

# The Potential for Contaminated Ground Water to Adversely Affect Chinook Salmon (*Oncorhynchus tshawytscha*) under Exposure Conditions Simulating the Hanford Reach of the Columbia River, Washington, USA

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## ABSTRACT

The Hanford Reach of the Columbia River is a free flowing stretch that exists within the Hanford Nuclear Reservation in south central Washington, USA. Operations at the reservation have resulted in contamination of ground water with chromium and other chemical and radiological constituents. Ground water discharges into the Columbia River occur in areas where chinook salmon (*Oncorhynchus tshawytscha*) frequently spawn and may influence fertilization, egg survival, and health of alevins and parr. This paper describes the problem and outlines the framework for research designed to determine the potential for chromium to adversely affect chinook salmon. This research is to be performed by the Biological Resources Division from October 1998 to December 1999.

## INTRODUCTION

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 tshawytscha*). 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 and others, 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 Hanford site continues to be contaminated with chemical and radiological constituents (Geist and others, 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 and others, 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 and others, 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 and others 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 3-4gm in weight (Olson and Foster, 1956).

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. 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 hexavalent 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.

The early life stages of chinook salmon are most likely to come in constant contact with elevated chromium and these stages have been shown to be the most sensitive to contaminants (McKim, 1977). Chromium may hamper fertilization success by directly acting on the fertilized egg to cause death of the embryo (Billard and Roubaud, 1985). Or chromium may react with the sperm and egg individually to impede fertilization. If fertilization is successful, chromium may affect the survival of early lifestages (Olson and Foster, 1956; Benoit, 1976). While it has been documented that elevated concentrations of chromium reduce survival (Buhl and Hamilton, 1991), and to a lesser extent, growth (Olson and Foster, 1956; Benoit, 1976), information has not been gathered on the relevance of recovery periods on these toxicological effects. In the Hanford Reach, chromium that moves from the ground water upwellings becomes diluted extensively. Thus, as young fry begin to emerge from the redds, they may no longer be exposed to elevated concentrations of chromium. The effects of chromium exposure to alevins, as monitored by post-exposure recovery of fry during later development, will mimic the exposure situation present in the Hanford Reach.

Chinook salmon will be present in the Hanford Reach for 5-7 months, and it is important to understand health effects as related to chromium exposure. It is unclear what the exposure concentration might be through contaminated surface water or diet, but long-term health effects from continuous exposure is not well understood in either early life or parr stages (Geist and others, 1994). An understanding of the physiological responses (pathology) associated with chromium exposure can be used to supplement fish population or water and sediment monitoring. Evaluations based on the residue concentrations and physiological condition (e.g.

increased lipid peroxidation) of fish integrate the actual exposure to pollutants (dose) and effects of these exposures on fish survival and growth (Farag and others, 1994; Farag and others, 1995). Further associations of tissue chromium accumulation, oxidative stress, and growth reduction would add more weight to a determination of fish health impairment. This weight of evidence approach uses all of the information gathered to determine the health status of a fish population.

We will meet two objectives by investigating the health status of salmon exposed to chromium during the early and parr stages. First, the effects of chromium on survival and growth of chinook salmon will be explained in terms of the health status of individual fish. Additionally, tools can be developed in the laboratory under controlled conditions, that can later be used to assess the health of fish in the Hanford Reach.

The objectives of this proposed 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. This objective will be accomplished in three tasks: Task 1, (Fertilization), the potential for chromium to adversely affect gametes and their fertilization in chinook salmon; Task 2, (Early Life Stage), determine the effects of chromium on the early development of chinook salmon; Task 3, (Fish Health), determine degree of fish health impairment of chinook salmon exposed to chromium.

## **EXPERIMENTAL PROCEDURES**

### **General**

Experimental water will simulate that of the Columbia River surface and pore water in the Hanford Reach and 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<sub>3</sub>; 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 and others, 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) and the State of Washington. 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.

Gametes and 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 performed 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 and others, 1995).

### **Task 1: Fertilization**

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 the following 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 and others, 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<sub>3</sub>; 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.

## **Task 2: Early Life Stage**

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. 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 the following times: after hatching, mid-way through yolk absorption, at swim-up, and at 30-days post swim-up. This fish will be 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 at swim-up and at 30-days post swim-up for histological analyses. At the end of the exposure and at 30 days post swim-up all surviving alevins in each treatment will be measured for total length and weighed to determine growth.

### **Task 3: Parr Health**

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 lifestage experiment. Because fry at the end of the early lifestage 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 and others, 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 Gutteridge, 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 and others, 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<sup>R</sup> incubator at a temperature of  $10 \pm 2^{\circ}\text{C}$  and hardness of approximately 150 mg  $\text{CaCO}_3/\text{L}$ . 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. 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  $\mu\text{g}/\text{L}$  chromium (hereafter, referred to 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  $\mu\text{g}/\text{L}$  Chromium, and four with 24  $\mu\text{g}/\text{L}$ ) 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 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, lipid peroxidation, 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.

