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1.0. Title Page

QUALITY ASSURANCE PROJECT PLAN FOR THE HANFORD 100 AREA AQUATIC ASSESSMENT PROJECT

STUDY PLAN "P99-10-01; (HANFORD); THE POTENTIAL FOR CHROMIUM TO ADVERSELY AFFECT CHINOOK SALMON (*ONCORHYNCHUS TSHAWYSTCHA*) UNDER EXPOSURE CONDITIONS SIMULATING THE HANFORD REACH OF THE COLUMBIA RIVER, WASHINGTON, USA.

UNITED STATES GEOLOGICAL SURVEY; BIOLOGICAL RESOURCES DIVISION, ENVIRONMENTAL AND CONTAMINANTS RESEARCH CENTER

Submitted To:

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2.0. Document Approval Form

Quality Assurance Project Plan Approval Form
USGS, BRD, Environmental and Contaminants Research Center

Project Title:	The Potential for Chromium to Adversely Affect Chinook Salmon (Oncorhynchus tshawystcha) under Exposure Conditions Simulating the Hanford Reach of the Columbia River, Washington, USA.
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NOTE: THE ORIGINAL VERSIONS OF ALL APPENDIXES TO THIS QAPP, COMPLETE WITH APPROPRIATE SIGNATURES, ARE ON FILE AT THE US GEOLOGICAL SURVEY, BIOLOGICAL RESOURCES DIVISION, ENVIRONMENTAL AND CONTAMINANTS RESEARCH CENTER, COLUMBIA, MO.

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4.0. Introduction

This Quality Assurance Project Plan (QAPP) complies with the quality assurance policy established by the United States Environmental Protection Agency (USEPA) on monitoring and measurement projects. The USEPA policy stipulates that every monitoring and measurement project must have a written and approved QAPP. A QAPP is a written document, which presents in specific terms, the policies, organization (where applicable), objectives, functional activities, and specific quality assurance (QA) and quality control (QC) activities designed to achieve the data quality goals of a specific project (s) or continuing operation (s). The QAPP is prepared by the responsible Program Office, Regional Office, laboratory, contractor, grantee, or other organization.

This document describes the sixteen elements of a QAPP. These QAPP elements describe procedures which will be used to document and report precision, accuracy, and completeness of environmentally-related measurements. Environmentally-related measurements are defined as all field and laboratory investigations that generate data. These include (1) the measurement of chemical, physical, or biological parameters in the environment, (2) the determination of the presence or absence of pollutants in waste streams, (3) the assessment of health and ecological effects studies, (4) conduct of clinically and epidemiological investigations, (5) performance of engineering and process evaluations, (6) study of laboratory simulation of environmental events, and (7) study or measurement on pollutant transport and fate, including diffusion models.

5.0. Project Description

5.1. Background Information

The Environmental Contaminants Research Center (ECRC) Research Study Plan 418: "The Potential for Chromium to Adversely Affect Chinook Salmon (*Oncorhynchus tshawystcha*) under Exposure Conditions Simulating the Hanford Reach of the Columbia River, Washington, USA" (Appendix A) describes a cooperative research project between the U.S. Geological Survey, Biological Resources Division (USGS-BRD), Environmental and Contaminants Research Center, the U.S. Fish and Wildlife Service Upper Columbia River Basin Field Office, and the Hanford Natural Resource Trustees with participation by the U.S. Department of the Interior, U.S. Department of Commerce, U.S. Department of Energy, the Yakama Nation, the Confederated Tribes of the Umatilla Indian Reservation, the Nez Perce Tribe, and the States of Oregon and Washington. The goal of the project is to assess the injury to chinook salmon inhabiting the Hanford Reach of the Columbia River as a result of exposure to chromium.

5.1.1. Summary

The project consists of three separate research tasks as follows:

Task 1. Fertilization: Examine the potential for chromium to adversely affect gametes and the fertilization process in chinook salmon.

Task 2. Early Life StageToxicity: Determine the effects of chromium on the early development of chinook salmon.

Task 3. Fish Health: Determine degree of health impairment of chinook salmon exposed to chromium.

The research activities within the project are described in the task-specific sections below.

Task 1: Fertilization; Examine the potential for chromium to adversely affect gametes and the fertilization process in chinook salmon. The objective of this task is to determine the toxicity of chromium to chinook salmon ovum, sperm, and the fertilization process. Chinook salmon gametes will be exposed to chromium in laboratory toxicity tests to evaluate survival and fertilization efficiency. Experimental water will simulate that of the Columbia River surface and pore water in the Hanford Reach which are known to be associated with the location of spawning redds (Hope and Peterson 1996, Geist 1997). Experimental water will be adjusted to a hardness of 80 mg/L as $CaCO_3$; pH, alkalinity, and conductivity will be in a range consistent with Columbia River conditions. Experimental water temperature will match seasonal conditions: December through March, 5°C; March through July, 10°C (Wiggins et al. 1997). Geist (1997) documented that the hyporheic zone (where river water and ground water mix) is generally warmer than the river water. However, data from samples collected between November and March indicate that the temperature of the hyporheic zone minus the river water is only 1°C. Experimental water will be prepared by blending laboratory well water with deionized water produced by reverse osmosis. Experimental water produced in this way will eliminate the use of surface water and the potential for fish pathogens to be introduced to the experiment and influence test results. Experimental water will be produced in 5,600L batches and analyzed to insure quality is within 5% of the experimental design in terms of hardness, alkalinity, conductivity, and pH. Unless otherwise indicated, experimental water was used. Photoperiod will be adjusted to simulate time of year of the exposure.

The range of chromium concentrations tested in the experimental water will be from 0 to 120 μg/L. This range of concentrations are above and below the chronic AWQC for chromium, 11 μg/L (USEPA 1986, State of Washington (173-201A-040)). This concentration range is also representative of concentrations in pore water sampled from the intergravel substrates in locations where salmon spawn (Giest 1997, Hope and Peterson 1996).

Gametes will be obtained from reproductively mature chinook salmon from the McNenny State Fish Hatchery, Spearfish, South Dakota. The salmon from which the gametes are obtained will be certified disease free prior to any testing. The disease free status is essential in assuring that toxicity testing is on healthy test organisms, increases reliability of results, and is a recommended standard procedure (ASTM 1993). This source of chinook salmon has been used in past Natural Resource Damage Assessments (Blackbird Mine Site, Idaho; Marr et al. 1995).

Gametes will be taken from chinook salmon brood stock between October and November of 1998. This is the normal time for gametogenesis in fall adult chinook salmon and the stock will be checked weekly for ovum and sperm formation. We will use a pooled source of eggs and sperm from a minimum of three females and three males to perform three tests: 1) toxicity of

chromium to the ovum, the ovum survival test; 2) toxicity of chromium to sperm, the sperm survival test; and 3) toxicity of chromium to fertilization, the fertilization test.

A physiological saline (PS) solution will be used in the ovum and fertilization test; and a physiological saline solution with sperm extender (PS/SE) will be used in the sperm test (Billard and Roubaud 1985). The physiological saline solution will consist of a standard 1% NaCl solution buffered to pH 9.0; the PS/SE will be the same solution with KCl added (30 mM) to prevent the spermatozoa from becoming motile. Ova and sperm can survive for several hours in these solutions. In all three tests, there will be six treatment concentrations of chromium: 0, 5, 11, 24, 54, and 120 μg/L. The six chromium treatments will be incorporated into the PS or the PS/SE so that when diluted with the appropriate amount of sperm or ovum the desired concentration of chromium is achieved. Each treatment will be replicated four times for a total of 24 treatments.

Ovum survival test. Ova will be divided into 24 treatment lots of 150-200 eggs, each and mixed with 10 ml of PS containing the appropriate chromium concentration. After 15 min, the liquid will be removed from each treatment and replaced with 10ml fresh uncontaminated PS solution followed by insemination with 1ml of intact sperm. This will be a 15 min ovum exposure.

Sperm survival test. Sperm will be diluted with PS/SE containing the appropriate chromium concentration (1ml sperm:10ml PS/SE) to obtain 24 treatment lots. The sperm, PS/SE, and chromium will be mixed, and left standing for 15 min. Sperm will be separated from the PS/SE exposure treatments by centrifuging for 10 min at 1800g, followed by replacement of 10ml fresh uncontaminated PS/SE. The exposed sperm will be used to inseminate 24 lots of about 150-200 ova, each previously diluted in 10ml of PS. This will be a 15 min sperm exposure.

Fertilization test. Ova (150-200), sperm (1ml), and 10ml of PS containing the appropriate chromium concentrations will be mixed together to achieve the 24 treatment lots. This will be a 65 min exposure of egg and sperm during fertilization and water hardening. In all three tests, ova and sperm will be mixed for 5 min followed by rinsing and water hardening in Hanford experimental water according to standard procedures (Piper et al. 1982). Water hardening will last for one hour and is the process by which water is absorbed into the eggs and fills the perivitelline space between the shell and yoke. The eggs become turgid during this process and additional water exchange is minimal during further development. In the fertilization test, exposure to chromium will continue through water hardening. After water hardening, eggs will be rinsed and transferred into incubators. Eggs from all three tests will be incubated in McNenny hatchery water (temperature, 11°C; hardness, 360 mg/L as $CaCO_3$; alkalinity, 210 mg/L; and pH, 7.6) for 10 days. The eggs will be cleared in 10% acetic acid solution for 2 min and percent fertilization will be determined. The embryo of fertilized eggs will turn an opaque white and become visible through the translucent chorion. At 10 days the embryo will have a definite optic lobe developed with an elongated somite and will be easily distinguished from an unfertilized germinal disk.

Exposure water will be monitored once per week for dissolved oxygen, pH, alkalinity, hardness, and conductivity (ECRC SOPs B4.14, B4.15, B4.16, and B4.9). More frequent monitoring will be performed if conditions dictate. Samples of exposure water will be taken

weekly to monitor total chromium exposure concentrations. One hundred mL samples of exposure water from each treatment will be filtered using a Nalgene® 300 filter holder. Each filtered sample will be transferred to a pre-cleaned, 125 ml I-Chem® polyethylene bottle, acidified to 1% HNO_3, and analyzed with ICP-MS (See ECRC SOP C5.212). At each time of total chromium sampling, one additional sample will be extracted from the low, middle, and high chromium exposures and analyzed for speciation of chromium (SOP P.454). For analysis of chromium in tissue, samples will be lyophilized (SOP C5.36), acid digested with microwave heating (SOP C5.94), and analyzed by either ICP-MS (SOP C5.212) or graphite furnace atomic absorption spectrophotometry (SOP C5.163).

