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The Potential for Chromium to Affect the Fertilization Process of Chinook Salmon (*Oncorhynchus tshawytscha*) in the Hanford Reach of the Columbia River, Washington, USA

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Abstract. The Hanford Nuclear Reservation in south central Washington was claimed by the federal government as a site for the production of plutonium. During the course of production and operation of the facilities at Hanford, radionuclides and chromium were discharged directly into the river and also contaminated the groundwater. This study was designed to assess the effects of chromium (Cr) on Chinook salmon (*Oncorhynchus tshawytscha*) fertilization under exposure conditions similar to those of the Hanford Reach of the Columbia River. Chinook salmon gametes were exposed to aqueous Cr concentrations ranging from 0 to 266 μg Cr l^{-1}. The current ambient water-quality criteria (AWQC) established for the protection of aquatic life (United States Environmental Protection Agency [USEPA] 1986) is 11 μg Cr l^{-1}. Cr has been measured in pore water from bottom sediments of the Columbia River at concentrations >600 μg Cr l^{-1}. Under exposure conditions designed to closely mimic events that occur in the river, the fertilization of Chinook salmon eggs was not affected by concentrations of Cr ranging from 11 to 266 μg Cr l^{-1}. Data suggest that the instantaneous nature of fertilization likely limits the potential effects of Cr on fertilization success. As a result, the current AWQC of 11 μg Cr l^{-1} is most likely protective of Chinook salmon fertilization.

Chromium (Cr) may hamper fertilization success by directly acting on the fertilized egg to cause death of the embryo, or it may react with the sperm and egg individually to impede fertilization (Billard and Roubaud 1985). Salmonid sperm are inactive in the testes and only become activated when the milt is mixed with water. Once activated, the sperm experience a period of vigorous motility that lasts for approximately 15 to 20 seconds (Erhdall 1994). After the initial activity, the motility becomes less, and sperm are more vibratory for an additional 1 to 2 minutes (Brown 1957; Terner 1986; Cosson *et al.* 1985; Ehrdahl 1994). As a result, the recommended procedure for fertilization used by fish culturists is to allow contact of sperm and egg for 1 minute after the addition of an activating medium such as water (Erdall 1994).

Chinook salmon in the Hanford Reach of the Columbia River spawn in deep and fast-velocity water. Groves and Chandler (1999) reported that redds of fall Chinook salmon were located in 0.2 to 6.5 m depth with substrate velocities of 0.1 to 2.0 m/s. The depth of the substrates, coupled with spawning behavior where substrate are disturbed during redd preparation, could potentially expose eggs and sperm to Cr present in the pore water. This study was designed to assess the effects of Cr on Chinook salmon fertilization under exposure conditions similar to those of the Hanford Reach of the Columbia River.

The Hanford Nuclear Reservation in south central Washington is a 900-km^2 area claimed by the federal government in 1943 as a site for the production of plutonium (Geist 1995). Because of national security concerns, public access and river development projects were restricted until 1971 (Geist 1995). The 90-km section within the Hanford Reservation was not developed. 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). Although upstream dams regulate flows within the Hanford Reach, it is the last unimpounded stretch of the mainstem Columbia River. As a result, the use of the Hanford Reach for fall Chinook salmon spawning and rearing has dramatically increased since 1960 (Dauble and Watson 1990; Becker 1985). The 10-year average adult escapement increased from 27,660 (1964 to 1973) to 54,661 (1983 to 1992). This increase is pronounced compared with the remainder of the mid- and upper Columbia River where Chinook salmon runs have decreased during the same time period (Schaller *et al.* 1999).

During operation of the Hanford facilities, large quantities of Columbia River water were used to cool nuclear reactors, and cooling water was treated with sodium dichromate to prevent corrosion, biofouling, and mineral collection within the pipes (Peterson *et al.* 1996). During operations, cooling water with associated radionuclides and Cr were discharged directly to the river and also entered ground water through

