivity ranged from 122 to 163 μS, and relative values reflected the percentage of groundwater in the test solutions.

For water samples from the exposure aquaria, total chromium was consistently lower than hexavalent chromium during the pre-test, with the greatest difference noted at the 54 µg/L level in Table 3.1. Variability among replicates was high for some treatments. For example, the total chromium results for the 0 µg/L target concentration (river water control) were influenced by one suspect value of 7.8 µg/L (the other two measurements were 1.2 and 1.7 µg/L). The hexavalent chromium concentration from the 266 µg/L concentration group was influenced by a suspect value of 430 µg/L; all other results in this group ranging from 243-268 µg/L. There was good correlation between the total and hexavalent chromium results (r^2=0.94), with the hexavalent results showing slightly higher values.

Table 3.1. 96-Hour Pre-Test: Summary of Water Sample Results from Exposure Aquariums (NR = no result)

(Control 0 µg/L)			(11 µg/L Target)			(24 µg/L Target)		
Date/Tank	Cr	Cr^{+6}	Date/Tank	Cr	Cr^{+6}	Date/Tank	Cr	Cr^{+6}
12-9 1C	7.84	NR	12-9 2A	9.45	10.9	12-9 3A	20.0	NR
12-11 1B	1.73	NR	12-9 2C	8.90	9.63	12-9 3C	17.4	NR
12-12 1C	1.22	NR	12-11 2B	8.59	9.25	12-11 3B	18.4	NR
Mean	**3.60**	**NR**	12-11 2D	7.51	7.74	12-11 3D	17.1	NR
StdDev	**3.68**	**NR**	12-12 2A	9.57	10.1	12-12 3A	17.1	NR
			12-12 2C	7.69	8.65	12-12 3C	18.0	NR
			Mean	**8.62**	**9.37**	**Mean**	**18.0**	**NR**
			StdDev	**0.87**	**1.09**	**StdDev**	**1.11**	**NR**
(54 µg/L Target)			(120 µg/L Target)			(266 µg/L Target)		
Date/Tank	Cr	Cr^{+6}	Date/Tank	Cr	Cr^{+6}	Date/Tank	Cr	Cr^{+6}
12-9 4A	47.2	69.8	12-9 5A	108	NR	12-9 6A	250	NR
12-9 4C	47.5	86.6	12-9 5C	92.6	NR	12-9 6C	254	268
12-11 4B	41.5	63.7	12-11 5B	97.7	NR	12-11 6B	216	240
12-11 4D	33.3	58.8	12-11 5D	94.7	NR	12-11 6D	215	430
12-12 4A	41.2	45.9	12-12 5A	98.5	NR	12-12 6A	218	243
12-12 4C	38.6	58.6	12-12 5C	89.2	NR	12-12 6C	214	254
Mean	**41.5**	**63.9**	**Mean**	**96.8**	**NR**	**Mean**	**228**	**287**
StdDev	**5.36**	**13.6**	**StdDev**	**6.64**	**NR**	**StdDev**	**19.0**	**80.8**

3.3 Early Life-Stage Test

Exposure conditions during the study were within the specifications described in the project protocol unless specifically noted (Quality Assurance Project Plan 2000). Important test conditions for the early life-stage test are summarized below:

3.3.1 Water Chemistry

The temperature range for all exposure aquariums throughout the duration of the test was 3.4 to 7.5°C, or slightly outside the guidelines of 5 ± 2°C. However, the elevated temperatures occurred only during Days 0 and 1 of the exposure. The mean temperature for individual treatments ranged from 5.45 to 5.61°C (Figure 3.1). The average temperature of each exposure aquarium and CTU (e.g., 130 days of exposure at 5°C equals 650 CTU) are provided in Appendix A, Table A.3. Average CTU for each exposure concentration ranged from 1065 to 1087 CTU (Figure 3.2). The highest CTU value was for the 266 µg/L treatment and reflected a higher relative volume of groundwater versus chilled water diluent.

Dissolved oxygen concentrations ranged from 9.2 to 14.1 mg/L. Water from aquariums at the 266 µg/L target concentration consistently showed a decrease in dissolved oxygen compared with the other concentration groups. The pH ranged from 7.0 to 8.0 with no apparent differences between concentration groups. Alkalinity ranged from 64 to 80 mg/L (as $CaCO_3$), and hardness ranged from 35 to 87 mg/L (as $CaCO_3$) (Table A.4). Conductivity results ranged from 124 to 211 µS and were positively correlated with the percentage of groundwater in the test solution.

Mean concentrations of total chromium and hexavalent chromium in individual exposure aquariums ranged from ~2 to 13% of nominal or target concentrations (Table A.5). The greatest variation between the measured concentration and the target concentration was in the 120 µg/L treatment because of the relatively lower values during that last sampling periods. The overall values for total chromium and hexavalent chromium (Table 3.2) were within the range specified in the project plan (Quality Assurance Project Plan 2000). There was excellent correlation between the total chromium and hexavalent chromium results (r^2=0.98), with the hexavalent chromium results showing slightly higher values. The general agreement between measured total chromium and hexavalent chromium concentration with the target concentrations confirmed that essentially all of the chromium was in the hexavalent form. The results also indicate proper delivery from the Mount Brungs diluter, good mixing characteristics in the test apparatus, and the absence of a chemically reducing or adsorbing environment in the exposure aquariums. All average exposure concentrations were within the protocol range (Table 3.2, target value ±20%); therefore, the target concentrations will be used from this point forward.

The results for quality control samples were within acceptable ranges (Quality Assurance Project Plan 2000). Procedural blanks and detection limits ranged from 0.036 to 0.049 µg/L for total chromium and 0.65 µg/L for hexavalent chromium. A standard reference material was available only for the total chromium analysis, and the percent difference between the certified value and the measured value ranged from 2% to 14%. The analytical recoveries for matrix spikes ranged from 92% to 111% for total

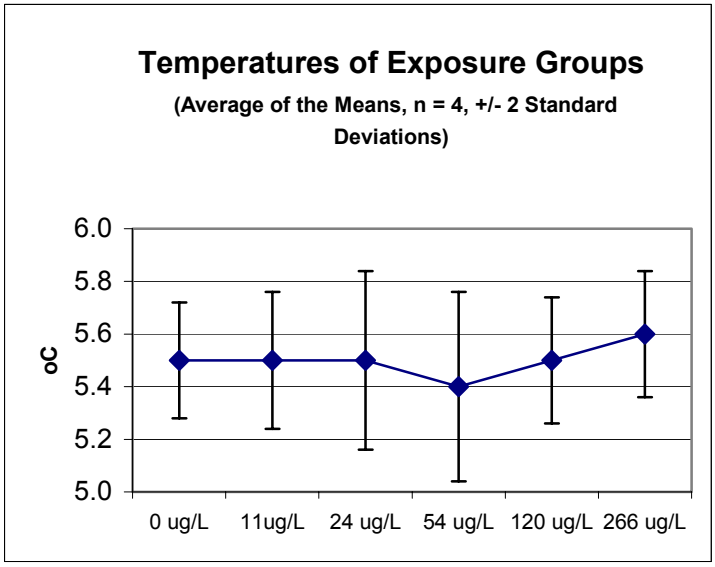

Figure 3.1. Temperatures of Exposure Groups (early life-stage test, chinook salmon)