Task 2: Early Life Stage; Determine the effects of chromium on the early development of chinook salmon. The objectives of this task are to (1) determine the effects of chromium on chinook salmon egg survival, egg hatching and alevin survival, growth, and behavioral development, and (2) evaluate the bioconcentration of chromium by chinook salmon and determine effects chromium exposure on DNA strand breakage, and lipid peroxidation (see task 3 for further details of physiology measurements). Chinook salmon eyed eggs and alevins will be exposed to chromium in an early life-stage toxicity test. Experimental water will simulate that of the Columbia River surface and pore water in the Hanford Reach which are known to be associated with the location of spawning redds (Hope and Peterson 1996, Geist 1997). Experimental water will be adjusted to a hardness of 80 mg/L as $CaCO_3$; pH, alkalinity, and conductivity will be in a range consistent with Columbia River conditions. Experimental water temperature will match seasonal conditions: December through March, 5°C; March through July, 10°C (Wiggins et al. 1997). Geist (1997) documented that the hyporheic zone (where river water and ground water mix) is generally warmer than the river water. However, data from samples collected between November and March indicate that the temperature of the hyporheic zone minus the river water is only 1°C. Experimental water will be prepared by blending laboratory well water with deionized water produced by reverse osmosis. Experimental water produced in this way will eliminate the use of surface water and the potential for fish pathogens to be introduced to the experiment and influence test results. Experimental water will be produced in 5,600L batches and analyzed to insure quality is within 5% of the experimental design in terms of hardness, alkalinity, conductivity, and pH. Unless otherwise indicated, experimental water was used. Photoperiod will be adjusted to simulate time of year of the exposure.

The range of chromium concentrations tested in the experimental water will be from 0 to 120 μg/L. This range of concentrations are above and below the chronic AWQC for chromium, 11 μg/L (USEPA 1986, State of Washington (173-201A-040)). This concentration range is also representative of concentrations in pore water sampled from the intergravel substrates in locations where salmon spawn (Giest 1997, Hope and Peterson 1996). Specific concentration are stated with each task.

Eyed embryos of chinook salmon will be obtained from the McNenny State Fish Hatchery, Spearfish, South Dakota. These eggs will be certified disease free prior to any testing. The disease free status is essential in assuring that toxicity testing is on healthy test organisms, increases reliability of results, and is a recommended standard procedure (ASTM 1993). This source of chinook salmon eggs has been used in past Natural Resource Damage Assessments (Blackbird Mine Site, Idaho; Marr et al. 1995).

Eyed eggs of chinook salmon will be exposed to 5, 11, 24, 54, and 120 $\mu g/L$ of chromium and a control treatment with no chromium added. The test will be conducted in a modified Mount and Brungs (1967) flow-through diluter system (ECRC SOP F20.E18,"Construction, Operation, Calibration, And Maintenance of the Proportional Diluter"). Temperature will be maintained at 5±2 °C by chilling the exposure water before it enters the diluter and submerging the exposure aquaria in a temperature-controlled water bath.

To initiate the test, two groups of 50 eggs each will be placed into 177-mL glass hatching containers and suspended into each of four exposure aquaria. The aquaria will be covered with black plastic to shield the eggs from light during incubation, and gentle aeration will be used to provide continuous circulation of the exposure water. On the median hatch date, the alevins will be released into the exposure aquaria. On the median swim-up date, the chromium exposure will be discontinued and the alevins will be maintained in the aquaria in chromium-free water until 30 days after the medium swim-up date.

During the exposure, egg mortality and hatching will be monitored and recorded daily. Dead eggs will be removed from the hatching containers and discarded. Alevin mortality and deformities will be monitored daily and dead alevins will be removed from the aquaria and discarded. The development of alevins will be monitored daily to document the sequence and timing of critical developmental stages including; hatch, onset of movement, side plough, upright plough, free swimming, and exogenous feeding following Dill (1977). The tanks will be video taped weekly to develop an accurate count of these developmental patterns among the test populations. Quantitative measures of the form and frequency of movements will be made during the alevin/free swimming transition.

Samples of alevins containing 15 fish each will be taken from each of the four replicate exposures at 0, 7, 14, and 28 days post median hatch date and analyzed for tissue residues of chromium, DNA strand breakage, and lipid peroxidation (see Task 3 for further details of physiology measurements). Two fish from each replicate will be collected on days 0 and 24 for histological analyses. At the end of the exposure (30 days post median-hatch) all surviving alevins in each treatment will be measured for total length and weighed to determine growth.

Exposure water will be monitored once per week for dissolved oxygen, pH, alkalinity, hardness, and conductivity (ECRC SOPs B4.14, B4.15, B4.16, and B4.9). More frequent monitoring will be performed if conditions dictate. Samples of exposure water will be taken weekly to monitor total chromium exposure concentrations. One hundred mL samples of exposure water from each treatment will be filtered using a Nalgene® 300 filter holder. Each filtered sample will be transferred to a pre-cleaned, 125 ml I-Chem® polyethylene bottle, acidified to 1% HNO_3, and analyzed with ICP-MS (See ECRC SOP C5.212). At each time of total chromium sampling, one additional sample will be extracted from the low, middle, and high chromium exposures and analyzed for speciation of chromium (SOP P.454). For analysis of chromium in tissue, samples will be lyophilized (SOP C5.36), acid digested with microwave heating (SOP C5.94), and analyzed by either ICP-MS (SOP C5.212) or graphite furnace atomic absorption spectrophotometry (SOP C5.163).

<u>Task 3: Fish Health; Determine degree of fish health impairment of chinook salmon exposed to chromium</u>. The objectives of this task are to (1) gather data on chromium-induced physiological responses to aid in the interpretation of growth and survival responses documented

during the early life-stage test (Task 2 above) and (2) compare laboratory-derived chromium-induced effects on health parameters of chinook salmon with health parameters measured on field-collected salmon from the Hanford Reach of the Columbia river. Parr of chinook salmon will be exposed to chromium in a 60-day flow-through toxicity test. Experimental water will simulate that of the Columbia River surface and pore water in the Hanford Reach which are known to be associated with the location of spawning redds (Hope and Peterson 1996, Geist 1997). Experimental water will be adjusted to a hardness of 80 mg/L as $CaCO_3$; pH, alkalinity, and conductivity will be in a range consistent with Columbia River conditions. Experimental water temperature will match seasonal conditions: December through March, 5°C; March through July, 10°C (Wiggins et al. 1997). Geist (1997) documented that the hyporheic zone (where river water and ground water mix) is generally warmer than the river water. However, data from samples collected between November and March indicate that the temperature of the hyporheic zone minus the river water is only 1°C. Experimental water will be prepared by blending laboratory well water with deionized water produced by reverse osmosis. Experimental water produced in this way will eliminate the use of surface water and the potential for fish pathogens to be introduced to the experiment and influence test results. Experimental water will be produced in 5,600L batches and analyzed to insure quality is within 5% of the experimental design in terms of hardness, alkalinity, conductivity, and pH. Unless otherwise indicated, experimental water was used. Photoperiod will be adjusted to simulate time of year of the exposure.

The range of chromium concentrations tested in the experimental water will be from 0 to 120 μg/L. This range of concentrations are above and below the chronic AWQC for chromium, 11 μg/L (USEPA 1986, State of Washington (173-201A-040)). This concentration range is also representative of concentrations in pore water sampled from the intergravel substrates in locations where salmon spawn (Giest 1997, Hope and Peterson 1996). Specific concentration are stated with each task.

Eyed embryos of chinook salmon will be obtained from the McNenny State Fish Hatchery, Spearfish, South Dakota. These eggs will be certified disease free prior to any testing. The disease free status is essential in assuring that toxicity testing is on healthy test organisms, increases reliability of results, and is a recommended standard procedure (ASTM 1993). This source of chinook salmon eggs has been used in past Natural Resource Damage Assessments (Blackbird Mine Site, Idaho; Marr et al. 1995).

The goals of this experiment are two-fold. First, data gathered from this experiment will further explain toxicological responses on growth and survival documented during the early life-stage experiment. Because fry at the end of the early life-stage experiment are small, it will be difficult to interpret the results in terms of individual tissue responses. And, therefore, explain the mechanistic processes involved during chromium action on fish. Second, this experiment will provide useful information to interpret effects of chromium on fish in the Hanford Reach. Health parameters used in the laboratory can also be performed on fish collected in the field.

Measurements will be performed to assess physiological impairment caused by chromium. For example, researchers have documented that chromium causes lipid peroxidation (Susa et al. 1996). Lipid peroxidation results in damage to polyunsaturated fatty acids located in the cell membrane. This damage can decrease fluidity, increase leakiness, and inactivate

membrane-bound enzymes. An ultimate result may be cell death and tissue damage (Halliwell and Gutterirdge 1985, Wills 1985). Chromium can form intermediates that react with DNA (Outridge and Scheuhammer 1993). These reactions have been associated with DNA damage measured in the form of DNA strand breakage (Aiyar et al. 1990). Therefore, lipid peroxidation and DNA strand breakage will be measured, in addition to histology and tissue metal accumulation, to document physiological impairment during this study.

Eyed eggs will be maintained in a Heath[R] incubator at a temperature of 10 ± 2 °C and hardness of approximately 150 mg $CaCO_3$/L. Mortalities will be documented and removed daily. At hatch, the fish will be moved to flow-through culture tanks with a flow of 4 L/min. The fish will be fed at least a 5% wet weight ration of a commercial biodiet daily. The daily food ration will be split between two feedings.

The experimental phase will begin during the parr stage of fish by randomly distributing 35 fish in each of 12 test chambers receiving experimental water with a flow-through proportional diluter system (Jackson SOP: F.P19). The circular chambers will have a 20-L capacity with dimensions of 43.2 cm X 35.6 cm and a volume of 20,510 cubic cm. The fish will be allowed to acclimate in the experimental chambers for at least five days before the start of the experiment. Thus, the experiment will be conducted for a period of 100 days beginning with parr fish. Eyed embryos, larvae, and parr will be handled so as to minimize stress in accordance with the ECRC-Columbia Animal Welfare Plan and the Region 6 U.S. Fish and Wildlife Service, Fish Health Policy.

Chromium in stock solutions will be delivered to eight test chambers via automatic pipettes (Micromedic Systems AP, Model #25000FW). Two test concentrations of 11 and 24 µg/L chromium (referred to from this point as 11 and 24 will be maintained in each of four replicate chambers. Four chambers without chromium added will be used as controls. Thus, a total of 12 experimental (four control, four with 11 µg/L Chromium, and four with 24 µg/L) units will be maintained. Each chamber will receive 8 L/hr for 10 volume additions per day. Experimental units will be checked daily for mortality and observations on behavior.