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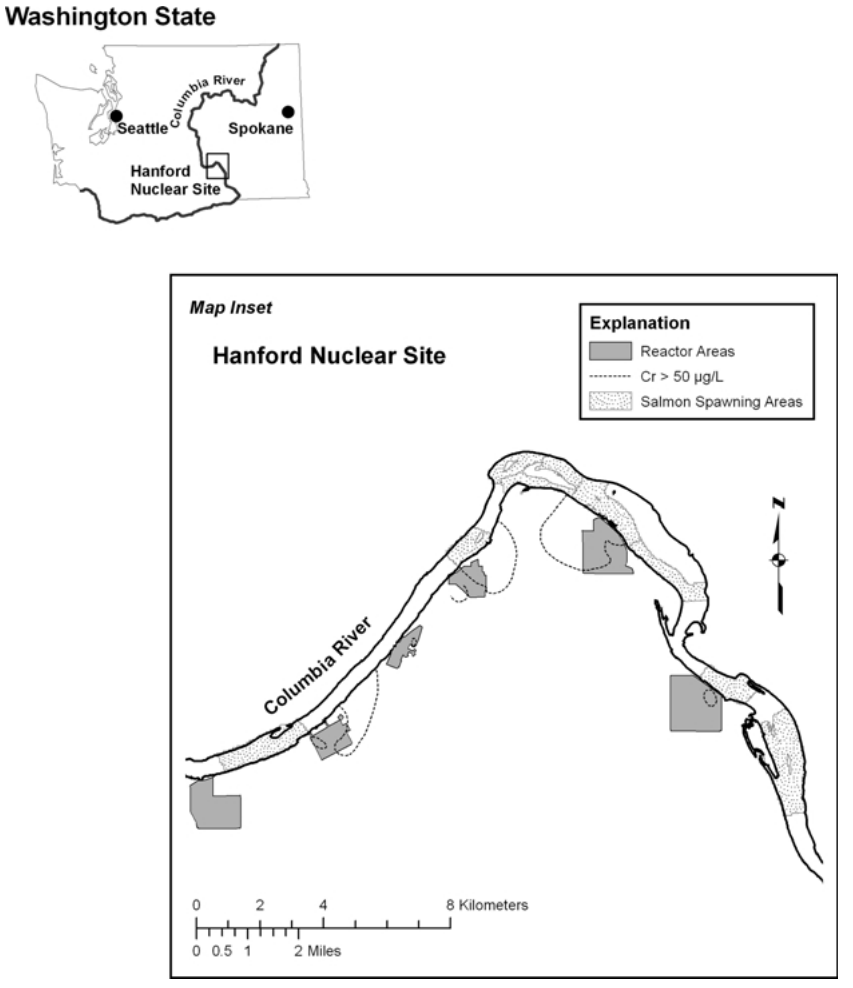

Fig. 1. Map of the Hanford Reach of the Columbia River, Washington. Crosshatched areas within the river indicate locations of Chinook salmon spawning redds. Groundwater plumes with levels of chromium exceeding 50 µg l^{-1} are indicated by hatched contour lines. Shaded polygons show the locations of nuclear reactor areas.

leakage of pipes and seepage from retention areas (Fig. 1). Peak aquifer discharges occur during low river flows in fall and winter, and minimum aquifer discharges occur during high river flows in spring and summer (Geist *et al*. 1994). Adult Chinook salmon spawn in variable water depths, water velocities, and substrate types (Swan *et al*. 1988) in close proximity to the areas where contaminated ground water is entering the river (Fig. 1). Spawning in the Hanford Reach begins in mid-October, peaks in mid-November, and ends in late November (Dauble and Watson 1997). Redd counts conducted during the last several decades indicate that the majority of Chinook salmon spawning occurs throughout only a few kilometers of river in the Hanford Reach (Dauble and Watson 1997). This intensive habitat use in proximity to contaminated plumes of groundwater strongly suggests that Chinook salmon are at risk of exposure.

Cr associated with groundwater and seeps is a contaminant of major concern. The concentrations of Cr in the groundwater upwellings (Hope and Peterson 1996) exceed the AWQC of 11 µg Cr l^{-1} for the protection of aquatic life as established by the USEPA (1986). Hexavalent Cr [Cr(VI)] concentrations ranging from nondetectable to >600 µg l^{-1} have been measured in pore water samples collected from the 100 Areas (Hope and Peterson 1996). The Department of Energy currently has activities underway to pump and treat Cr at the Hanford facility and to decrease the amounts of Cr(VI) released into the Hanford Reach. However, the critical nature of the Hanford Reach as a spawning habitat for the Chinook salmon makes it essential to determine the potential for Cr in the groundwater to adversely impact Chinook salmon. Although some data exist on the effects of CR(VI) on salmon (Olson and Foster 1956; Buhl and Hamilton 1991), previous studies did not investigate the direct effects on fertilization.