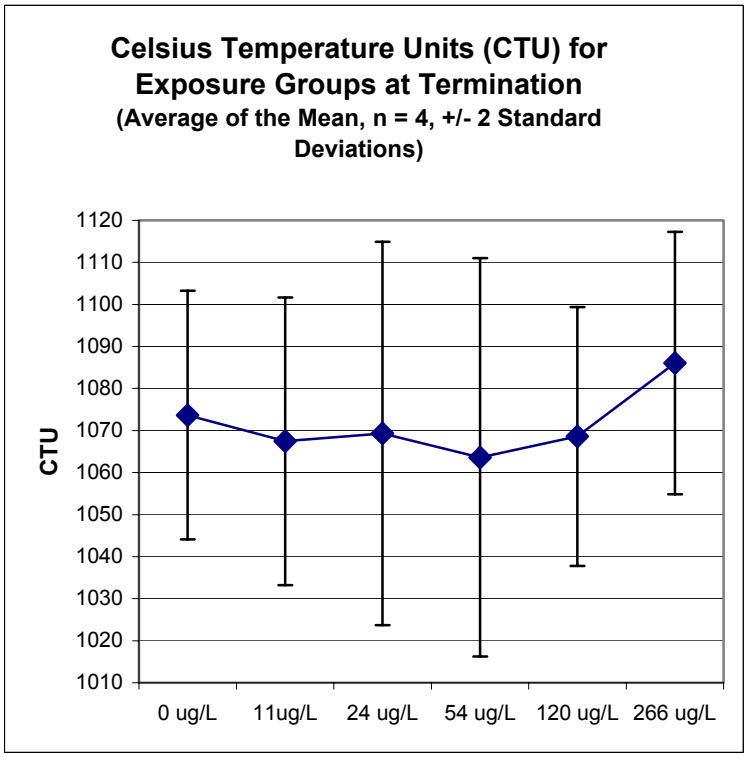

Figure 3.2. Celsius Temperature Units for Exposure Groups at Termination

Table 3.2. Summary of Total Chromium and Hexavalent Chromium Water Sample Results from Exposure Aquaria (μg/L)

Average Total Chromium and Hexavalent Chromium					Protocol Range
Target Concentration	Total Cr	Std Dev	Cr^{+6}	Std Dev	
Control	0.79	0.82	0.8	0.1	Control
11 μg/L	10.1	1.7	10.6	1.25	8.25-13.75
24 μg/L	22.4	2.75	24.8	1.5	18-30
54 μg/L	49	6.65	48.3	4.48	40.5-67.5
120 μg/L	104	17.7	NS	NS	90-150
266 μg/L	259	29.1	262	15.3	200-332

Weekly samples from 12/22/1999 to 3/28/2000.
(n=13 each tank for total chromium).
(n=3 for the control and 24 μg/L treatments for hexavalent chromium).
(n=9 for the 11 and 54 μg/L treatments for hexavalent chromium).
(n=12 for the 266 μg/L treatment for hexavalent chromium).
(n=0 for the 120 μg/L treatment for hexavalent chromium).
NS = No sample.

chromium and 95% to 113% for hexavalent chromium. For replicate analysis, the percent differences ranged from 0% to 9% for total chromium and 0% to 5% for hexavalent chromium.

3.3.2 Toxicological Response

Exposure to chromium-containing groundwater did not affect hatching success or the time required for exposure groups to reach median hatch (Table 3.3). Median hatch occurred from Day 41 to Day 47. The midpoint for the overall hatch occurred on Day 45. For individual exposure aquariums, 62.5% of the median hatch dates occurred over the Day 45 to Day 47 period. Overall survival to median hatch was nearly 99% overall, with no apparent effect of chromium concentrations on survival. The number of deformed (spinal curvatures) individuals that survived past hatching was low in treatment groups and the control (Appendix A, Table A.6).

Overall survival remained high throughout the swim-up and termination periods (Table 3.3). There were no statistical differences (ANOVA) in the days required to reach median swim up between any exposure group and the control group. The dates to median swim up ranged from Day 95 to Day 100, with the overall mean occurring on Day 98. Survival was similar in all exposure groups and exceeded 98% at both swim up and termination. At swim up and termination, there were no statistically significant differences (ANOVA, p=0.05, Appendix B, Tables B.3.1 and B.3.5) in survival between the control group and any of the exposure groups. There were no observable differences in behavior (e.g., feeding patterns, startle response, schooling behavior, response to light) between exposure groups. There were no observable differences in developmental milestones (median hatch and median swim up, Table 3.3) between exposure groups.

Table 3.3. Percentage of Survival at Median Hatch, Median Swim Up, and Termination and Dates to Median Hatch and Median Swim Up (early life-stage test, chinook salmon)

Target Concentration	Days to Hatch	Days to Swim Up	Percent Survival at Hatch	Percent Survival Hatch to Swim Up	Percent Survival Hatch to 30 Days Post Swim Up
1A (0 µg/L)	42	95	99	99	98
1B	47	99	99	98	98
1C	43	95	98	98	96
1D	45	100	100	100	100
Mean	***44.3***	***97.3***	***99.0***	***98.8***	***98.0***
Standard Deviation	***2.2***	***2.6***	***0.8***	***1.0***	***1.6***
2A (11 µg/L)	46	99	100	99	99
2B	46	99	100	100	98
2C	42	96	99	99	98
2D	47	100	99	99	99
Mean	***45.3***	***98.5***	***99.5***	***99.3***	***98.5***
Standard Deviation	***2.2***	***1.7***	***0.6***	***0.5***	***0.6***
3A (24 µg/L)	46	99	100	100	100
3B	47	99	100	100	100
3C	41	95	100	100	100
3D	47	100	99	99	99
Mean	***45.3***	***98.3***	***99.8***	***99.8***	***99.8***
Standard Deviation	***2.9***	***2.2***	***0.5***	***0.5***	***0.5***
4A (54 µg/L)	42	96	100	100	100
4B	47	99	99	98	98
4C	46	99	99	99	99
4D	47	100	100	99	99
Mean	***45.5***	***98.5***	***99.5***	***99.0***	***99.0***
Standard Deviation	***2.4***	***1.7***	***0.6***	***0.8***	***0.8***
5A (120 µg/L)	43	96	100	100	100
5B	47	100	100	100	100
5C	42	95	100	100	100
5D	45	100	100	99	98
Mean	***44.3***	***97.8***	***100.0***	***99.8***	***99.5***
Standard Deviation	***2.2***	***2.6***	***0.0***	***0.5***	***1.0***
6A (266 µg/L)	43	96	99	98	98
6B	45	99	99	99	99
6C	43	95	100	99	98
6D	46	100	99	99	99
Mean	***44.3***	***97.5***	***99.2***	***98.7***	***98.5***
Standard Deviation	***1.5***	***2.4***	***0.5***	***0.5***	***0.6***

Average lengths at test termination (Day 132) were consistent among treatments. The 54, 120, and 266 µg/L treatment groups had slightly lower average fork length than the controls at study termination (Table 3.4 and Figure 3.3). However, these differences were <2% across treatments and were not significantly different (ANOVA, p=0.05, Appendix B, Table B.1.1). The average weight of chinook salmon at test termination (Day 132) was also consistent among exposure groups (Table 3.4 and Figure 3.4). The 54, 120, and 266 µg/L exposure groups had slightly lower average weights than the control group. However, there were no statistically significant differences in weights among groups (ANOVA; p=0.05, Appendix B, Table B.2.1).