At Day 60 and at the termination of the experiment, samples will be collected for fish health measurements. An external necropsy assessment will be made on all sacrificed fish (UW SOP P.1) and lengths and weights will be recorded. One whole fish will be collected from each replicate chamber for measurements of tissue metal accumulation. DNA strand breakage, lipid peroxidation, histological anomalies, and tissue metal accumulation will be evaluated in 2 to 4 fish from each replicate. Gill lamellae, liver (free of the gall bladder), kidney, and intestine will be removed immediately from the 10 individual fish. Samples for histology will be collected from 2 fish from each replicated chamber and fixed in 10% neutral buffered formalin. It should be noted that spleen and skin samples will also be collected for histological examinations. Samples for DNA strand breakage, lipid peroxidation, and tissue metals will be collected from four fish from each replicate, frozen with liquid nitrogen, and stored at -90°C. At a later date, these samples will be ground with liquid nitrogen and composited by tissue to result in one sample from each replicate chamber. Aliquots of these composites will be measured for DNA strand breakage (Jackson SOP F20.29), lipid peroxidation (Jackson SOP F20.25), and tissue metals.

Samples will be collected from the remaining two fish for additional measurements of

DNA strand breakage. For example, both the anterior and posterior portions of the kidney will be sampled to distinguish between effects related to immune functions of the kidney (i.e. anterior section) to effects on the excretory processes (i.e. posterior portion). This information can be used to explain the mechanisms of observed toxicity. These data are also necessary to make comparisons between data gathered from samples of whole, ground tissue and those from specific locations within a tissue. Whole tissue samples would be less labor intensive to collect in field situations. However, it must be documented that this method is sufficiently sensitive to correspond with other toxicological effects. Fish will not be fed for 24 h prior to sampling.

Exposure water will be monitored once per week for dissolved oxygen, pH, alkalinity, hardness, and conductivity (ECRC SOPs B4.14, B4.15, B4.16, and B4.9). More frequent monitoring will be performed if conditions dictate. Samples of exposure water will be taken weekly to monitor total chromium exposure concentrations. One hundred mL samples of exposure water from each treatment will be filtered using a Nalgene® 300 filter holder. Each filtered sample will be transferred to a pre-cleaned, 125 ml I-Chem® polyethylene bottle, acidified to 1% HNO_3, and analyzed with ICP-MS (See ECRC SOP C5.212). At each time of total chromium sampling, one additional sample will be extracted from the low, middle, and high chromium exposures and analyzed for speciation of chromium (SOP P.454). For analysis of chromium in tissue, samples will be lyophilized (SOP C5.36), acid digested with microwave heating (SOP C5.94), and analyzed by either ICP-MS (SOP C5.212) or graphite furnace atomic absorption spectrophotometry (SOP C5.163).

5.1.2. Overview

The Hanford Nuclear Reservation in south central Washington is a 900 square kilometer area claimed by the federal government in 1943 as a site for the production of plutonium (Figure 1)(Geist 1995). The location was ideal because it was remote, sparsely populated, and most importantly, had a readily available supply of cold water from the Columbia River. Because of national security concerns, public access and river development projects were restricted until 1971 (Dauble and Watson, 1997). Extensive dam building and development occurred throughout the Columbia River Basin from 1943 to 1971 and led to severely reduced populations of chinook salmon (*Ocorhynchus tshawystcha*). The 90 km section within the Hanford Reservation was not developed, and today, the Hanford Reach remains a free flowing stretch of the Columbia River and is the only remaining area where significant mainstem spawning occurs in the Columbia River (Dauble and Watson 1990). The Hanford Reach of the Columbia River is regulated by upstream dams, but is the last unimpounded stretch of the mainstem Columbia River.

Large quantities of Columbia River water were used to cool nuclear reactors and cooling water was treated with sodium dichromate to prevent corrosion and mineral collection within the pipes (Peterson et al. 1996). During operations, cooling water with associated radionuclides and chromium were discharged directly to the river and also entered ground water through leakage of pipes and seepage from retention areas. Today, groundwater at the Handford site continues to be contaminated with chemical and radiological constituents (Geist et al. 1994). The hydraulic head of the ground water aquifers in the 100 Area (National Priority List Site) are higher in elevation than that of the Columbia River and results in discharge from the aquifer into the Columbia River through shoreline springs and seeps (Figure 1). The ground water is hydraulically

connected to the river with peak aquifer discharges occurring during low river flows (fall and winter) and minimum aquifer discharges occurring during high river flows (spring and summer) (Geist et al. 1994).

The use of the Hanford Reach for fall chinook spawning and rearing has dramatically increased since 1960 (Becker 1985, Dauble and Watson 1990). The 10 year average adult escapement increased from 27,660 (1964-1973) to 54,661 (1983-1992). This increase is pronounced when compared with the rest of the mid and upper Columbia River where chinook runs have declined during the same time period. Spawning occurs in close proximity to the 100 Area where contaminated ground water is entering the River. Adult chinook spawn in variable water depths, water velocities, and substrate types (Swan et al. 1988). Spawning in the Hanford Reach begins in mid-October, peaks in mid-November, and ends in late November (Dauble and Watson 1997). Egg and fry development within the redds take place from mid-October to May during low river flows that result in peak aquifer discharges. Based on the mid-November peak redd abundance and ambient temperatures, eggs would become eyed in early December, hatch in late December, and alevins would emerge from the redds in late February. Upon emergence, fry move out of the main river channel into shallow, slow moving, near shore and backwater habitat (Dauble and Watson 1990, Dauble et al. 1989). Juveniles remain in the Hanford Reach from February to mid-July feeding on macroinvertebrates (Becker 1973). Outmigrating begins in May and is usually completed by July at 5-7 months of age, 60-70mm in length, and 3-4 gm in weight (Olson and Foster 1956).

5.2. Current Site Information

One contaminant of major concern, and associated with the 100 Area groundwater and seeps, is chromium. The concentrations of chromium in the groundwater upwellings (Hope and Peterson 1996) exceed the chronic ambient water quality criteria (AWQC, 11 μg/L) for the protection of aquatic life, established by the U.S. Environmental Protection Agency (USEPA 1986) and the State of Washington (WAC-173-201A-040). The Department of Energy currently has activities underway to pump and treat chromium at the Hanford facility, and reduce the amounts of hexavalent chromium released into the Hanford Reach. However, the critical nature of the Hanford Reach as spawning habitat for the chinook salmon, makes it essential to determine if current water quality standards protect chinook salmon (Geist 1997). In particular, additional information is needed to determine if the current standards protect early life stage survival and development. While some data do exist on the effects of hexavelent chromium on salmon (Olson and Foster 1956, Buhl and Hamilton 1991), previous studies did not investigate the direct effects on fertilization, effects on alevin exposure only, recovery of exposed alevins, or physiological impairment.

5.3. Project Objectives

The objective of this project is to assess the effects of chromium on chinook salmon under exposure conditions similar to those of the Hanford Reach of the Columbia River (also see Task-specific objectives above).

5.4. Project Schedule

PROJECT SCHEDULE		
ITEM	START	END
Study Design and Organization	Apr 98	Sep 98
TASK 1: Fertilization		
perform tests	Nov 98	Dec 98
data analyses	Jan 99	Mar 99
TASK 2: Early Life Stage		
perform test	Nov 98	Mar 99
tissue analyses	Mar 99	Sep 99
data analyses	Oct 99	Dec 99
TASK 3: Parr Health		
perform tests	Mar 99	Jun 99
tissue analyses	Jun 99	Dec 99
data analyses	Jan 00	Mar 00
TASK 4: Reports		
Submit draft report	Mar 00	Jun 00
Submit final report		90 days after receipt of comments

6.0. Project Organization and Responsibilities

The Project's organization and responsibilities are summarized in figures 2 through 5. The qualifications personnel to perform research and/or administrative and management activities associated with the Project are summarized in Appendix B in the form of curriculum vitae.

Figure 2. Distribution of overall responsibilities for project P99-10-01

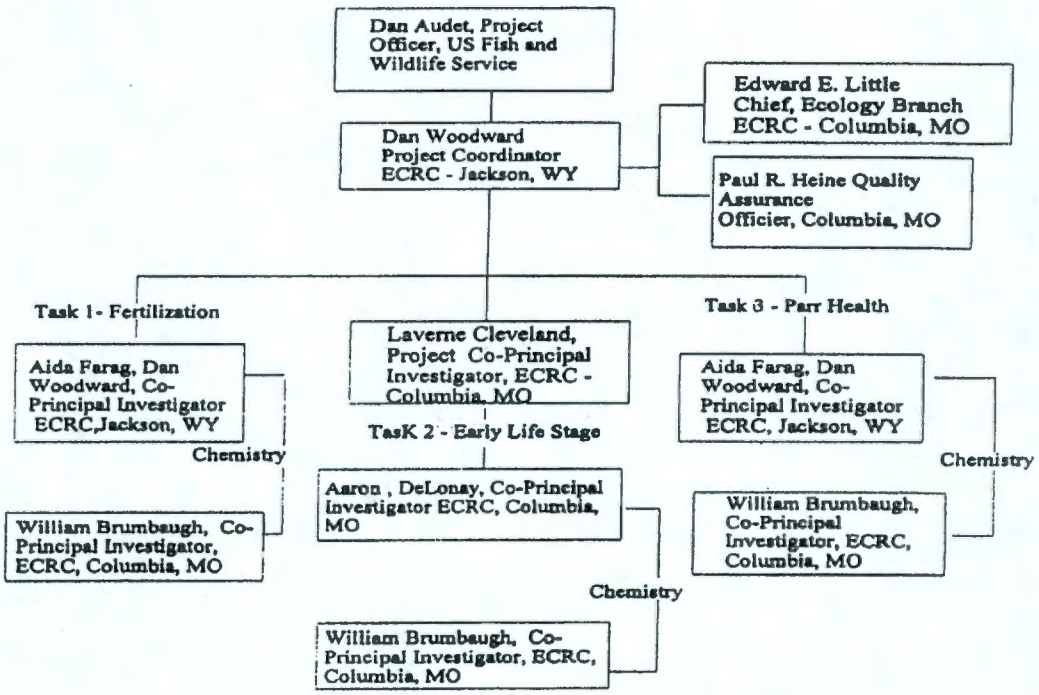


Figure 3. Distribution of responsibilities for project P99-10-01 Task 1: Fertilization