Materials and Methods

Experimental water was formulated to simulate Columbia River surface and pore water in the Hanford Reach as well as conditions known to be associated with the location of spawning redds (Hope and Peterson 1996), and unless otherwise indicated, was used throughout the experiment. The water was prepared by blending laboratory well water with deionized water produced by reverse osmosis. Experimental water produced in this way eliminated the use of surface water and removed the potential for fish pathogens to be introduced into the experiment and influence test results. Water for the fertilization exposure during water hardening was produced in 4–l batches. Experimental water was adjusted to a range of hardness of 78 to 82 mg l^{-1} as $CaCO_3$ at a pH of 7.7 to 7.8, alkalinity of 82 to 83 mg l^{-1} as $CaCO_3$, and conductivity of 161 to 170 µh which are values consistent

Table 1. Mean percent fertilization SE of Yellowstone cutthroat trout and Chinook salmon[a]

Yellowstone cutthroat			Chinook salmon		
Chromium (μg l^{-1})	*N*	% Fertilization	Chromium (μg l^{-1})	*N*	% Fertilization
0	8	47.9[a] (5.7)	0	4	66.7[a] (2.3)
11	4	54.9[a] (4.2)	11	4	67.2[a] (3.1)
24	4	50.3[a] (7.4)	24	NS	NS
54	4	51.4[a] (5.7)	54	NS	NS
120	4	39.0[a] (5.9)	120	4	62.6[a] (2.8)
266	4	51.6[a] (2.7)	266	4	69.1[a] (1.1)

[a] The eggs and sperm were exposed to various concentrations of hexavalent Cr during fertilization, and the eggs were hardened in water that contained similar concentrations of Cr. Means within columns with the same letter are not significantly different (ANOVA, followed by Tukey means comparisons, $p \leq 0.05$).
ANOVA = Analysis of variance.
NS = no sample.

Table 2. Measured concentrations of total Cr and Cr (VI) in the water used to expose eggs and sperm to Cr (VI) during fertilization

Yellowstone cutthroat			Chinook salmon		
Nominal Cr (μg l^{-1})	Chromium 1% NaCl solution (μg l^{-1})	Cr in exposure water	Nominal Cr (μg l^{-1})	Chromium 1% NaCl solution (μg l^{-1})	Cr in exposure water
Total Cr					
0	1.1	<0.5	0	1.6	3.0
11	11.5	10.2	11	12.9	13.9
24	21.4	23.4	24	25.7	26.8
54	55.2	51.2	54	53.0	55.4
120	126.0	117.0	120	113.0	117.0
266	259.0	279.0	266	261.0	251.0
Cr (VI)					
120	135	130			

Cr (VI) = hexavalent chromium.

with Columbia River conditions. The exposure waters were analyzed to ensure that quality was within 5% of the nominal experimental values in terms of hardness, alkalinity, conductivity, and pH. Experimental water temperatures were 10°C to 13°C and were slightly lower than the seasonal conditions of 14°C to 18°C documented during October and November in the Columbia River (Hope and Peterson 1996). The photoperiod was adjusted to simulate time of year of exposure.

Cr exposure concentrations ranged from 0 to 266 μg l^{-1}. These concentrations were above and below the chronic AWQC for chromium of 11 μg l^{-1} (USEPA 1986) and were representative of concentrations in pore water sampled from the intergravel substrates in locations where salmon spawn (Hope and Peterson 1996).

Gametes of fall-run Chinook salmon were obtained from the McNenny State Fish Hatchery in Spearfish, SD. Salmon from the McNenny Hatchery were selected for use in this study because Chinook salmon are a species of concern in the Hanford Reach where they are exposed to significant concentrations of Cr as well as several other contaminants during development and residence in the Reach (Geist *et al.* 1994). The potential effects of these contaminants on Chinook salmon are unknown. A history of preexposure to environmental contaminants of test organisms captured on-site could potentially bias or confound test results (American Society for Testing and Materials 2000a). Selection of a fall-run Chinook salmon stock from the McNenny Hatchery eliminated the potential confounding effects caused by possible pre-exposure. Also, evolutionary adaptation has resulted in stocks of salmon that exhibit distinct differences in life history and reproductive site fidelity. These ecologic adaptations would not likely result in significant differences in the tolerance or sensitivity to anthropogenic contaminants, such as Cr(VI) of relatively recent origin (Mayer and Ellersieck 1986). Evidence of adaptation to environmental concentrations of Cr may constitute a biologic effect of exposure and result in altered viability of natural populations. Finally, adult brood fish from the McNenny Fish Hatchery were examined and tested for disease and parasite infection during spawning, and the eggs are certified disease free before experimentation. The disease-free status is essential to assure that toxicity experiments are performed on healthy organisms, increases reliability of results, and is a recommended standard procedure (ASTM 2000b). Yellowstone cutthroat trout (*Oncorhynchus clarki* spp.) were used as a surrogate species during additional fertilization experiments and were also certified as disease free.