Table 3.4. Salmon Fork Lengths and Weights at Termination

Target Concentration	0.0 µg/L	11 µg/L	24 µg/L	54 µg/L	120 µg/L	266 µg/L
Number of Fish (all replicates)						
Replicate 1	26	30	31	30	32	26
Replicate 2	29	27	30	29	31	27
Replicate 3	27	29	20	28	32	25
Replicate 4	32	29	30	29	29	28
Total	**114**	**115**	**111**	**116**	**124**	**106**
Fork Length (mm)						
Average Replicate 1	41.6	41.8	39.9	40.6	39.8	40.3
Average Replicate 2	40	40	40.6	39.6	39.7	39.6
Average Replicate 3	39.3	41.2	41.2	40.4	40.2	41.1
Average Replicate 4	39.9	39.3	39.7	39.8	39.2	39.1
Average of the Means	**40.2**	**40.6**	**40.4**	**40.1**	**39.7**	**40.0**
Standard Deviation	**0.983**	**1.13**	**0.686**	**0.476**	**0.411**	**0.869**
Weight (g)						
Average Replicate 1	0.632	0.627	0.55	0.576	0.53	0.551
Average Replicate 2	0.547	0.551	0.575	0.523	0.526	0.519
Average Replicate 3	0.508	0.606	0.594	0.547	0.525	0.564
Average Replicate 4	0.531	0.509	0.526	0.52	0.49	0.475
Average of the Means	**0.555**	**0.573**	**0.561**	**0.542**	**0.518**	**0.527**
Standard Deviation	**0.054**	**0.053**	**0.030**	**0.026**	**0.019**	**0.040**

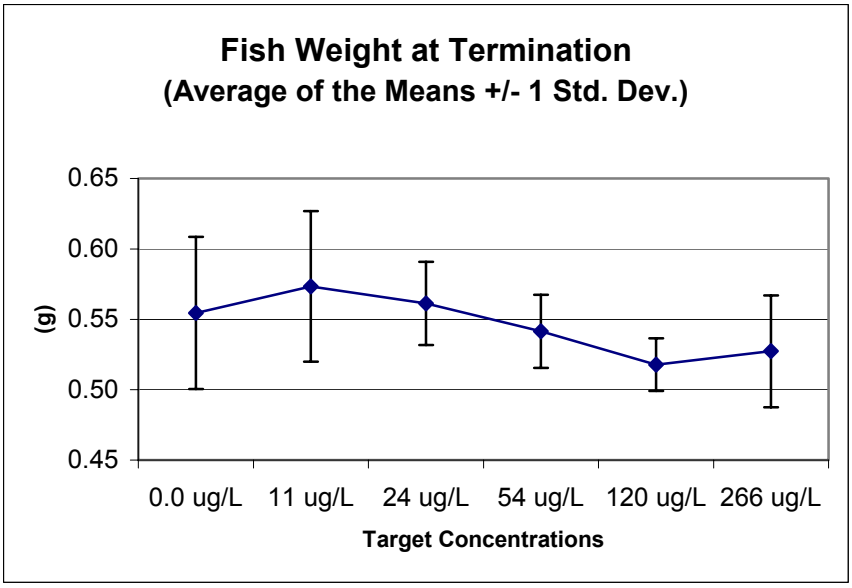

Figure 3.3. Average Fork Length of Surviving Salmon at Termination

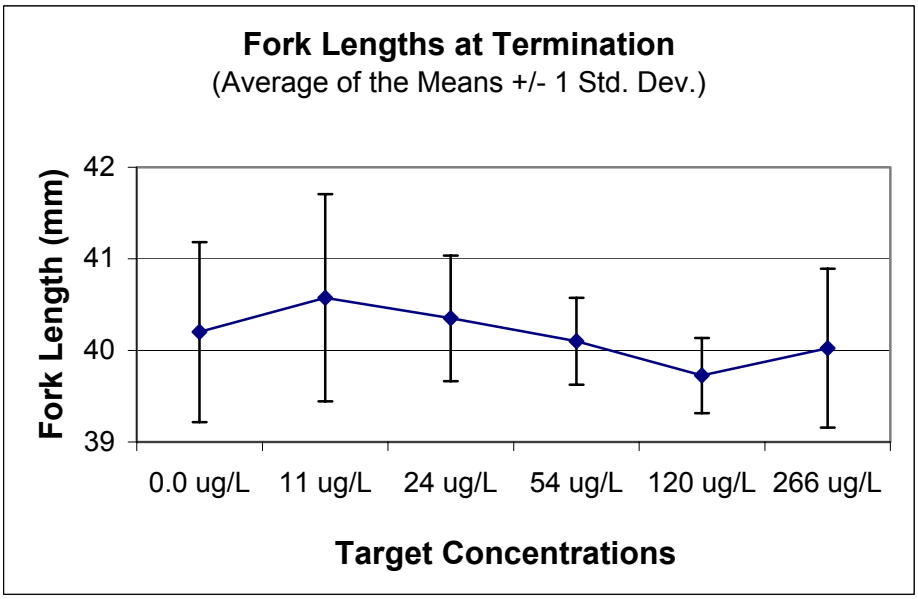

Figure 3.4. Average Weight of Surviving Salmon at Termination

Whole-body concentrations of chromium for fish samples collected at hatch, halfway between hatch and swim up, at swim up, and at termination are given in Table 3.5. At hatch, the fish tissue concentrations of chromium were statistically higher (ANOVA, p=0.05, Appendix B, Tables B.4.1 and B.4.8) than

Table 3.5. Chromium Concentrations in Salmon Tissues (µg/g dry wt) at Four Life Stages for Six Exposure Concentrations (±1 standard deviation)

Exposure Concentration	Control	11 µg/L	24 µg/L	54 µg/L	120 µg/L	266 µg/L
Hatch	0.314	0.429	0.461	0.488	0.643	0.654
	0.369	0.426	0.488	0.504	0.534	0.719
	0.448	0.425	0.649	0.67	0.616	0.681
	0.539	0.678	0.672	0.603	0.634	0.868
Mean	***0.418***	***0.490***	***0.568***	***0.566***	***0.607***	***0.731***
Std Dev	***0.098***	***0.126***	***0.108***	***0.086***	***0.050***	***0.095***
1/2 to Swim Up	0.312	0.447	0.506	0.669	1.00	1.67
	0.233	0.361	0.611	0.627	1.07	1.54
	0.273	0.372	0.591	0.705	1.11	1.54
	0.244	0.365	0.488	0.675	1.01	1.39
Mean	***0.266***	***0.386***	***0.549***	***0.669***	***1.05***	***1.54***
Std Dev	***0.035***	***0.041***	***0.061***	***0.032***	***0.052***	***0.114***
Swim Up	0.409	0.878	0.922	1.59	1.83	2.87
	0.397	0.627	0.977	1.41	1.87	2.34
	0.372	0.597	---[(a)]	1.61	2.23	2.33
	0.394	0.578	0.85	1.57	1.96	3.05
Mean	***0.393***	***0.670***	***0.916***	***1.54***	***1.97***	***2.65***
Std Dev	***0.015***	***0.140***	***0.064***	***0.091***	***0.180***	***0.368***
Termination	0.548	0.705	0.974	1.12	1.9	2.05
	0.587	0.623	0.986	1.35	1.7	1.93
	0.531	0.679	0.945	1.37	1.96	2.38
	0.502	0.681	0.906	1.13	1.48	2.15
Mean	***0.542***	***0.672***	***0.953***	***1.24***	***1.76***	***2.13***
Std Dev	***0.036***	***0.035***	***0.036***	***0.136***	***0.217***	***0.191***

(a) The initial result was >20 µg/g. The sample was redigested and analyzed with a result of 2.25 µg/g. This value was not included in the mean.

the control group for all exposure groups, except for the 11 µg/L group. For the fish samples analyzed at halfway between hatch and swim up, at swim up, and at termination, all exposure groups were elevated compared with the control. Furthermore, for the fish samples analyzed at halfway between hatch and swim up, at swim up, and at termination, each successively higher exposure group had chromium tissue concentrations that were elevated (e.g., the 266 µg/L exposure group has statistically higher tissue concentrations than the 120 µg/L exposure group, the 120 µg/L exposure group has statistically higher tissue values than the 54 µg/L exposure group, etc.). At termination, there was a statistically significant decrease in chromium levels in fish tissue for the 266 µg/L and 54 µg/L exposure groups compared with the tissue levels at swim up when the chromium exposures were ended. At termination, the 120 µg/L exposure group was numerically lower than at swim up, but the difference was not statistically significant. The tissue concentrations of chromium were higher at termination than at swim up for the control, 11 µg/L exposure group, and the 24 µg/L exposure group, with the control group having a statistically significant increase.