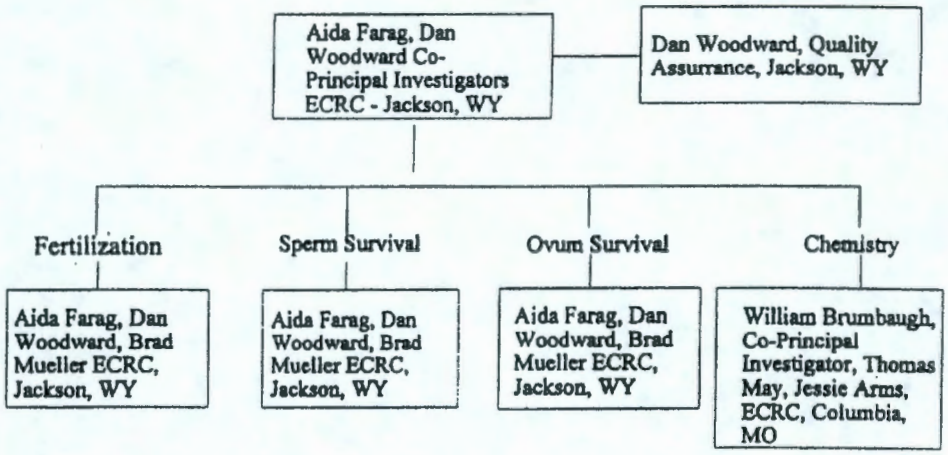


Figure 4. Distribution of responsibilities for project P99-10-01 Task 2: Early Life Stage

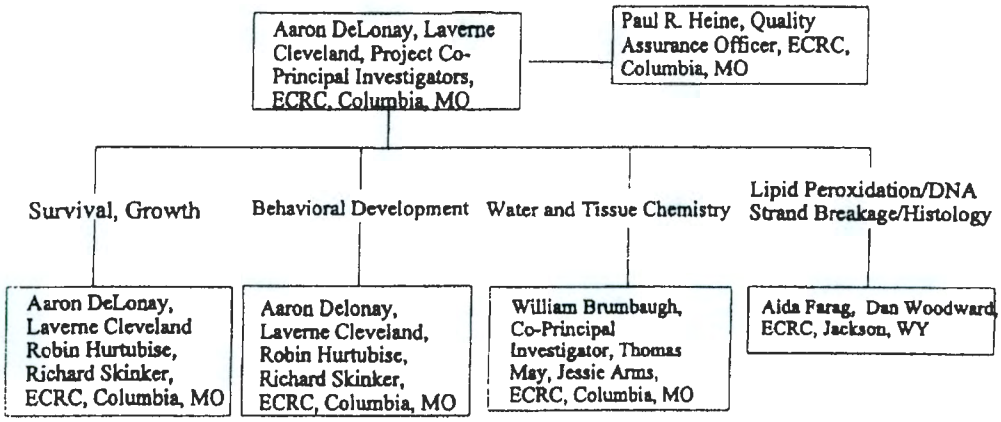
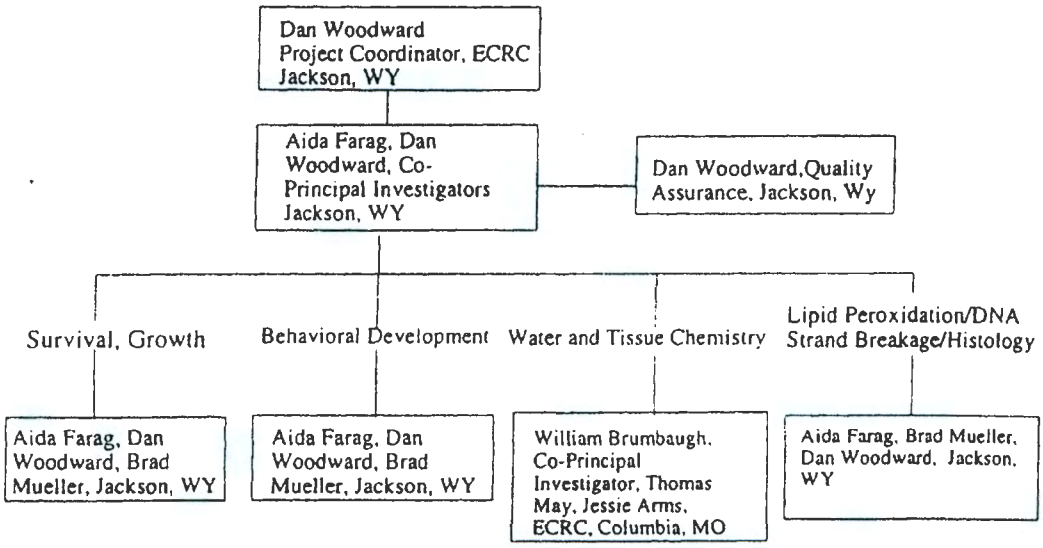


Figure 5. Distribution of responsibilities for project P99-10-01 Task 3: Parr Health



6.1. Program Management

Dan Woodward of the ECRC Field Research Station at Jackson, Wyoming will serve as Coordinator for the Project and will report to the ECRC Ecology Branch Chief and the Project Officer for the U.S. Fish and Wildlife Service (Figure 2). Quality Assurance and Quality Control (QA/QC) functions for the Project will be the responsibility of Paul R. Heine , the ECRC QA Officer. The QA Officer or his designee will conduct all QA/QC inspections and audits and will report to the Project Coordinator and Ecology Branch Chief. Aida Farag and Daniel Woodward will serve as co-principal investigators for studies under Task 1 and 3 of the Project (Figure 2). Aaron DeLonay and Laverne Cleveland will serve as co-principal investigators for the study under Task 2 of the Project and will report to the Project Coordinator (Daniel Woodward). William Brumbaugh will serve as Co-principal Investigator of chemical analyses for the studies under Task 1, 2, and, 3 of the Project and will report to the respective Co-principal Investigator for each task (Figure2).

6.2. Quality Assurance Responsibility

Individuals identified in Figures 2-5 as quality assurance personnel (Paul Heine, Dan Woodward) will be responsible for conducting laboratory checks, identifying and controlling non-conformance, and initiating corrective actions whenever QA/QC limits are exceeded.

6.3. Laboratory Responsibilities

Under Task 1 of the Project Aida Farag, Dan Woodward, and Brad Mueller will be responsible for conducting the fertilization, sperm survival, and ovum survival tests and Bill Brumbaugh will be responsible for chemical analyses for each test under Task 1 (Figure 3). Under Task 2 of the Project Aaron DeLonay, LaverneCleveland, Robin Hurtubise, and Richard Skinker will be responsible for conducting the early life stage tests and collection of data on growth, survival, and behavioral development and Aida Farag and Dan Woodward will be responsible for lipid peroxidation, DNA strand breakage, and histological analyses (Figure 4). Bill Brumbaugh will be responsible for all water and tissue chemical analyses under Task 2 of the Project (Figure 4). Under Task 3 of the project, Aida Farag, Dan Woodward, and Brad Mueller will be responsible for conducting the parr health study and collection of data on parr growth, survival, behavioral development, lipid peroxidation, DNA strand breakage, and histology (Figure 5). All tissue and water analyses for chromium under Task 4 will be performed by William Brumbaugh (Figure 5).

7.0. Quality Assurance Objectives for Measurement Data

7.1. Precision, Accuracy, and Test Acceptability

7.1.1. Task 1- Fertilization; Task 2 - Early life Stage, Task 3- Parr Health

Precision describes the degree to which data generated from replicated treatments differ. Accuracy is the difference between measured data values and the true data values. Precision and accuracy will be determined for tests under each Task by replicating treatments a minimum of four times. Treatment replication allows the estimation of standard deviation and coefficient of variation (CV) which are used as indicators of precision and accuracy. The acceptability of toxicity tests conducted under Task 1, Task 2, and Task 3 will be assessed by their precision and responses observed for control treatments used in all tests.

7.1.2. Chemical Analyses

Precision and accuracy for chemical analyses performed for tests under Task 1, Task 2, and Task 3, will be assessed by use of replicate analyses, certified reference materials, matrix spikes, procedural blanks, and control samples. Water and tissue samples will be analyzed for chromium according to ECRC SOPs C5.212, P.454, C5.36, C5.94 and C5.163.

8.0. Analytical, Toxicity Testing, and Sampling Procedures

8.1. Analytical Procedures

8.1.1. Water Quality

Exposure water will be monitored for dissolved oxygen, pH, alkalinity, hardness, and conductivity following standard methods (APHA, 1975 and ECRC SOPs B4.14, B4.15, B4.16, and B4.9.

8.1.1.1. Chromium Water Analyses

One hundred mL samples of exposure water will be filtered using a Nalgene® 300 filter holder. Each filtered sample will be transferred to a pre-cleaned, 125 ml I-Chem® polyethylene bottle, acidified to 1% HNO3, and analyzed with ICP-MS according to ECRC SOP C5.212. Water samples will be analyzed for chromium speciation according to ECRC SOP P.454.

8.1.1.2. Chromium Tissue Analyses

Tissue samples will be collected from each chromium treatment, placed in polyethylene bags and frozen at -4 °C pending analysis. To analyze tissue samples for chromium the tissue samples will be lyophilized according to ECRC SOP C5.36 , acid digested with microwave heating (ECRC SOP C5.94), and analyzed by either ICP-MS (ECRC SOP C5.212) or graphite furnace atomic absorption spectrophotometry (ECRC SOP C5.163).

8.1.1.3. Lipid Peroxidation

Samples for lipid peroxidation will be collected from the chromium treatments, frozen with liquid nitrogen, and stored at -90°C pending analysis according to Jackson SOP F20.25.

8.1.1.4. DNA Strand Breakage

Samples for DNA strand breakage will be collected from the chromium treatments, frozen with liquid nitrogen, and stored at -90°C pending analysis according to Jackson SOP F20.29.

8.1.1.5. Histology

Samples for histology will be collected from the chromium treatments and fixed in 10% neutral buffered formalin pending histological examinations. Gill tissue, liver tissue (free of the gall bladder), kidney, intestines, spleen, and skin will be examined for histological anomalies.

8.2. Toxicity Testing Procedures

The laboratories in which studies will be conducted will be restricted to authorized personnel. Protocol P99-10-01 used for conducting research described in Task 1, Task 2, and Task 3 of the Project is included as Appendix C. A list of Standard Operating Procedures (SOPs) used for conducting the Protocol is attached to the Protocol (Appendix C). These SOPs are established methods which are followed during the conduct of research activities under the Protocol. The use of SOPS insures that (1) sound, scientifically acceptable technical guidelines will be used to perform all research activities described in the Protocol, (2) that work performed and data generated will be of the highest attainable quality, and (3) that all work performed will

be thoroughly documented for purposes such as litigation. Examples of ECRC SOPs are presented in Appendix D.

