A Comparison of Toxicology Studies of Vulnerability of Early Life Stages of Salmon to Hexavalent Chromium at Hanford-14140

Michael Gochfeld * and Joanna Burger **,

*Rutgers--Robert Wood Johnson Medical School, Environmental and Occupational Health Sciences Institute and Consortium for Risk Evaluation with Stakeholder Participation, Piscataway, New Jersey 08854

** Rutgers University, Division of Life Sciences, 604 Allison Road, Environmental and Occupational Health Sciences Institute and Consortium for Risk Evaluation with Stakeholder Participation, Piscataway, New Jersey 08854-8082

ABSTRACT

Laboratory and field ecotoxicology studies clarify levels of pollutants that adversely affect organisms at various life stages. We examined three well-controlled laboratory studies of hexavalent chromium (Cr-VI) toxicity to fertilization, eggs and juvenile Chinook Salmon (*Oncorhynchus tshawytscha*), to identify effect levels for several endpoints. These studies are relevant to Cr-VI contamination of ground and surface water. At the Department of Energy's Hanford Site, ground water with chromium flows towards the Columbia River, upwelling through the gravel (pore water) into the river, posing a potential threat to biota, including the eggs and newly hatched salmon. Three ecotoxicological studies assessed fertilization and hatching [1], and pathophysiology, growth, and survival of alevin [2] and parr [3] eggs and young salmon. Farag et al. [1,3] used salmon eggs from a remote hatchery to avoid any possible evolutionary adaptations to chromium and used deionized water spiked with chromate to minimize effects of other ground water constituents. Patton et al. [2] used salmon eggs from nearby Priest Rapids hatchery to incorporate possible adaptation to historic exposures, and used chromate-rich Hanford ground water diluted with Columbia River water to achieve natural exposure conditions. Both choices were justifiable, but since the three studies tested different life stages, the methodological implications for toxicology and exposure, have not been tested on a particular life stage. Farag et al. [1] studied fertilization and hatchability on the one hand and free-swimming parr on the other, while Patton et al. tested the alevin stage, during which the newly hatched fish remain in gravel exposed to pore water, and do not feed. Fertilization, hatching, and alevin survival were not reduced at 260-266 µg/L (ppb) of Cr-VI in the water (=NOAEL for eggs and alevins). Older fish (parr) had metabolic and histologic changes of uncertain significance at levels as low as 24 ppb, but this stage does not encounter levels above 10 ppb in the rapidly flowing river water. LOAELs for some endpoints were 100-120 µg/L. At 266 µg/L,

survival was significantly reduced for parr (97% to 70%), but there was no survival effect for the alevin. These results suggest that Cr-VI in Hanford pore water are too low to impact Chinook Salmon populations. Funded by the DOE through a contract to Consortium for Risk Evaluation with Stakeholder Participation (CRESP-DE-FG-26-00NT-40938).

INTRODUCTION

Salmon are iconic wildlife species of the Pacific Northwest. Salmon, wild and farmed, rank third in the United States per capita seafood consumption, behind shrimp and canned tuna [4]. The several species of wild salmon spawn in the gravel beds of freshwater streams and rivers, requiring clear, well-oxygenated flows. After hatching the juvenile salmon pass through several stages (alevin, fry, parr, smolt). The young salmon (as parr) migrate downstream to estuaries and, as smolt enter the sea. After several years maturing at sea, the adults return to spawn in their natal streams. Wild salmon populations support huge commercial and recreational fisheries, and salmon are a vital and historic part of the culture and diet of Northwestern Tribes [5]. Salmon populations in the Columbia River have fluctuated dramatically over the past 70 years [6], with an overall decline [7]. Salmon populations have been heavily impacted by fishing, by dams that interfere with upriver and down river migration [8], and by development diminishing suitable spawning sites. There is also concern about the possible negative impact of environmental contaminants on the health of individual salmon and salmon populations.

Chinook (or King) Salmon (*Oncorhynchus tshawytscha*) populations and 'runs' in various rivers are listed as endangered or as a species of special concern. The species has a complicated and variable life cycle [9]. Adult fish spend several (up to 8) years at sea, before migrating upriver to spawn. The life stages include eggs laid in gravel nests called redds, alevin that remain in the gravel for weeks, becoming fry after "swim-up". Fry become parr after about 10 weeks, and finally smolt that enter the sea [9]. Eggs take up to three months to hatch. Alevins (recently-hatched fish) remain in the gravel for several weeks, absorbing their yolk sac. They do not eat. Fry are young fish that have just emerged from the redds after "swim-up"; they eat voraciously. Parr grow rapidly through the fingerling stage, and most migrate downriver to the estuaries.