Analysis of tissue concentration levels showed an increase in chromium levels with increasing concentrations of the solution in the aquaria (Figure 3.5). However, tissue concentrations dropped from the time of swim up until study termination for exposures ≥54 µg/L ($p=0.08$, Appendix B, Table B.4.9). This decrease can be attributed to a change in exposure conditions because, following swim up, the water in the aquaria was restored to background levels of chromium to mimic conditions in the Columbia River.

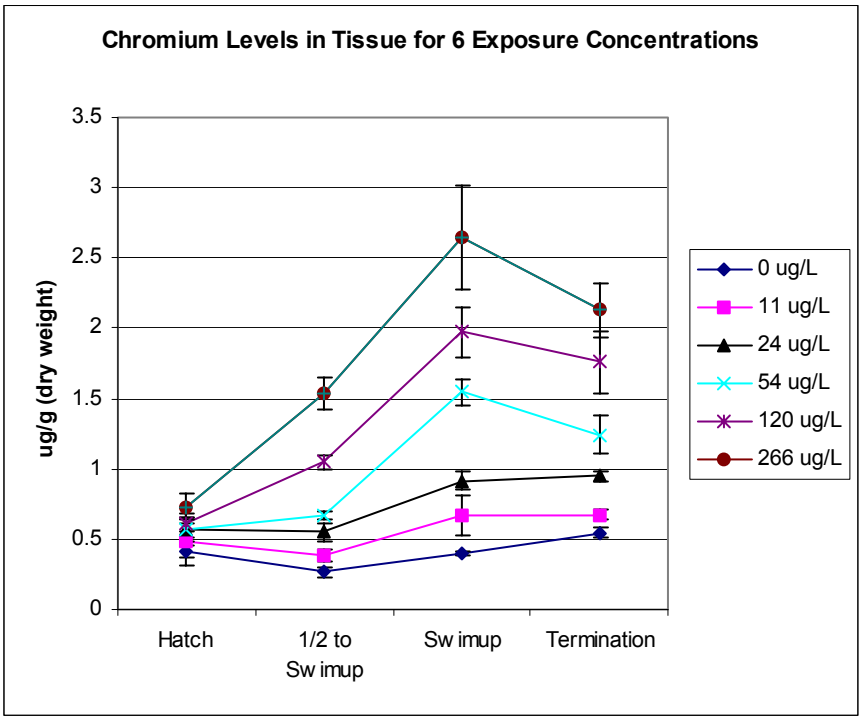

Figure 3.5. Chromium Concentrations in Fish Tissue at Four Life Stages (mean ±1 standard deviation)

The estimated bioconcentration factor (BCF) ranged from 7.9 to 52 for the 266 μg/L and 11 μg/L treatments, respectively (Table 3.6). Both the uptake rate (K1) and elimination rate (K2) constants decreased as the exposure concentration increased. The temporal pattern among treatment groups suggested that elimination rates for aqueous concentrations of chromium ≥54 μg/L are sufficiently slow to result in elevated tissue chromium (Table 3.6). Whether the fish reached "steady-state" with respect to tissue concentrations is unknown because of the limited number of sample intervals.

Table 3.6. Bioconcentration Factors (BCF) Estimated from Least Squares Non-Linear Regression for Each Chromium Exposure Concentration (μg/L)

Exposure Concentration	K1 (uptake rate)	K2 (elimination rate)	K1/K2 (BCF)
Control	(a)	(a)	(a)
11	99	1.9	52
24	41	1.2	33
54	14	0.68	21
120	6.4	0.48	13
266	3.4	0.43	7.9

(a) K1 and K2 not estimable.

4.0 Discussion

Adult chinook salmon spawn in the Hanford Reach from mid-October through early November as water temperatures decline to <15°C (Dauble and Watson 1997). Eggs incubate in the gravel-cobble substrate of the river bottom (redds) and hatch there in late winter (~January). The young alevins develop and subsist on their yolk until they emerge from redds in early March (Becker 1973; 1985). It is during this sensitive development stage that exposure to groundwater contaminants is of principal concern (Geist et al. 1994). After emerging from redds, subyearling fall chinook salmon rear in shallow nearshore areas for two to three months before migrating to the Pacific Ocean (Dauble et al. 1989; Becker 1973).

This laboratory study showed that the survival, development, and growth of fall chinook salmon from the eyed-egg stage to swim-up stage were not adversely affected by exposures to hexavalent chromium from 11 to 266 μg/L. Survival was high for all treatment levels and controls, exceeding 98% from hatch through swim up. In addition, there was no difference among the lengths and weights of fish among all treatment groups at test termination. The USGS recently completed early life-stage exposures for fall chinook salmon at chromium concentrations ranging from 5 to 120 μg/L (Farag et al. 2000) and found no significant adverse effects on fish survival, growth, and physiology under exposure conditions similar to those used for this study.

We saw no effects on growth (i.e., length or weight) under the described test conditions. Farag et al. (2000), based on a limited data set, suggested a trend toward reduced weight for some groups of chromium-exposed salmon. However, they observed no obvious pattern in the growth response, i.e., there was neither a dose-dependent size relationship nor decreased growth with time. Stevens and Chapman (1984) based on chronic tests with trivalent chromium, reported significant reduction in growth of juvenile steelhead occurred, but only at concentrations also producing significant mortality. In contrast, Olson and Foster (1956) found that growth rate was a more sensitive index of toxicity than mortality for both chinook salmon and rainbow trout exposed to hexavalent chromium.

Our highest test concentration was ~50% of the maximum value of hexavalent chromium (632 μg/L) reported by Hope and Peterson (1996a) for pore water sampled near the former reactor site at 100-D/DR but similar to the maximum value (246 μg/L) measured along the shoreline adjacent to the 100-H reactor (Hope and Peterson 1996b). Thus, exposure scenarios were similar to those expected in the Hanford Reach at locations where groundwater and surface water mix. One difference was the temperature regime, which was held constant at 5°C. However, the number of days and temperature during which developing embryos were exposed to chromium was similar to conditions expected to occur in the Hanford Reach (Figure 4.1).