9.0. Sample Custody Procedures

Principal Investigators will be the custodians for all samples collected during the conduct of the Protocol and a strict chain of custody will be maintained. All samples collected for chromium water analysis, chromium tissue analysis, lipid peroxidation, DNA strand breakage, and histology will be labeled with a unique sample identification number and sealed with tamper resistant tape to discourage tampering. All samples will be stored in a secured coolers or freezers as appropriate, which will be continuously monitored for temperature. As necessary, samples will be shipped between the Jackson Field Station and the Columbia Center or to other analytical facilities in secured containers by overnight express courier service. A ECRC chain-of-custody form will accompany all samples which are shipped or otherwise transferred.

Data files and records will be maintained by the Principal Investigators during the conduct of studies and will be made available to the Project Coordinator upon request and at the completion of studies. Files and records will include all project documents, correspondences (telephone, mail, e-mail, and FAX), raw data records, laboratory logs, and project deliverables. Data from all studies will be submitted to ECRC Information Technology personnel for local archiving within four weeks after the studies are completed as outlined in ECRC SOP B5.63. Study files will be permanently archived as outlined in ECRC SOP B5.147. Data will be transmitted to the Hanford Project Officer in the form of a final report.

10.0. Calibration Procedures and Frequency

The instruments used for measuring chemical parameters such as chromium exposure water and tissue concentration, pH, dissolved oxygen, temperature, conductivity, alkalinity, and hardness are calibrated and standardized according to instrument manufacturers procedures. A list of these SOPs are attached to the Protocol (Appendix C). Records of calibration data, performance checks, and instrument service are maintained in permanent bound log notebooks or in computer files in a manner that the history of performance of the instruments may be easily reviewed. Standards such as NBS–certified thermometers for devices used to measure temperature, class-s weights for balances, and conductivity, hardness, and alkalinity standards and National Institute of Standards and Technology quality control solutions are used to insure that instruments function properly. Analytical reagents are labeled and dated upon receipt and are protected from deterioration. All instruments used to measure water quality are calibrated weekly, except dissolved oxygen and pH meters which are calibrated before being used.

11.0. Internal Quality Control Checks and Frequency, Performance and System Audits

The Quality Assurance Officer will conduct quality control audits at the end of all studies. All audit reports will be forwarded to the Principal Investigators and Project Coordinators for documentation of corrective action. The completed audit report will then be sent to the Quality Assurance officer for review. All studies will include control treatments. All study treatments will be replicated to provide a measure of precision as standard deviations and coefficients of variation. The acceptability of tests will be assessed by their precision and the response (e.g.,

survival, growth) of organisms exposed to the control treatments. For acceptable tests survival of organisms exposed to the control treatments should be ≥ 85 % and variation among replicated treatments should not exceed 20 %. All deviations from the Protocol will be documented in the form of a Protocol amendment which will undergo the same approval process as the Protocol. Results of chromium exposure water and tissue analyses will be accompanied by data validation information including duplicate results, standard reference material recoveries, spike results, procedural blank results, detection limits, calibration data, and summaries of deviations from the Protocol due to laboratory or sample problems.

12.0. Data Reduction, Validation, Assessment, and Reporting Procedures

Effects parameters will be calculated for each toxicity test. Effects parameters will include percentage survival, percentage inhibition of growth and mean weight loss for gain, mean behavioral responses, mean increases in lipid peroxidation, mean occurrences of DNA strand breakages, mean occurrences of histological anomalies, no observable effect concentrations (NOEC), lowest observable effect concentration. When appropriate each effect parameter will be accompanied by an indicator of variance associated with the effect (e.g., standard deviations, standard errors, coefficients of variations). Effects parameters will be determined by comparing the responses of organisms exposed to the chromium treatments to that of organisms exposed to the control treatments or comparison of responses among chromium treatments.

Response differences between treatments will be determined by use of appropriate statistical procedure such as analysis of variance (ANOVA) with means separation test (e.g., Fishers LSD, Duncan's, Tukeys). Statistical analyses will be performed with computer programs such as Statistical Analysis Systems (SAS) programs (SAS 1985).

All data will be evaluated by the Principal Investigator for each test, the Project Coordinator, and the Quality Assurance Officer. The results of tests will be reported to the Project Officer in the form of a final report.

13.0. Preventive Maintenance Procedures and Schedules

Records are kept on all analytical equipment. Calibration checks are used to determine when probes, diffusion membranes, batteries, and buffer solutions need to be changed. Reference standards are replaced on their expiration dates. Refer to the list of SOPs in Appendix C for additional information.

14.0. Corrective Actions

Corrective actions will be implemented whenever research equipment is not in compliance. Details on corrective actions are described for specific research equipment and research activities in ECRC SOPs. Corrective actions are based on data acceptability limits (e.g., analytical standards values not within specified range) or identification of equipment defects. Measures will be implemented to correct defects underlying non compliance, document the results of corrective actions, and monitor activities or equipment until compliance is established. In cases of non compliance, corrective actions will be immediate and most often will be implemented the analyst. Corrective actions may also result from QA Audits. Most often issues requiring corrective actions are related to equipment malfunction or failure or deviations from

QA objectives.

15.0. Quality Assurance Reports to Management

Written QA reports will be initiated at the request of the Project Coordinator or Project Officer or as a result of a QA audit. The QA officer will be responsible for all QA reports. The reports will include information such as a description of discrepancies or problems, descriptions of variances from QA objectives, implications of the variances, discrepancies, or problems, required corrective actions, and a schedule for meeting corrective actions.

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17.0. Appendix A. Environmental and Contaminants Research Center Research Study Plan 418

ENVIRONMENTAL AND CONTAMINANTS RESEARCH
CENTER RESEARCH STUDY PLAN 418

Study Plan Title: The Potential for Chromium to Adversely Affect Chinook Salmon (*Oncorhynchus tshawystcha*) under Exposure Conditions Simulating the Hanford Reach of the Columbia River, Washington, USA.

BRD SIS Project Number:

Date Prepared: 08/17/98

Date Revised:

BRD Center: Environmental and Contaminants Research Center

Facility Contact: Wilbur L. Mauck

Project Contact: Daniel Woodward

Date Initiated: 1998

I. Rational and Justification: Extensive dam building and development occurred throughout the Columbia River Basin from 1943 to 1971 and led to severely reduced populations of chinook salmon (*Ocorhynchus tshawystcha*). An area that did not experience development is a 90 km section within the Hanford Nuclear Reservation which was claimed by the federal government in 1943 as a site for the production of plutonium. Currently, the Hanford Reach remains a free flowing stretch of the Columbia River and is the only remaining area where significant mainstem chinook salmon spawning occurs in the Columbia River (Dauble and Watson 1990). The Hanford Reach of the Columbia River is regulated by upstream dams, but is the last unimpounded stretch of the Columbia River. The use of the Hanford Reach for fall chinook spawning and rearing has dramatically increased since 1960 (Becker 1985, Dauble and Watson 1990). The 10 year average adult escapement increased from 27,660 (1964-1973) to 54,661 (1983-1992). This increase is pronounced when compared with the rest of the mid and upper Columbia River where chinook runs have declined during the same time period.

Plutonium production within the Hanford Reservation required the use of large quantities of Columbia River water to cool nuclear reactors. The cooling water was treated with sodium dichromate to prevent corrosion and mineral collection within cooling system pipes (Peterson et al. 1996). During operations, cooling water with associated radionuclides and chromium were discharged directly to the Columbia River and also entered ground water through leakage of pipes and seepage from retention areas. Currently, groundwater at the Hanford site continues to be contaminated with chemical and radiological constituents (Geist et al. 1994).

The Hanford Reach of the Columbia River is critically important as spawning habitat for the chinook salmon and it is essential to determine if current water quality standards protect

chinook salmon (Geist 1997). Chromium is one contaminant of major concern and it is associated with the 100 Area (National Priority List Site) groundwater and seeps. The concentrations of chromium in the groundwater upwellings (Hope and Peterson 1996) exceed the chronic ambient water quality criteria of (AWQC) of 11 μg/L for the protection of aquatic life, established by the U.S. Environmental Protection Agency (USEPA 1986) and the State of Washington (WAC-173-201A-040). In particular, additional information is needed to determine if the current standards protect survival and development early-life stages of chinook salmon. Previous studies on the effects of hexavelent chromium on chinook salmon (Olson and Foster 1956, Buhl and Hamilton 1991) did not investigate direct effects on fertilization, effects on health status of alevins, recovery of exposed alevins, or physiological impairment.

II. Objectives (what): The objectives of this study are to assess the effects of chromium on chinook salmon under exposure conditions similar to those of the Hanford Reach of the Columbia River.

III. Listing of Studies:

A. **Study 1: Fertilization: The potential for chromium to adversely affect gametes and their fertilization in chinook salmon.**

1. Principal Investigator(s): Daniel F. Woodward and Aida Farag
2. Specific Objectives: **To determine the toxicity of chromium to chinook salmon ovum, sperm, and the fertilization process.**
3. Experimental Design or Methodological Approach: Experimental water will simulate that of the Columbia River surface and pore water in the Hanford Reach which are known to be associated with the location of spawning redds (Hope and Peterson 1996, Geist 1997). Experimental water will be adjusted to a hardness of 80 mg/L as $CaCO_3$; pH, alkalinity, and conductivity will be in a range consistent with Columbia River conditions. Experimental water temperature will match seasonal conditions: December through March, 5°C; March through July, 10°C (Wiggins et al. 1997). Geist (1997) documented that the hyporheic zone (where river water and ground water mix) is generally warmer than the river water. However, data from samples collected between November and March indicate that the temperature of the hyporheic zone minus the river water is only 1°C. Experimental water will be prepared by blending laboratory well water with deionized water produced by reverse osmosis. Experimental water produced in this way will eliminate the use of surface water and the potential for fish pathogens to be introduced to the experiment and influence test results. Experimental water will be produced in 5,600L batches and analyzed to insure quality is within 5% of the experimental design in terms of hardness, alkalinity, conductivity, and pH. Unless otherwise indicated, experimental water was used. Photoperiod will be adjusted to simulate time of year of the exposure.

   The range of chromium concentrations tested in the experimental water will be from 0 to 120 μg/L. This range of concentrations are above and below the chronic

AWQC for chromium, 11 μg/L (USEPA 1986, State of Washington (173-201A-040)). This concentration range is also representative of concentrations in pore water sampled from the intergravel substrates in locations where salmon spawn (Giest 1997, Hope and Peterson 1996).