Statistical analyses were performed with SAS software version 6.11 (SAS, Cary, NC), SYSSTAT (SPSS, Chicago, IL), and Toxstat V3.5 (Western EcoSystems Technology, 1996, 2003 Central Avenue, Cheyenne, WY). Multiple analysis of variance followed by Tukey means comparisons was performed on all data that meet the assumptions of homogeneity and normality. Percent fertilization was the dependent variable. Four replicates were tested in each experiment (unless otherwise indicated).

Gametes were taken from Yellowstone cutthroat brood stock at the Jackson National Fish Hatchery during May 1999 and from the Chinook salmon brood stock during October 1999. These are normal times for gametogenesis in spring spawning cutthroat trout and fall spawning Chinook salmon. The stocks were checked weekly for ovum and sperm formation before the experiments. Pooled sources of eggs and sperm were obtained from 7 female and 8 male cutthroat trout and from 3 female and 10 male Chinook salmon.

A standard 1% NaCl (saline) solution was used to maintain active ova and sperm during the fertilization experiment. Six treatment

concentrations of Cr–0, 11, 24, 54, 120, and 266 μg Cr l^{-1}–were prepared in 1% NaCl. Each treatment was replicated four times for a total of 24 treatments for cutthroat trout. Four treatment concentrations were used for Chinook salmon (0, 11, 120, and 266 μg Cr l^{-1}) for a total of 16 treatments. Ova and sperm were mixed for 1 minute, rinsed, and allowed to water harden.

Thirty ml ova (300 to 400) were added to a 15.2-cm diameter plastic fertilization container. Then 1 ml sperm and 30 ml 1% NaCl containing the appropriate Cr concentration was added to the fertilization container. The mixture was swirled gently for 1 minute (Erdahl 1994) to allow fertilization. We used a 1% NaCl solution rather than physiologic saline as described in previous studies because the latter was a more difficult matrix to analyze for Cr, particularly for the separation of the hexavalent species by cation exchange. During 1998, we compared fertilization success in 1% NaCl solutions and physiologic saline solutions and found no difference (unpublished data).

After the fertilization process, the eggs were rinsed 3 times with 25 ml Hanford experimental water with the appropriate concentration of Cr. Then the eggs were allowed to harden in Hanford experimental water with Cr for 1.5 hours according to the procedures of Piper *et al.* (1982). Water hardening 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. After water hardening, the eggs were rinsed 3 times with 80 ml Hanford experimental water lacking Cr and transferred into incubators.

Eggs from the cutthroat trout were incubated at the USGS Jackson Field Research Station in water with a temperature of 10°C, hardness of 158 mg/L as $CaCO_3$, alkalinity of 150 mg l^{-1} as $CaCO_3$, and pH of 7.3. Eggs from Chinook salmon were incubated in the McNenny Hatchery water at a temperature of 11°C, hardness of 360 mg l^{-1} as $CaCO_3$, alkalinity of 210 mg l^{-1} as $CaCO_3$, and pH of 7.6. After 1 week, the eggs were cleared in 10% acetic acid solution for at least 2 minutes and percent fertilization was determined. The embryos of fertilized eggs turn opaque white and become visible through the translucent chorion. By day 10, the embryos developed a definite optic lobe with an elongated somite and could be easily distinguished from an unfertilized germinal disk.

Treatment water was monitored for pH, alkalinity, hardness, and conductivity. One hundred ml water was taken from each treatment to monitor total Cr exposure concentrations. These water samples were filtered with a 0.45-μm polycarbonate filter using a Nalgene 300 filter holder. Each filtered sample was transferred to a precleaned, 125-ml I-Chem polyethylene bottle, acidified to 1% HNO_3, and analyzed by inductively coupled plasma-mass spectrometry (ICP-MS). Each time water samples were taken for total Cr analyses, one additional sample was taken from the low-, middle-, and high-Cr treatments for analyzes of Cr(VI). For these analyses, the sample was buffered to pH 5 and passed through a strong cation-exchange resin (AG-50W-X8) that sequestered only the Cr(III) species, which after buffering was assumed to be present as $Cr(OH)_2^+$ (Cranston and Murray 1978). The eluent containing the Cr(VI) was then acidified to 1% HNO_3 and analyzed by ICP-MS for total Cr. The samples collected for Cr(VI) analysis that contained 1% NaCl were diluted five-fold with deionized water before buffering to prevent overloading of the cation-exchange column by sodium ions. In addition, all samples containing 1% NaCl were digested with nitric acid and microwave heating in open vessels to decrease chloride concentrations for ICP-MS analysis.