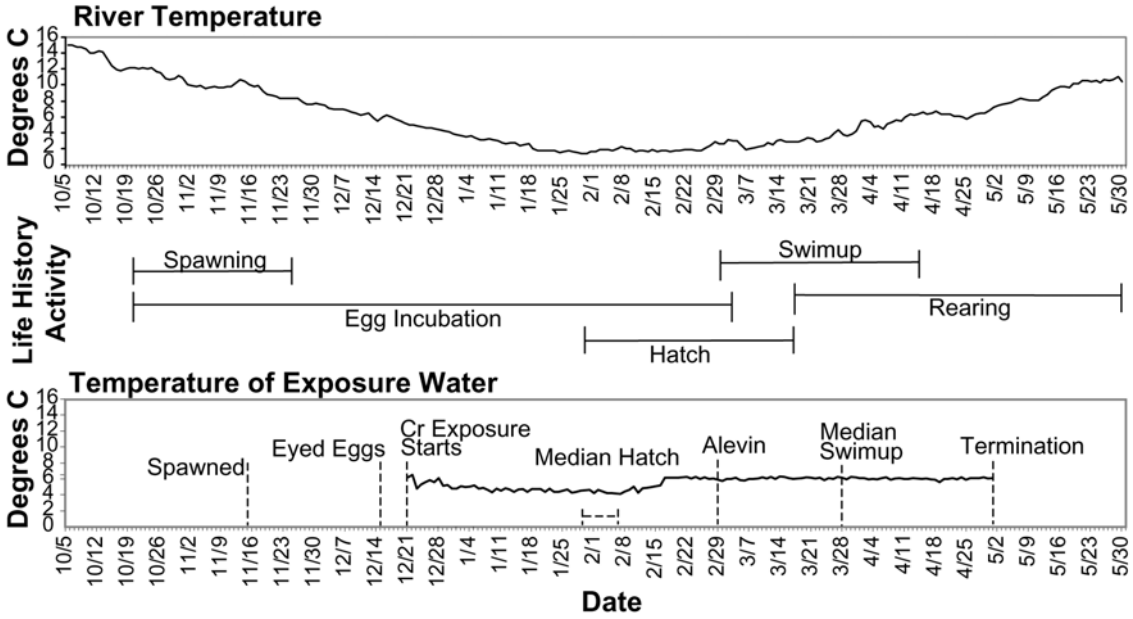

Figure 4.1. Life Stage and Temperature Comparisons for Hanford Reach Fall Chinook Salmon and Laboratory Test Exposures. Life history activity intervals are shown for comparison purposes only and do not represent actual dates.

Our results differ from those of Olson and Foster (1956), who reported increased mortality for juvenile fall chinook salmon exposed to 77 and 180 µg/L chromium 100 and 55 days post-hatch, respectively. Other studies also indicated that fish are more sensitive during the period of increased metabolism and maximum growth. For example, Eisler (1986) reported a 96-hr LC50 of 200 µg/L for salmon fingerlings. One explanation for the general lack of response to chromium at selected test concentrations was that fish were exposed from only the eyed-egg stage through swim up. For comparison with other chronic exposures reported here, we terminated our tests at 63 days post-hatch, or 98 days from the eyed-egg stage (Table 4.1). This duration was similar to Olson and Foster (1956) but longer than Farag et al. (2000), who had slightly warmer exposure temperatures.

Table 4.1. Comparison of Exposure Intervals for Chromium Toxicity Tests Involving Early Life-Stages of Chinook Salmon

Interval	PNNL	USGS	Olson and Foster[(a)]
Hatch	45 d	32 d	46 d
Swim up	98 d	83 d	101 d
Termination	132 d	113 d	~280 d

(a) Olson and Foster (1956) started their test immediately after the eggs were fertilized, and chromium exposures were continuous through termination. The other two tests were initiated at the eyed-egg stage, and chromium exposures occurred only through median swim up.

Whole-body concentrations of chromium in fall chinook salmon had a typical dose-response pattern; i.e., those subjected to the highest exposure concentrations and longest exposure intervals had higher tissue concentrations. The estimated values were consistent with other compounds having fairly high solubility, i.e., rapidly transported across biological membranes. Tissue concentrations of chromium were not elevated significantly above controls at 11 μg/L, suggesting fish effectively regulated their body burden during the 98-day exposure period. In contrast, at swim up, mean tissue concentrations were elevated ~2x those of controls for the 24 μg/L concentration and increased to ~5x controls at 266 μg/L. Elevated tissue concentrations occurred for concentrations ≥120 μg/L, but not until the midpoint between hatch and swim up. Buhler et al. (1969) reported, in studies with adult rainbow trout, that tissue concentrations reached equilibrium with water in 2 to 4 days at 2.5 mg/L hexavalent Cr. Freshwater fish can regulate the essential elements, such as chromium, over a wide range of ambient concentrations (Leland and Kuwabara 1985). This mechanism allows some fish to excrete a higher than normal proportion of their metal intake under contaminated conditions, helping maintain trace metals concentrations in the body at normal levels.

It was noteworthy that whole-body chromium concentrations following the recovery period were only slightly lower than those measured at swim up. This pattern suggests that elimination of chromium was slow. We found it interesting that tissue concentrations reported by Farag et al. (2000) were less for similar exposure intervals (e.g., whole body concentrations at median swim up were 1.04 μg/g chromium versus 1.97 μg/g chromium for the 120 μg/L exposure treatment). Whole body concentrations reported by Farag et al. (2000) were also lower than our values across all treatments at test termination. Whether this difference is due to differences in growth rates of fish or exposure conditions is unclear. With the exception of hardness, which might have contributed to slightly higher uptake rates of chromium, there were no substantial differences in measured water quality parameters that could explain the different tissue burdens. The Farag et al. (2000) early life stage test had a hardness range of 79 to 82 mg/L as $CaCO_3$ for all exposure concentrations, where this study had average hardness values (mg/L as $CaCO_3$ ± 1 standard deviation) during the chromium exposure period of 57 ± 14, 59 ± 13, and 71 ± 16, respectively for chromium treatments of 0, 24, and 266 μg/L (Table A.4). Hardness values recorded for this study were more variable (range 35-87 mg/L as $CaCO_3$) because of seasonal changes in the Columbia River and the amount of dilution with well water. The hardness parameter was not manipulated across all exposure conditions as was done with in Farag et al. (2000), yet likely provided a more realistic exposure scenario.

In conclusion, this study indicates that growth and survival of fall chinook salmon developing in the river bottom substrate (i.e., redds) would not be affected by chromium concentrations up to 266 μg/L. This assessment builds from the assumption that the development period, temperature regimes, and corresponding exposure interval to the swim-up stage (i.e., when the alevins absorb their yolk sac, emerge from redds, and begin exogenous feeding in the water column) are similar to those used during laboratory testing. Collectively, these data support that current cleanup criteria of 10 μg/L are adequate for protection of fall chinook salmon populations in the Hanford Reach.

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Appendix A

Analytical and Toxicological Results

Table A.1. Analytical Results and Collection Dates for Chromium-Containing Groundwater Used as the Toxicant Source for Both 96-Hour (11/30/99 data only) and Early Life-Stage Evaluations

Date	Sample Number	µg/L Total Chromium	µg/L Hexavalent Chromium
11/30/99	1130A	2420	2350
	1130B	2420	2420
	1130C	2350	2320
12/3/99	123A	2310	Not Sampled
	123B	2240	Not Sampled
	123C	2300	Not Sampled
12/13/99	1213A	2384	Not Sampled
	1213B	2308	Not Sampled
	1213C	2281	Not Sampled
12/29/99	1229A	2680	Not Sampled
	1229B	2630	Not Sampled
	1229C	2710	Not Sampled
1/11/00	111A	2980	Not Sampled
	111B	2930	Not Sampled
	111C	2570	Not Sampled
1/25/00	0125A	2690	Not Sampled
	0125B	2690	Not Sampled
	0125C	2700	Not Sampled
2/7/00	0207A	2580	Not Sampled
	0207B	2550	Not Sampled
	0207C	2580	Not Sampled
2/21/00	221A	2280	Not Sampled
	221B	2282	Not Sampled
	221C	2280	Not Sampled
3/7/00	0307A	2190	Not Sampled
	0307B	2190	Not Sampled
	0307C	2110	Not Sampled
3/24/00	324A	2057	Not Sampled
	324B	2037	Not Sampled
	324C	2069	Not Sampled

Table A.2. Results (inorganics, anions, radionuclides) for Hanford Site Groundwater Monitoring Samples Collected on February 3, 2000 from 100-D Area Well 199-D5-43 (well water used for the toxicity evaluation)