Gametes will be obtained from reproductively mature chinook salmon from the McNenny State Fish Hatchery, Spearfish, South Dakota. The salmon from which the gametes are obtained will be certified disease free prior to any testing. The disease free status is essential in assuring that toxicity testing is on healthy test organisms, increases reliability of results, and is a recommended standard procedure (ASTM 1993). This source of chinook salmon has been used in past Natural Resource Damage Assessments (Blackbird Mine Site, Idaho; Marr et al. 1995).

Gametes will be taken from chinook salmon brood stock between October and November of 1998. This is the normal time for gametogenesis in fall adult chinook salmon and the stock will be checked weekly for ovum and sperm formation. We will use a pooled source of eggs and sperm from a minimum of three females and three males to perform three tests: 1) toxicity of chromium to the ovum, the ovum survival test; 2) toxicity of chromium to sperm, the sperm survival test; and 3) toxicity of chromium to fertilization, the fertilization test.

A physiological saline (PS) solution will be used in the ovum and fertilization test; and a physiological saline solution with sperm extender (PS/SE) will be used in the sperm test (Billard and Roubaud 1985). The physiological saline solution will consist of a standard 1% NaCl solution buffered to pH 9.0; the PS/SE will be the same solution with KCl added (30 mM) to prevent the spermatozoa from becoming motile. Ova and sperm can survive for several hours in these solutions. In all three tests, there will be six treatment concentrations of chromium: 0, 5, 11, 24, 54, and 120 μg/L. The six chromium treatments will be incorporated into the PS or the PS/SE so that when diluted with the appropriate amount of sperm or ovum the desired concentration of chromium is achieved. Each treatment will be replicated four times for a total of 24 treatments.

Ovum survival test. Ova will be divided into 24 treatment lots of 150-200 eggs, each and mixed with 10 ml of PS containing the appropriate chromium concentration. After 15 min, the liquid will be removed from each treatment and replaced with 10ml fresh uncontaminated PS solution followed by insemination with 1ml of intact sperm. This will be a 15 min ovum exposure.

Sperm survival test. Sperm will be diluted with PS/SE containing the appropriate chromium concentration (1ml sperm:10ml PS/SE) to obtain 24 treatment lots. The sperm, PS/SE, and chromium will be mixed, and left standing for 15 min. Sperm will be separated from the PS/SE exposure treatments by centrifuging for 10 min at 1800g, followed by replacement of 10ml fresh uncontaminated PS/SE. The exposed sperm will be used to inseminate 24 lots of about 150-200 ova, each previously diluted in 10ml of PS. This will be a 15 min sperm exposure.

Fertilization test. Ova (150-200), sperm (1ml), and 10ml of PS containing the appropriate chromium concentrations will be mixed together to achieve the 24 treatment lots. This will be a 65 min exposure of egg and sperm during fertilization and water

hardening. In all three tests, ova and sperm will be mixed for 5 min followed by rinsing and water hardening in Hanford experimental water according to standard procedures (Piper et al. 1982). Water hardening will last for one hour and is the process by which water is absorbed into the eggs and fills the perivitelline space between the shell and yoke. The eggs become turgid during this process and additional water exchange is minimal during further development. In the fertilization test, exposure to chromium will continue through water hardening. After water hardening, eggs will be rinsed and transferred into incubators. Eggs from all three tests will be incubated in McNenny hatchery water (temperature, 11°C; hardness, 360 mg/L as $CaCO_3$; alkalinity, 210 mg/L; and pH, 7.6) for 10 days. The eggs will be cleared in 10% acetic acid solution for 2 min and percent fertilization will be determined. The embryo of fertilized eggs will turn an opaque white and become visible through the translucent chorion. At 10 days the embryo will have a definite optic lobe developed with an elongated somite and will be easily distinguished from an unfertilized germinal disk.

Exposure water will be monitored once per week for dissolved oxygen, pH, alkalinity, hardness, and conductivity (ECRC SOPs B4.14, B4.15, B4.16, and B4.9). More frequent monitoring will be performed if conditions dictate. Samples of exposure water will be taken weekly to monitor total chromium exposure concentrations. One hundred mL samples of exposure water from each treatment will be filtered using a Nalgene® 300 filter holder. Each filtered sample will be transferred to a pre-cleaned, 125 ml I-Chem® polyethylene bottle, acidified to 1% HNO_3, and analyzed with ICP-MS (See ECRC SOP C5.212). At each time of total chromium sampling, one additional sample will be extracted from the low, middle, and high chromium exposures and analyzed for speciation of chromium (SOP P.454). For analysis of chromium in tissue, samples will be lyophilized (SOP C5.36), acid digested with microwave heating (SOP C5.94), and analyzed by either ICP-MS (SOP C5.212) or graphite furnace atomic absorption spectrophotometry (SOP C5.163).

4. Listing of Critical Data: **Objective: To determine the toxicity of chromium to chinook salmon ovum, sperm, and the fertilization process.**.
Data gathered from this objective will include chinook salmon ova survival, sperm survival, and percent fertilization at chromium concentrations representative of concentrations in pore water sampled from the intergravel substrates in locations where salmon spawn.
5. Statistical Treatment: Percent data for fertilization, ova survival, and sperm survival will be arc sine transformed and analyzed with Analysis of Variance (ANOVA) followed by Tukey means comparisons test. Statistical significance will be assigned at $P \leq 0.05$. Acceptance or rejection of test results will be determined from statistical analyses and peer review of the methods, data, and results.
6. Acceptance or Rejection Criteria for Results: Acceptance or rejection criteria for test results are outlined in ECRC Standard Operating Procedures and standardized methods for specific studies. Appropriate Standard Operating Procedures and standardized methods used for these tests are cited in ECRC Protocol P99-10-01 (Attachment I).
7. Special Safety Requirements: Safety requirements for all research activities at the ECRC

are given in the ECRC Safety Plan.

8. Endpoint of Study, based on Accomplishments: This study will provide an assessment of the effects of chromium on the reproductive process, growth, survival, behavior, health status, and histopathology of chinook salmon. This study will end with the analysis and interpretation of the data gathered and the submission of a final report to the funding agency, the U.S. Fish and Wildlife Service. Data from this research will also be published in peer-reviewed journals.

9. Schedule of Study and the Outputs Expected:

STUDY SCHEDULE AND OUTPUTS		
ITEM	START	END
Study Design and Organization	Apr 98	Sep 98
perform tests	Nov 98	Dec 98
data analyses	Jan 99	Mar 99
Submit draft report	Mar 00	Jun 00
Submit final report		90 days after receipt of comments

10. Place Where Data will be Stored and Archived: File folders of original data will be maintained at the ECRC Field Research Station, Jackson, WY and the ECRC, Columbia, MO according to standard operating procedures. Files from this study will be permanently archived by the ECRC QA/QC Officer as outlined in ECRC SOP B5.147.

11. Relationship to Cooperator Needs: The data gathered from this study will (1) provide an assessment of injury to chinook salmon exposed to chromium in the Hanford area of the Columbia River Basin, (2) will be useful in recovering damages for lost resources and in evaluating remedial options, including immobilization, treatment, and the no-action alternative; and (3) will be useful to Trustees, including the U.S. Fish and Wildlife Service, Hanford Natural Resource Trustees, the U.S. Department of the Interior, and the States of Oregon and Washington in efforts to manage the Columbia River salmon population.

12. Literature Cited: See literature cited section of Study 3 below.

13. Signatures:

Prepared by: _____ Date:_____
Laverne Cleveland
Principal Investigator

Approved by: _____ Date:_____
Edward E. little
Branch Chief

Approved by: _____ Date:_____
Aida Farag
Principal Investigator

Approved by: _____ Date:_____
Daniel F. Woodward
Principal Investigator

Approved by: _____ Date:_____
I. Eugene Greer
Animal Care and Use Committee Chair

Approved by: _____ Date:_____
Paul R. Heine
Quality Assurance and Safety Officer

Approved by: _____ Date:_____
Wilbur L. Mauck
Center Director

B. Study 2: **Early Life Stage; Determine the effects of chromium on the early development of chinook salmon.**

1. Principal Investigator(s): Aaron DeLonay and Laverne Cleveland
2. Specific Objectives: (1) **To determine the effects of chromium on chinook salmon egg survival, egg hatching and alevin survival, growth, and behavioral development; (2) To evaluate the bioconcentration of chromium by chinook salmon and determine effects chromium exposure on DNA strand breakage, and lipid peroxidation (see Study 3 for further details of physiology measurements).**
3. Experimental Design or Methodological Approach: Experimental water will simulate that of the Columbia River surface and pore water in the Hanford Reach which are known to be associated with the location of spawning redds (Hope and Peterson 1996, Geist 1997). Experimental water will be adjusted to a hardness of 80 mg/L as $CaCO_3$; pH, alkalinity, and conductivity will be in a range consistent with Columbia River conditions. Experimental water temperature will match seasonal conditions: December through March, 5°C; March through July, 10°C (Wiggins et al. 1997). Geist (1997) documented that the hyporheic zone (where river water and ground water mix) is generally warmer than the river water. However, data from samples collected between November and March indicate that the temperature of the hyporheic zone minus the river water is only 1°C. Experimental water will be prepared by blending laboratory well water with deionized water produced by reverse osmosis. Experimental water produced in this way will eliminate the use of surface water and the potential for fish pathogens to be introduced to the experiment and influence test results. Experimental water will be produced in 5,600L batches and analyzed to insure quality is within 5% of the experimental design in terms of hardness, alkalinity, conductivity, and pH. Unless otherwise indicated, experimental water was used. Photoperiod will be adjusted to simulate time of year of the exposure.

The range of chromium concentrations tested in the experimental water will be from 0 to 120 μg/L. This range of concentrations are above and below the chronic AWQC for chromium, 11 μg/L (USEPA 1986, State of Washington (173-201A-040)). This concentration range is also representative of concentrations in pore water sampled from the intergravel substrates in locations where salmon spawn (Giest 1997, Hope and Peterson 1996). Specific concentration are stated with each task.

Eyed embryos of chinook salmon will be obtained from the McNenny State Fish Hatchery, Spearfish, South Dakota. These eggs will be certified disease free prior to any testing. The disease free status is essential in assuring that toxicity testing is on healthy test organisms, increases reliability of results, and is a recommended standard procedure (ASTM 1993). This source of chinook salmon eggs has been used in past Natural Resource Damage Assessments (Blackbird Mine Site, Idaho; Marr et al. 1995).

Eyed eggs of chinook salmon will be exposed to 5, 11, 24, 54, and 120 μg/L of chromium and a control treatment with no chromium added. The test will be conducted in a modified Mount and Brungs (1967) flow-through diluter system (ECRC SOP F20.E18,"Construction, Operation, Calibration, And Maintenance of the Proportional

Diluter"). Temperature will be maintained at 5±2 °C by chilling the exposure water before it enters the diluter and submerging the exposure aquaria in a temperature-controlled water bath.