### **Analysis of Water and Tissue (All Tasks)**

Exposure water will be monitored once per week for dissolved oxygen, pH, alkalinity, hardness, and conductivity. 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 Nalgene7 300 filter holder. Each filtered sample will be transferred to a pre-cleaned, 125 ml I-Chem7 polyethylene bottle, acidified to 1% HNO<sub>3</sub>, and analyzed with ICP-MS. At each time of total chromium sampling, one additional sample will be extracted from the low, middle, and high chromium exposures for analysis of Cr(VI) (hexavalent chromium). The Cr(VI) water samples will be filtered as above, then immediately put on ice and shipped by overnight carrier (or hand delivered) to the analytical laboratory. Upon receipt, the analytical laboratory will immediately conduct extraction or ion-exchange separation of the Cr(VI) species. The treated sample containing only the Cr(VI) will then be acidified to 1% HNO<sub>3</sub> for analysis by ICP-MS or graphite furnace atomic absorption spectrophotometry. For analysis of chromium in tissue, samples will be lyophilized, acid digested with microwave heating, and analyzed by either ICP-MS or graphite furnace atomic absorption spectrophotometry.

## **RESULTS AND INTERPRETATION**

The data from these experiments will be used to determine the toxicity of chromium to fertilization, alevin survival, and to health of alevins and parr of chinook salmon. The results will be compared with environmental concentrations of chromium in the Columbia River to evaluate the potential for chromium to adversely affect salmon. This research is to be performed by the Biological Resources Division from October 1998 to December 1999.

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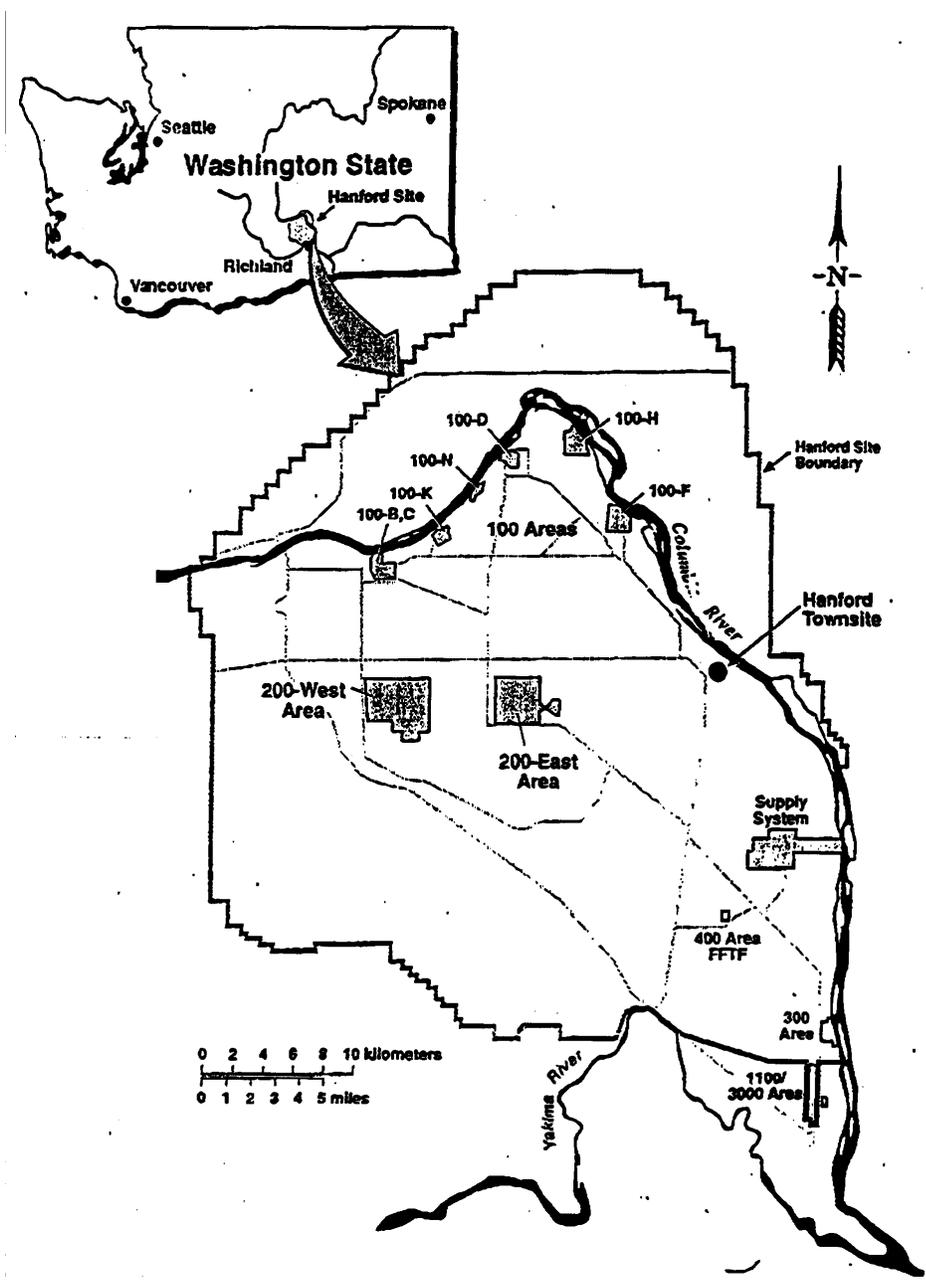


Figure 1. Map of the Hanford Reach of the Columbia River