Inorganics	**Replicate 1 (ug/L)**		**Replicate 2**	
Silver	0.8	U[a]	0.8	U
Aluminum	29.7		36.2	
Barium	66.9		66.6	
Beryllium	0.1	U	0.1	U
Calcium	75100.0		73000.0	
Cadmium	0.4	U	0.4	U
Cobalt	0.7	U	0.7	U
Chromium	2210.0		2210.0	
Copper	0.5	U	0.5	U
Iron	13.6		34.3	
Potassium	4190.0		4270.0	
Magnesium	17100.0		17000.0	
Manganese	2.3		2.6	
Sodium	9150.0		8950.0	
Nickel	1.4		1.2	
Lead	2.1	U	2.1	U
Antimony	2.1	U	2.1	U
Tin	2.6	U	2.6	U
Strontium	412.0		410.0	
Vanadium	6.8		6.8	
Zinc	35.3		38.6	
Anions	**Replicate 1 (mg/L)**			
Chloride	27.8			
Fluoride	0.5	U		
Nitrite	0.25	U		
Nitrate	49.0			
Sulfate	108.0			
Radionuclides	**Replicate 1 (pCi/L)**		**MDA[b]**	
Gross Alpha	0.99	U	1.6	
Gross Beta	4.6		2.0	
Tritium	359.0		170.0	

(a) U = Below detection limit.
(b) MDA = Minimal detectable activity.

Table A.3. Summary of Water Temperature Data (±1 standard deviation) for Exposure Aquaria (early life-stage evaluation)

Target Concentration Tank #	oC Mean (n = 133)	Standard Deviation	Celsius Temperature Units	Standard Deviation
(0 ug/L)				
1A	5.6	0.61	1090	NA
1B	5.4	0.78	1060	NA
1C	5.6	0.65	1083	NA
1D	5.4	0.76	1063	NA
Average of Means	**5.5**	**0.11**	**1074**	**14.8**
(11 ug/L)				
2A	5.5	0.75	1069	NA
2B	5.4	0.77	1063	NA
2C	5.6	0.58	1090	NA
2D	5.3	0.81	1049	NA
Average of Means	**5.5**	**0.13**	**1067**	**17.1**
(24 ug/L)				
3A	5.5	0.77	1068	NA
3B	5.4	0.80	1060	NA
3C	5.7	0.56	1101	NA
3D	5.3	0.83	1048	NA
Average of Means	**5.5**	**0.17**	**1069**	**22.8**
(54 ug/L)				
4A	5.7	0.63	1097	NA
4B	5.3	0.80	1051	NA
4C	5.4	0.71	1063	NA
4D	5.3	0.85	1044	NA
Average of Means	**5.4**	**0.18**	**1064**	**23.7**
(120 ug/L)				
5A	5.6	0.73	1079	NA
5B	5.3	0.84	1049	NA
5C	5.6	0.63	1082	NA
5D	5.5	0.73	1065	NA
Average of Means	**5.5**	**0.12**	**1069**	**15.4**
(266 ug/L)				
6A	5.7	0.67	1102	NA
6B	5.6	0.76	1084	NA
6C	5.7	0.68	1093	NA
6D	5.5	0.87	1065	NA
Average of Means	**5.6**	**0.12**	**1086**	**15.6**

NA = Not Applicable

Table A.4. Hardness and Alkalinity Data for Exposure Aquaria (mg/L as $CaCO_3$).

Table A.4. Hardness and Alkalinity Data for Exposure Aquaria (mg/L as $CaCO_3$).

	Hardness						Alkalinity					
(Cr Exposure Level)	0 ug/L	11 ug/L	24 ug/L	54 ug/L	120 ug/L	266 ug/L	0 ug/L	11 ug/L	24 ug/L	54 ug/L	120 ug/L	266 ug/L
Day 0	NS	NS	NS	NS	NS	NS	72	NS	68	NS	NS	68
Day 1	60	NS	60	NS	NS	77	NS	NS	NS	NS	NS	NS
Day 9	58	NS	64	NS	NS	79	68	NS	64	NS	NS	68
Day 16	63	NS	63	NS	NS	76	64	NS	68	NS	NS	72
Day 23	64	NS	63	NS	NS	76	68	NS	68	NS	NS	74
Day 29	38	NS	40	NS	43	48	72	70	70	72	72	68
Day 36	35	NS	41	NS	NS	47	68	64	68	66	68	68
Day 44	36	NS	39	NS	42	NS	64	68	66	70	68	76
Day 50	NS	38	NS	42	NS	47	70	72	74	72	68	76
Day 67	68	NS	67	NS	NS	80	72	NS	66	NS	NS	78
Day 74	NS	NS	NS	NS	NS	NS	78	NS	78	NS	NS	80
Day 79	66	NS	69	NS	NS	82	76	NS	70	NS	NS	76
Day 85	70	NS	70	NS	NS	87	72	72	73	74	76	87
Day 92	66	NS	72	NS	NS	84	69	NS	78	NS	NS	77
Mean	***57***	***NC***	***59***	***NC***	***NC***	***71***	***70***	***NC***	***70***	***NC***	***NC***	***74***
1 Standard Deviation	***14***	***NC***	***13***	***NC***	***NC***	***16***	***4***	***NC***	***4***	***NC***	***NC***	***6***
Post Exposure Recovery Period												
Day 107	NS	66	NS	64	NS	63	64	74	72	74	68	66
Day 113	NS	63	NS	64	NS	65	68	68	66	68	66	72
Day 128	62	NS	60	NS	NS	62	70	NS	65	NS	NS	74

NS = No Sample
NC = Mean and Standard Deviation were not calculated because of the low number of results.

Table A.5. Early Life-Stage Test: Results for Water Samples from Exposure Aquaria Analyzed for Total Chromium and Hexavalent Chromium (µg/L)

Date/Tank # Control (0µg/L)	Total Cr	Cr +6	Date/Tank # 11 µg/L Target	Total Cr	Cr +6	Date/Tank # 24 µg/L Target	Total Cr	Cr +6
12-22 1C	2.09	0.90	12-22 2C	10.90	NS	12-22 3C	24.9	23.9
12-28 1B	1.50	0.70	12-28 2B	13.30	NS	12-28 3B	26.6	26.5
1-05 1A	1.74	0.80	1-05 2A	13.00	NS	1-05 3A	25.4	23.9
1-11 1D	2.29	NS	1-11 2D	11.00	9.60	1-11 3D	26.3	NS
1-19 1C	0.51	NS	1-19 2C	8.68	8.11	1-19 3C	20.8	NS
1-25 1A	0.73	NS	1-25 2A	11.30	12.40	1-25 3A	23.8	NS
2-16 1B	0.28	NS	2-16 2B	8.41	10.40	2-16 3B	18.3	NS
2-22 1A	0.39	NS	2-22 2A	10.20	11.80	2-22 3A	21.6	NS
3-03 1B	0.23	NS	3-03 2B	9.59	11.30	3-03 3B	21.4	NS
3-08 1C	0.26	NS	3-08 2C	8.59	10.90	3-08 3C	21.9	NS
3-15 1D	0.05	NS	3-15 2D	7.85	9.82	3-15 3D	19.4	NS
3-22 1A	0.05	NS	3-22 2A	9.67	11.00	3-22 3A	20.3	NS
3-28 1B	0.09	NS	3-28 2B	9.36	NS	3-28 3B	19.9	NS
Mean	**0.79**	**0.80**	**Mean**	**10.14**	**10.59**	**Mean**	**22.4**	**24.8**
Standard Dev.	**0.82**	**0.10**	**Standard Dev.**	**1.70**	**1.29**	**Standard Dev.**	**2.75**	**1.50**