To initiate the test, two groups of 50 eggs each will be placed into 177-mL glass hatching containers and suspended into each of four exposure aquaria. The aquaria will be covered with black plastic to shield the eggs from light during incubation, and gentle aeration will be used to provide continuous circulation of the exposure water. On the median hatch date, the alevins will be released into the exposure aquaria. On the median swim-up date, the chromium exposure will be discontinued and the alevins will be maintained in the aquaria in chromium-free water until 30 days after the medium swim-up date.

During the exposure, egg mortality and hatching will be monitored and recorded daily. Dead eggs will be removed from the hatching containers and discarded. Alevin mortality and deformities will be monitored daily and dead alevins will be removed from the aquaria and discarded. The development of alevins will be monitored daily to document the sequence and timing of critical developmental stages including; hatch, onset of movement, side plough, upright plough, free swimming, and exogenous feeding following Dill (1977). The tanks will be video taped weekly to develop an accurate count of these developmental patterns among the test populations. Quantitative measures of the form and frequency of movements will be made during the alevin/free swimming transition.

Samples of alevins containing 15 fish each will be taken from each of the four replicate exposures at 0, 7, 14, and 28 days post median hatch date and analyzed for tissue residues of chromium, DNA strand breakage, and lipid peroxidation (see Task 3 for further details of physiology measurements). Two fish from each replicate will be collected on days 0 and 24 for histological analyses. At the end of the exposure (30 days post median-hatch) all surviving alevins in each treatment will be measured for total length and weighed to determine growth.

Exposure water will be monitored once per week for dissolved oxygen, pH, alkalinity, hardness, and conductivity (ECRC SOPs B4.14, B4.15, B4.16, and B4.9). More frequent monitoring will be performed if conditions dictate. Samples of exposure water will be taken weekly to monitor total chromium exposure concentrations. One hundred mL samples of exposure water from each treatment will be filtered using a Nalgene® 300 filter holder. Each filtered sample will be transferred to a pre-cleaned, 125 ml I-Chem® polyethylene bottle, acidified to 1% HNO_3, and analyzed with ICP-MS (See ECRC SOP C5.212). At each time of total chromium sampling, one additional sample will be extracted from the low, middle, and high chromium exposures and analyzed for speciation of chromium (SOP P.454). For analysis of chromium in tissue, samples will be lyophilized (SOP C5.36), acid digested with microwave heating (SOP C5.94), and analyzed by either ICP-MS (SOP C5.212) or graphite furnace atomic absorption spectrophotometry (SOP C5.163).

4. Listing of Critical Data: **Objective 1: To determine the effects of chromium on chinook salmon egg survival, egg hatching and alevin survival, growth, and**

behavioral development.

Data gathered from this objective will include chinook salmon egg survival, egg hatching, and alevin survival, growth, and behavioral development at chromium concentrations representative of concentrations in locations where salmon spawn.

(2) **To evaluate the bioconcentration of chromium by chinook salmon and determine effects chromium exposure on DNA strand breakage, and lipid peroxidation (see Study 3 for further details of physiology measurements).**

Data gathered from this objective will include chinook salmon chromium tissue residues, DNA strand breakage, lipid peroxidation and physiological development at chromium concentrations representative of concentrations in locations where salmon spawn.

5. Statistical Treatment: Data will be analyzed by use of Analysis of Variance (ANOVA) followed by Tukey means comparisons test. Percent data will be arc sine transformed before analysis. Statistical significance will be assigned at $P \leq 0.05$. Acceptance or rejection of test results will be determined from statistical analyses and peer review of the methods, data, and results.
6. Acceptance or Rejection Criteria for Results: Acceptance or rejection criteria for test results are outlined in ECRC Standard Operating Procedures and standardized methods for specific studies. Appropriate Standard Operating Procedures and standardized methods used for this test are cited in ECRC Protocol P99-10-01 (Attachment I).
7. Special Safety Requirements: Safety requirements for all research activities at the ECRC are given in the ECRC Safety Plan.
8. Endpoint of Study, based on Accomplishments: This study will provide an assessment of the effects of chromium on the reproductive process, growth, survival, behavior, health status, and histopathology of chinook salmon. This study will end with the analysis and interpretation of the data gathered and the submission of a final report to the funding agency, the U.S. Fish and Wildlife Service. Data from this research will also be published in peer-reviewed journals.
9. Schedule of Study and the Outputs Expected:

STUDY SCHEDULE AND OUTPUTS

ITEM	START	END
Study Design and Organization	Apr 98	Sep 98
perform test	Nov 98	Mar 99
tissue analyses	Mar 99	Sep 99
data analyses	Oct 99	Dec 99
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Submit final report		90 days after receipt of comments

10. Place Where Data will be Stored and Archived: File folders of original data will be maintained at the ECRC Field Research Station, Jackson, WY and the ECRC, Columbia, MO according to standard operating procedures. Files from this study will be

permanently archived by the ECRC QA/QC Officer as outlined in ECRC SOP B5.147.

11. Relationship to Cooperator Needs: The data gathered from this study will (1) provide an assessment of injury to chinook salmon exposed to chromium in the Hanford area of the Columbia River Basin, (2) will be useful in recovering damages for lost resources and in evaluating remedial options, including immobilization, treatment, and the no-action alternative; and (3) will be useful to Trustees, including the U.S. Fish and Wildlife Service, Hanford Natural Resource Trustees, the U.S. Department of the Interior, and the States of Oregon and Washington in efforts to manage the Columbia River salmon population.

12. Literature Cited: See "Literature Cited" section of study 3 below.

13. Signatures:

Prepared by: _____ Date:_____
Laverne Cleveland
Principal Investigator

Approved by: _____ Date:_____
Edward E. Little
Branch Chief

Approved by: _____ Date:_____
Aaron Delonay
Principal Investigator

Approved by: _____ Date:_____
I. Eugene Greer
Animal Care and Use Committee Chair

Approved by: _____ Date:_____
Paul R. Heine
Quality Assurance and Safety Officer

Approved by: _____ Date:_____
Wilbur L. Mauck
Center Director

C. Study 3: **Fish Health; Determine degree of fish health impairment of chinook salmon exposed to chromium.**

1. Principal Investigator(s): Daniel F. Woodward and Aida Farag
2. Specific Objectives: (1) **Gather data on chromium-induced physiological responses to aid in the interpretation of growth and survival responses documented during the early life-stage test (Study 2 above).**
   (2) **Interpret the physiological effects of chromium on fish and define parameters that can be measured on fish samples collected from the Hanford Reach.**
3. Experimental Design or Methodological Approach: Experimental water will simulate that of the Columbia River surface and pore water in the Hanford Reach which are known to be associated with the location of spawning redds (Hope and Peterson 1996, Geist 1997). Experimental water will be adjusted to a hardness of 80 mg/L as $CaCO_3$; pH, alkalinity, and conductivity will be in a range consistent with Columbia River conditions. Experimental water temperature will match seasonal conditions: December through March, 5°C; March through July, 10°C (Wiggins et al. 1997). Geist (1997) documented that the hyporheic zone (where river water and ground water mix) is generally warmer than the river water. However, data from samples collected between November and March indicate that the temperature of the hyporheic zone minus the river water is only 1°C. Experimental water will be prepared by blending laboratory well water with deionized water produced by reverse osmosis. Experimental water produced in this way will eliminate the use of surface water and the potential for fish pathogens to be introduced to the experiment and influence test results. Experimental water will be produced in 5,600L batches and analyzed to insure quality is within 5% of the experimental design in terms of hardness, alkalinity, conductivity, and pH. Unless otherwise indicated, experimental water was used. Photoperiod will be adjusted to simulate time of year of the exposure.

   The range of chromium concentrations tested in the experimental water will be from 0 to 120 μg/L. This range of concentrations are above and below the chronic AWQC for chromium; 11 μg/L (USEPA 1986, State of Washington (173-201A-040)). This concentration range is also representative of concentrations in pore water sampled from the intergravel substrates in locations where salmon spawn (Giest 1997, Hope and Peterson 1996). Specific concentration are stated with each task.

   Eyed embryos of chinook salmon will be obtained from the McNenny State Fish Hatchery, Spearfish, South Dakota. These eggs will be certified disease free prior to any testing. The disease free status is essential in assuring that toxicity testing is on healthy test organisms, increases reliability of results, and is a recommended standard procedure (ASTM 1993). This source of chinook salmon eggs has been used in past Natural Resource Damage Assessments (Blackbird Mine Site, Idaho; Marr et al. 1995).

   The goals of this experiment are two-fold. First, data gathered from this experiment will further explain toxicological responses on growth and survival documented during the early life-stage experiment. Because fry at the end of the early life-stage experiment are small, it will be difficult to interpret the results in terms of

individual tissue responses. And, therefore, explain the mechanistic processes involved during chromium action on fish. Second, this experiment will provide useful information to interpret effects of chromium on fish in the Hanford Reach. Health parameters used in the laboratory can also be performed on fish collected in the field.

Measurements will be performed to assess physiological impairment caused by chromium. For example, researchers have documented that chromium causes lipid peroxidation (Susa et al. 1996). Lipid peroxidation results in damage to polyunsaturated fatty acids located in the cell membrane. This damage can decrease fluidity, increase leakiness, and inactivate membrane-bound enzymes. An ultimate result may be cell death and tissue damage (Halliwell and Gutterirdge 1985, Wills 1985). Chromium can form intermediates that react with DNA (Outridge and Scheuhammer 1993). These reactions have been associated with DNA damage measured in the form of DNA strand breakage (Aiyar et al. 1990). Therefore, lipid peroxidation and DNA strand breakage will be measured, in addition to histology and tissue metal accumulation, to document physiological impairment during this study.

Eyed eggs will be maintained in a Heath^R incubator at a temperature of 10 $\pm$ 2 °C and hardness of approximately 150 mg $CaCO_3$/L. Mortalities will be documented and removed daily. At hatch, the fish will be moved to flow-through culture tanks with a flow of 4 L/min. The fish will be fed at least a 5% wet weight ration of a commercial biodiet daily. The daily food ration will be split between two feedings.

The experimental phase will begin during the parr stage of fish by randomly distributing 35 fish in each of 12 test chambers receiving experimental water with a flow-through proportional diluter system (Jackson SOP: F.P19). The circular chambers will have a 20-L capacity with dimensions of 43.2 cm X 35.6 cm and a volume of 20,510 cubic cm. The fish will be allowed to acclimate in the experimental chambers for at least five days before the start of the experiment. Thus, the experiment will be conducted for a period of 100 days beginning with parr fish. Eyed embryos, larvae, and parr will be handled so as to minimize stress in accordance with the ECRC-Columbia Animal Welfare Plan and the Region 6 U.S. Fish and Wildlife Service, Fish Health Policy.