Results

When added to the water column, Cr did not alter fertilization at concentrations equal to the water-quality criteria (11 μg Cr l^{-1}) established for the protection of aquatic life (USEPA 1986). Additionally, Cr did not affect salmon fertilization at concentrations up to 266 μg l^{-1}. No significant differences in percent fertilization were observed among the treatment and reference groups (Table 1). Fertilizations responses to Cr were similar for Yellowstone cutthroat trout and Chinook salmon.

Measured concentrations of total Cr and Cr(VI) were generally within 10% of the nominal concentrations (Table 2) and duplicate analyses were within ± 20%. The general agreement of Cr(VI) results with the nominal concentrations confirmed that the majority of Cr remained in the Cr(VI) form. Percent recoveries of reference solutions and digestion spikes were ≥ 97%. For speciated samples, recovery of a Cr(VI) spike was 100%, whereas recovery of Cr(III) spike was virtually 0%, which indicates quantitative elution of Cr(VI) and sequestration of Cr(III) by the cation exchange resin.

Discussion

Cr concentrations ranging from 11 to 266 μg l^{-1} did not affect the fertilization process of Chinook salmon and Yellowstone cutthroat trout. Therefore, the AWQC of 11 μg Cr l^{-1} (the current target of cleanup efforts in the Hanford Reach of the Columbia River) would most likely protect Chinook salmon fertilization. The ova of rainbow trout (*Oncorhynchus mykiss*) are less sensitive to Cr exposure than sperm (Billard and Roubaud 1985); however; fish sperm begin to die soon after release and are washed away quickly by river currents. Because of the instantaneous nature of fertilization, the limited contact of sperm with the exposure water likely limited the effects of Cr in the water column on fertilization success.

Our results differ from fertilization results obtained by Billard and Roubaud (1985), who documented that 5 μg Cr l^{-1} decreased fertilization percentages in rainbow trout. These different results may be related to the different species and methodologies used by Billard and Roubaud (1985) relative to the present study. The time allowed for exposure to Cr during fertilization was 1 minute during the current study versus 15 minutes for Billard and Roubard (1985). The shorter time used in the current study more closely mimicked fertilization events that may occur under river conditions where velocities of the water at the substrate are fast (Groves and Chandler 1999) and motility of sperm is short-lived (Erhdahl 1994). Furthermore, the ova were held in exposure water for 1.5 hours after fertilization during the current study to more closely mimic natural conditions in which eggs continue to absorb water for approximately 1.5 hours after fertilization. The ova were not exposed to Cr during water hardening in the study performed by Billard and Roubard (1985). Additionally, the fertilization process of the rainbow trout tested by Billard and Roubard (1985) may have been more sensitive to Cr exposure than the cutthroat trout and Chinook salmon tested in the present study. We are unaware of any literature that investigates these possible differences. However, we observed no difference in fertilization success with cutthroat trout, a species of salmonid closely related to rainbow trout.

Based on the results of the present study, aqueous Cr concentrations up to 266 μg l^{-1} in the Hanford Reach of the Columbia River do not appear to represent a significant hazard directly to the fertilization process of Chinook salmon. However, Cr concentrations ranging from nondetectable to

>600 μg l^{-1} have been measured in pore water samples collected from the 100 Areas (Hope and Peterson 1996). Thus, potential effects of these higher Cr concentrations on the fertilization process of Chinook salmon could also be evaluated. We found results similar to the present study during additional experiments with gametes of cutthroat trout exposed to concentrations of Cr as high as 1010 μg l^{-1} for 1 minute. However, the eggs were only exposed to 120 μg Cr l^{-1} for the 1.5-hour water-hardening portion of the experiment. Because of this difference between the fertilization and water-hardening exposure concentrations, these results are discussed as anecdotal support only, and the data are not listed.

The lack of fertilization effects observed in concentrations of 266 μg Cr l^{-1} during the current study, together with the anecdotal evidence of no effects at 1010 μg Cr l^{-1} (although water-hardening concentrations were much lower), suggest that concentrations of Cr in the Hanford Reach of the Columbia River will not directly affect gametes of Chinook salmon or alter fertilization success in the Reach.

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