54 µg/L Target	Total Cr	Cr +6	120 µg/L Target	Total Cr	Cr +6	266 µg/L Target	Total Cr	Cr +6
12-22 4C	52.5	NS	12-22 5C	108.0	NS	12-22 6C	267	267
12-28 4B	61.1	NS	12-28 5B	114.0	NS	12-28 6B	289	290
1-05 4A	58.0	NS	1-05 5A	147.0	NS	1-05 6A	282	264
1-11 4D	53.4	48.7	1-11 5D	110.0	NS	1-11 6D	317	282
1-19 4C	45.1	42.1	1-19 5C	110.0	NS	1-19 6C	269	233
1-25 4A	54.7	50.9	1-25 5A	113.0	NS	1-25 6A	287	260
2-16 4B	40.9	43.2	2-16 5B	120.0	NS	2-16 6B	232	247
2-22 4A	49.1	50.5	2-22 5B	93.0	NS	2-22 6B	232	264
3-03 4B	48.4	55.1	3-03 5B	87.9	NS	3-03 6B	236	263
3-08 4C	45.7	50.4	3-08 5C	90.7	NS	3-08 6C	241	247
3-15 4D	39.0	43.0	3-15 5D	87.8	NS	3-15 6D	244	269
3-22 4A	46.5	50.6	3-22 5A	89.1	NS	3-22 6A	233	258
3-28 4B	42.6	NS	3-28 5B	85.6	NS	3-28 6B	224	NS
Mean	**49.0**	**48.3**	**Mean**	**104.3**	NS	**Mean**	**258**	**262**
Standard Dev.	**6.65**	**4.48**	**Standard Dev.**	**17.68**	NS	**Standard Dev.**	**29.1**	**15.3**

NS = Not Sampled.

Table A.6. Early Life-Stage Test: Total Mortalities, Deformities (visible spinal curvatures), and Number of Tissue Samples Collected for Each Exposure Group

Group (Cr Level)	# Dead Eggs	# Dead Hatch to Swim Up	# Dead Swim Up to Termination	# Dead Accidental	# Survived but Deformed	Total Deformed (Dead + Surviving)	Tissue Samples	# Fish at Termination	Total Fish - Accidental Dead
1A (0 µg/L)	1	0	1	0	0	0	72	26	100
1B	1	1	0	0	0	1	69	29	100
1C	2	0	2	0	0	1	69	27	100
1D	0	0	0	0	1	1	69	33	102
Total	**4**	**1**	**3**	**0**	**1**	**3**	**279**	**115**	**402**
2A (11 µg/L)	0	1	0	0	0	0	69	30	100
2B	0	0	2	2	0	2	69	27	98
2C	1	0	1	0	0	0	69	29	100
2D	1	0	0	0	0	0	69	30	100
Total	**2**	**1**	**3**	**2**	**0**	**2**	**276**	**116**	**398**
3A (24 µg/L)	0	0	0	0	0	0	69	31	100
3B	0	0	0	0	0	0	69	30	99
3C	0	0	0	13	1	2	69	21	90
3D	1	0	0	0	0	0	69	30	100
Total	**1**	**0**	**0**	**13**	**1**	**2**	**276**	**112**	**389**
4A (54 µg/L)	0	0	0	0	1	1	69	31	100
4B	1	1	0	0	0	0	69	29	100
4C	1	0	0	0	0	0	69	28	98
4D	0	1	0	2	0	0	69	29	99
Total	**2**	**2**	**0**	**2**	**1**	**1**	**276**	**117**	**397**
5A (120 µg/L)	0	0	0	0	0	0	69	32	101
5B	0	0	0	0	0	0	69	31	100
5C	0	0	0	0	0	0	69	32	101
5D	0	1	1	0	0	1	69	29	100
Total	**0**	**1**	**1**	**0**	**0**	**1**	**276**	**124**	**402**
6A (266 µg/L)	1	1	0	0	0	0	72	26	100
6B	1	0	0	0	0	0	72	27	100
6C	0	1	1	4	0	1	69	25	96
6D	1	0	0	3	1	1	69	29	99
Total	**3**	**2**	**1**	**7**	**1**	**2**	**282**	**107**	**395**

Appendix B

Statistical Results

Appendix B

Statistical Results (early life-stage test, chinook salmon)

B.1 Fish Length at Termination

Table B.1.1. Mean Fish Length (mm) by Cr Concentration Level (µg/L)

Cr Conc. Level	N Obs	Mean	Std Dev.	Std. Error
Control	4	40.188	0.967	0.484
11	4	40.580	1.105	0.553
24	4	40.351	0.671	0.335
54	4	40.085	0.465	0.233
120	4	39.722	0.391	0.195
266	4	40.022	0.855	0.427

Table B.1.2. Analysis of Variance Table—Fish Length (mm) by Cr Concentration Level

Source	DF	SS	MS	FValue	ProbF
Model	5	1.721026	0.344205	0.56	0.7312
Error	18	11.11773	0.617652		
Corrected Total	23	12.83875			

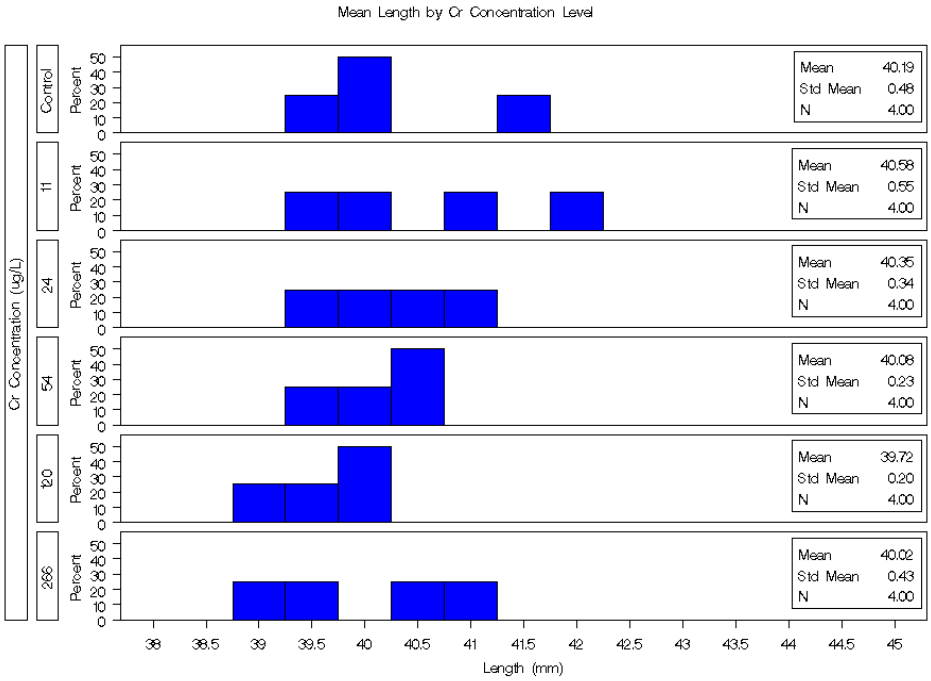

Figure B.1. Histogram of Mean Fish Length by Cr Concentration Level

B.2 Fish Weight at Termination

Table B.2.1. Mean Fish Weight (g) by Cr Concentration Level (µg/L)

Cr Conc. Level	N Obs	Mean	Std Dev.	Std. Error
Control	4	0.55451	0.05390	0.02695
11	4	0.57337	0.05337	0.02668
24	4	0.56138	0.02928	0.01464
54	4	0.54160	0.02608	0.01304
120	4	0.51781	0.01888	0.00944
266	4	0.52739	0.03933	0.01966

Table B.2.2. Analysis of Variance Table—Fish Weight (g) by Cr Concentration Level