Chromium in stock solutions will be delivered to eight test chambers via automatic pipettes (Micromedic Systems AP, Model #25000FW). Two test concentrations of 11 and 24 µg/L chromium (referred to from this point as 11 and 24 will be maintained in each of four replicate chambers. Four chambers without chromium added will be used as controls. Thus, a total of 12 experimental (four control, four with 11 µg/L Chromium, and four with 24 µg/L) units will be maintained. Each chamber will receive 8 L/hr for 10 volume additions per day. Experimental units will be checked daily for mortality and observations on behavior.

At Day 60 and at the termination of the experiment, samples will be collected for fish health measurements. An external necropsy assessment will be made on all sacrificed fish (UW SOP P.1) and lengths and weights will be recorded. One whole fish will be collected from each replicate chamber for measurements of tissue metal accumulation. DNA strand breakage, lipid peroxidation, histological anomalies, and tissue metal accumulation will be evaluated in 2 to 4 fish from each replicate. Gill

lamellae, liver (free of the gall bladder), kidney, and intestine will be removed immediately from the 10 individual fish. Samples for histology will be collected from 2 fish from each replicated chamber and fixed in 10% neutral buffered formalin. It should be noted that spleen and skin samples will also be collected for histological examinations. Samples for DNA strand breakage, lipid peroxidation, and tissue metals will be collected from four fish from each replicate, frozen with liquid nitrogen, and stored at -90°C. At a later date, these samples will be ground with liquid nitrogen and composited by tissue to result in one sample from each replicate chamber. Aliquots of these composites will be measured for DNA strand breakage (Jackson SOP F20.29), lipid peroxidation (Jackson SOP F20.25), and tissue metals.

Samples will be collected from the remaining two fish for additional measurements of DNA strand breakage. For example, both the anterior and posterior portions of the kidney will be sampled to distinguish between effects related to immune functions of the kidney (i.e. anterior section) to effects on the excretory processes (i.e. posterior portion). This information can be used to explain the mechanisms of observed toxicity. These data are also necessary to make comparisons between data gathered from samples of whole, ground tissue and those from specific locations within a tissue. Whole tissue samples would be less labor intensive to collect in field situations. However, it must be documented that this method is sufficiently sensitive to correspond with other toxicological effects. Fish will not be fed for 24 h prior to sampling.

Exposure water will be monitored once per week for dissolved oxygen, pH, alkalinity, hardness, and conductivity (ECRC SOPs B4.14, B4.15, B4.16, and B4.9). More frequent monitoring will be performed if conditions dictate. Samples of exposure water will be taken weekly to monitor total chromium exposure concentrations. One hundred mL samples of exposure water from each treatment will be filtered using a Nalgene® 300 filter holder. Each filtered sample will be transferred to a pre-cleaned, 125 ml I-Chem® polyethylene bottle acidified to 1% HNO_3, and analyzed with ICP-MS (See ECRC SOP C5.212). At each time of total chromium sampling, one additional sample will be extracted from the low, middle, and high chromium exposures and analyzed for speciation of chromium (SOP P.454). For analysis of chromium in tissue, samples will be lyophilized (SOP C5.36), acid digested with microwave heating (SOP C5.94), and analyzed by either ICP-MS (SOP C5.212) or graphite furnace atomic absorption spectrophotometry (SOP C5.163).

4. Listing of Critical Data: **Objective 1: Gather data on chromium-induced physiological responses to aid in the interpretation of growth and survival responses documented during the early life-stage test (Study 2 above).**
Objective 2: To compare laboratory-derived chromium-induced effects on health parameters of chinook salmon with health parameters measured on field-collected salmon from the Hanford Reach of the Columbia river.
Data gathered from these two objectives will include chinook salmon survival, behavior, lipid peroxidation, DNA strand breakage, histology and tissue metal accumulation at chromium concentrations representative of concentrations in locations where salmon spawn.

5. Statistical Treatment: Data will be analyzed by use of Analysis of Variance (ANOVA) followed by Tukey means comparisons test. Percent data will be arc sine transformed before analysis. Statistical significance will be assigned at $P \leq 0.05$. Acceptance or rejection of test results will be determined from statistical analyses and peer review of the methods, data, and results.
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13. Signatures:

Prepared by: _____ Date: _____
Laverne Cleveland
Principal Investigator

Approved by: _____ Date: _____
Edward E. little
Branch Chief

Approved by: _____ Date: _____
Aida Farag
Principal Investigator

Approved by: _____ Date: _____
Daniel F. Woodward
Principal Investigator

Approved by: _____ Date: _____
I. Eugene Greer
Animal Care and Use Committee Chair

Approved by: _____ Date: _____
Paul R. Heine
Quality Assurance and Safety Officer

Approved by: _____ Date: _____
Wilbur L. Mauck
Center Director

18.0. Appendix B. Curriculum vitae of ECRC research and administrative/management personnel

Resume
Midwest Science Center
June 9, 1997

Name: Robin D. Hurtubise _____ _____
Signature Date

Born: Yokosuka, Japan 09-14-69

Education:

Institution	Degree	Date
Southwest Missouri State University	B.S. Biology	May 1991
Southwest Missouri State University	M.S. Biology	May 1996

Training:	Course	Length	Sponsor	Date
Boater Safety Training		12 hrs.	Midwest Science Center	Summer 1996

Experience:

Biologist, Midwest Science Center, USGS, Columbia, MO. May 1997-present.

Research Science Technician, Midwest Science Center, NBS, Columbia, MO January 1993-present

Graduate Research Assistant, SMSU, Springfield, MO August 1994-June 1995.

Graduate Teaching Assistant, SMSU, Springfield, MO August 1993-June 1994.

Physical Science Technician, National Fisheries Contaminant Research Center, U.S. Fish and Wildlife Service, Columbia, MO August 1991-December 1992.

Publications:

M.S. Thesis. The Effects of Ultraviolet-b Radiation on Aquatic Invertebrates.

Hurtubise, R.D., E.E. Little, and J.E. Havel. Methods for Assessing the Impacts of UV-B Radiation on Aquatic Invertebrates. ASTM 1997. (In Review).

Hurtubise, R.D., J.E. Havel, and E.E. Little. The effects of ultraviolet-B radiation on freshwater invertebrates: Experiments with a solar simulator. Limnology and Oceanography. (In Review).

RESUME

National Fisheries Contaminant Research Center

March 18, 1997

Name: Brad G. Mueller _____ _____
Signature Date

Born: St. Louis, MO April 16, 1964

EDUCATION:

Institution	Degree	Date
University of Missouri-Columbia	B.S., Fisheries, Wildlife	1987

TRAINING:

Course	Credit hrs	Sponsor	Date
Fish Physiology	2	University of MO	1989
Limnology	3	University of MO	1989
Aquatic Toxicology	2	University of MO	1990
Aquatic Entomology	3	University of MO	1991
Chironomid Identification	0	University of MO	1993
Hydrolab Training course	0	Hydrolab (Austin Tx)	1994
Boat safety	0	MSC	1994
Hazmat Training	0	MSC	1994
Boat Operator training	0	MSC	1996

Biological Technician, National Fisheries Contaminant Research Center, Columbia, MO, 1988 to present.

Lab Technician, Virginia Polytechnic and State University, Blacksburg, VA, 1987 to 1988.

Fisheries Technician, Missouri Department of Conservation, Sedalia, MO, 1987.

PUBLICATIONS:

RESUME
Midwest Science Center
January 10, 1997

Name: Aaron J. DeLonay

Signature Date

Born: Wausau, WI June 5, 1965

EDUCATION:

Institution	Degree	Date
University of Wisconsin-Stevens Point	B.S., Water Resources Aquatic Biology	1987
University of Missouri-Columbia	M.S., Fisheries & Wildlife	1991

TRAINING:

Course	Length	Sponsor /Date
Standard First Aid	4 hours	American Red Cross / 1991
Adult CPR	4 hours	American Red Cross /1992
Driver Safety Training	8 hours	Columbia Safety Council/1992
Natural Resource Damage Assessment	3 days	U.S. FWS/1994
Endangered Species Training	8 hours	U.S. FWS/1996
Boater Safety Training	3 days	U.S. DOI (MSC)/1996

EXPERIENCE:

Ecologist, Population Ecology, Midwest Science Center, Columbia, MO, 1993 to present.

Ecologist, Behavioral Toxicology Section, National Fisheries Contaminant Research Center, U.S. Fish & Wildlife Service, Columbia, MO, 1990 to 1993.

Graduate Research Assistant, University of Missouri, Columbia, MO, 1988 to 1990.

Research Aide, University of Wisconsin-Stevens Point, Stevens Point, WI, September to December 1987.

Fisheries Intern, Northern Highland Fisheries Research Area, Wisconsin Department of Natural Resources, Woodruff, WI, May to August 1987.

Teaching Assistant, UW-Stevens Point Treehaven Field Station, Tomahawk, WI, May to August 1986.

PUBLICATIONS: Author or co-author of 12 publications.

RESUME
Midwest Science Center
February 25, 1997

Name: William G. Brumbaugh _____ _____
Signature Date

Born: Painesville, Ohio January 28, 1956

EDUCATION

Institution	Degree	Date
University of Missouri - Columbia	B.S., Chemistry	1978
University of Missouri - Columbia	M.S., Analytical Chemistry	1983
University of Missouri - Columbia	PhD, Analytical Chemistry	1997

TRAINING

Course	Hours	Sponsor	Date
Statistics	6	NFCRC	5/80
Writing Position Descriptions	6	OPM	6/81
Trace Analysis	3 cred.	UMC/NFCRC	9/81
Environmental Chemistry	3 cred.	UMC/NFCRC	9/83
EEO Training	3	OPM	3/84
Advanced Graphite Furnace AA	16	Perkin-Elmer	7/84
Technical Writing	24	Shipley	3/89
Multiculturism	6	OPM	6/91
Total Quality Management	16	OPM	5/92
Environmental Chemical Analysis	3 cred.	UMC/MSC	9/94
Supramolecular Chemistry	3 cred.	UMC/MSC	1/95
Mass Spectrometry Topics	3 cred.	UMC/MSC	9/95
Organic Spectroscopy Topics	3 cred.	UMC/MSC	1/96

EXPERIENCE:

Leader, Inorganic Chemistry Methods Development, Midwest Science Center, Columbia, Mo, 1990 to present.

Research Chemist, Midwest Science Center (formerly the National Fisheries Contaminant Research Center), Columbia, MO, 1984 to 1990.