Source	DF	SS	MS	FValue	ProbF
Model	5	0.008874	0.001775	1.16	0.3671
Error	18	0.027583	0.001532		
Corrected Total	23	0.036457			

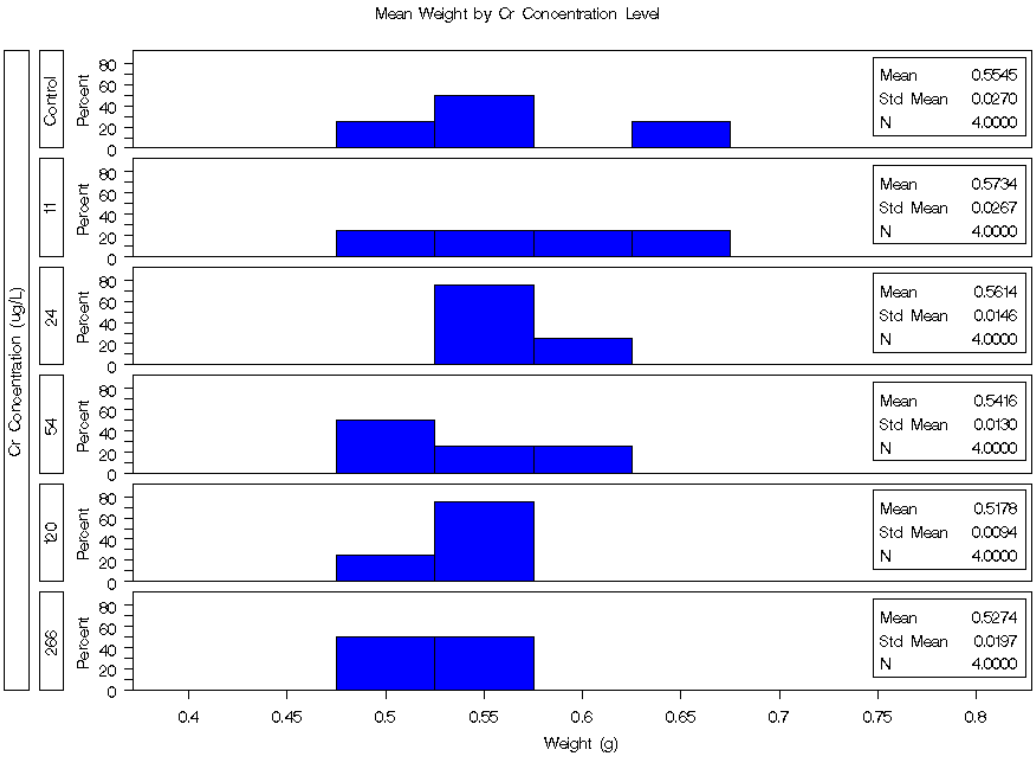

Figure B.2. Histogram of Mean Fish Weight by Cr Concentration Level

B.3 Survival Analysis

Table B.3.1. Summary of Non-Parametric Tests for Survival Rates from Kaplan-Meier (KM) Survival Model on the Six Cr Concentrations. The KM model accounts for censored observations that were removed for tissue analysis.

Test	ChiSq	DF	ProbChiSq
Log-Rank	7.7179	5	0.1725
Wilcoxon	7.0894	5	0.2141
-2Log(LR)	8.7073	5	0.1213

Table B.3.2. Summary Table of Censored and Uncensored Values by Cr Concentration Level (μg/L)

Cr Conc. Level	Total	Failed	Censored	Percent Censored
0	404	8	396	98.02
11	401	6	395	98.50
24	387	1	386	99.74
54	398	4	394	98.99
120	401	2	399	99.50
266	392	6	386	98.47
Total	2,383	27	2,356	98.87

Table B.3.3. Table of Mean and Median Survival by Cr Concentration Level (μg/L)

Cr Conc. Level	NObs	Mean	Median	StdDev	StdErr
Control	4	0.9847	0.9847	0.0059	0.0029
11	4	0.9803	0.9800	0.0159	0.0079
24	4	0.9850	0.9851	0.0058	0.0029
54	4	0.9975	1.0000	0.0050	0.0025
120	4	0.9900	0.9898	0.0081	0.0040
266	4	0.9950	1.0000	0.0099	0.0050

Table B.3.4. Table of Median Scores (number of points above the median) for Survival Classified by Cr Concentration Level (µg/L)

Cr Conc. Level	N	Sum of Scores	Expected Sum Under H0	Std. Dev. Under H0	Mean Score
Control	4	0.50	2	0.892805	0.125
11	4	1.00	2	0.892805	0.250
24	4	2.00	2	0.892805	0.500
54	4	4.00	2	0.892805	1.000
120	4	1.50	2	0.892805	0.375
266	4	3.00	2	0.892805	0.750

Table B.3.5. Non-Parametric One-Way Analysis of Median Scores

Chi-Square	8.8864
DF	5
Pr > Chi-Square	0.1137

B.4 Tissue Concentration of Hexavalant Chromium by Time in Study

Hatch

Table B.4.1. Analysis of Variance Table—Tissue Concentration (µg/g) by Cr Concentration Level at Hatch

Source	DF	SS	MS	FValue	ProbF
Model	5	0.226295	0.045259	4.84	0.0056
Error	18	0.168256	0.009348		
Corrected Total	23	0.39455			

Table B.4.2. Table of Predicted Means of Cr Tissue Concentration by Cr Concentration Level (µg/L) at Hatch

Cr Conc. Level	Mean Cr Tissue Concentration (µg/g)
Control	0.418
11	0.490
24	0.568
54	0.566
120	0.607
266	0.731

Hatch to Swim-Up

Table B.4.3. Analysis of Variance Table—Tissue Concentration (µg/g) by Cr Concentration Level at Hatch to Swim Up

Source	DF	SS	MS	FValue	ProbF
Model	5	4.47347	0.894694	228.86	<0.0001
Error	18	0.070369	0.003909		
Corrected Total	23	4.543839			

Table B.4.4. Table of Predicted Means of Cr Tissue Concentration by Cr Concentration Level (µg/L) at Hatch to Swim Up

Cr Conc. Level	Mean Cr Tissue Concentration (µg/g)
Control	0.266
11	0.386
24	0.549
54	0.669
120	1.048
266	1.535

Swim-Up

Table B.4.5. Analysis of Variance Table—Tissue Concentration (µg/g) by Cr Concentration Level (µg/L) at Swim Up

Source	DF	SS	MS	FValue	ProbF
Model	5	14.49711	2.899423	82.57	<0.0001
Error	17	0.596983	0.035117		
Corrected Total	22	15.0941			

Table B.4.6. Table of Predicted Means of Cr Tissue Concentration by Cr Concentration Level (µg/L) at Swim Up

Cr Conc. Level	Mean Cr Tissue Concentration (µg/g)
Control	0.393
11	0.670
24	0.916
54	1.545
120	1.973
266	2.648

Termination

Table B.4.7. Analysis of Variance Table—Tissue Concentration (µg/g) by Cr Concentration Level (µg/L) at Termination

Source	DF	SS	MS	FValue	ProbF
Model	5	7.787932	1.557586	88.29	<0.0001
Error	18	0.317555	0.017642		
Corrected Total	23	8.105487			

Table B.4.8. Table of Predicted Means of Cr Tissue Concentration by Cr Concentration Level (µg/L) at Termination

Cr Conc. Level	Mean Cr Tissue Concentration (µg/g)
Control	0.542
11	0.672
24	0.953
54	1.243
120	1.760
266	2.128

Table B.4.9. Table of Factor Contrasts for Swim Up Versus Termination

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
Swim up vs. Termination	1	0.26845007	0.26845007	3.15	0.0795

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