The Columbia River is subject to natural seasonal variation in water height and current velocity, which is significantly modified by water release practices at the dams [8]. The Hanford Reach (about 80 km) between Priest Rapids Dam and Richland city, is the largest "natural" stretch remaining on the Columbia River, and is an important spawning ground for the fall run Chinook Salmon. The nine deactivated plutonium-production reactors are arrayed along the Hanford Bight (the northernmost point of the Hanford Reach). Here, hexavalent chromium (Cr-VI), formerly used to control corrosion in the reactor cooling water, is now a contaminant of ground

water, with several documented plumes. As the Cr-VI groundwater plumes move toward the Columbia River they reach the gravel riverbed. Some of the ground water seeps out along the shoreline under low-flow conditions, and some occurs as upwellings through the gravel riverbed (hyporheic zone). The continued movement of the Cr-VI plume eventually enters the free-flowing river where it is rapidly diluted and carried downstream.

The key aspects of the Chinook Salmon life history that makes it vulnerable to chemicals in groundwater (including chromium) are the time spent in the gravel as eggs or alevin (exposed to groundwater upwellings called 'pore water'), and the time spent as fry or parr probably feeding close to the gravel, where they might be exposed to chromium in water or from the food chain.

The current Washington State Ambient Surface Water Criteria for chronic exposure to hexavalent chromium is 10 µg/L (=10 ppb), while the Environmental Protection Agency (EPA) chronic ambient water quality criterion for chromium is 11 µg/L. The standard applies equally to total chromium and hexavalent chromium (Cr-VI)(see below). The drinking water standard for chromium is 100 µg/L, and a separate standard for Cr-VI in drinking water is currently under development [10]. Chromium concentrations in the river, itself, are typically below any detection level, but in the pore water, levels occasionally exceed 100 µg/L.

Porewater samples analyzed along the Hanford Reach contained an average of 20 µg/L in 1995 (n=141 samples) and 15 µg/L in 2009 (n=124 samples). The maximum concentration was 632 µg/L. Hence it can be important to understand what levels of Cr-VI in pore water would harm the eggs and alevin. The Washington Department of Ecology's [11] current position is that "Research to date shows no negative impact to salmon from chromium concentrations." The authors of the ecotoxicological studies examined here, concur that the value of 10 µg//L [1-3] hereinafter referred to as Farag studies and Patton study) would be protective of salmon, both directly and indirectly by protecting the food chain on which the juvenile salmon depend.

The Farag and Patton studies were conducted in the 1990s. Dauble et al. [12], provided a detailed methodological summary of Farag and Patton studies and results (prior to their peer-review publications), and concluded that exposure up to 266 µg/L showed no effect on fertilization rate, viable hatch, growth or survival. The present paper examines the three studies with respect to effect levels.

METHODS

We examined three laboratory studies of the impact of Cr-VI on early life stages of Chinook Salmon. We looked for evidence relevant to LOELs (lowest observed effect levels) and LOAELs (lowest observed adverse effect levels). In some cases, authors may document

measurable anatomic, biochemical, or physiologic change of uncertain consequence as a LOEL, while other changes in growth and behavior may have survival consequences and be deemed "adverse" indicating a LOAEL. Doses which resulted in no measurable effect or no measurable adverse effect would be deemed NOEL and NOAEL respectively. Estimates of these levels are dependent on dose and duration of exposure and vary depending by the endpoints chosen. Studies had to include adequate data on dose and duration of exposure. In this paper we compare the most informative studies to address public concerns about chromium and salmon populations.

RESULTS

Two groups of researchers headed by A. Farag of the U.S. Geologic Survey [1,3] and G. Patton of the Pacific Northwest National Laboratory [2 directly addressed the toxicity of Cr-VI to salmon. Farag et al. [1] studied fertilization and hatching using an acute exposure of the gametes and fertilized eggs. Patton et al. [3] dosed eggs from the eyed-stage through the alevin stage up to the time of swim-up. Farag et al. [2] studied free-swimming parr beginning dosing 60 days after swim-up. The experimental conditions for the latter two studies are described in Table 1.

Table 1 Comparison of the Farag et al. [3] and Patton et al. [2] methods for studying hexavalent chromium exposure of early life stages of Chinook Salmon and results. .

	Farag et al. [3]	**Patton et al [2]**	**Issues and Questions**
Conducted by	USGS "off-site study"	PNNL "on-site study"	
Source of fish	McNenny Hatchery, Spearfish SD	Priest Rapids Hatchery, WA (eggs from fish from Hanford Reach)	Are these different genetic stocks with different exposure histories?
Life stages dosed	parr or fry stage, begin 60 days post swim-up, continue dosing for 134 days	begin at eyed egg stage and end at swim-up at about 98 days.	Before swim-up the alevins do NOT feed, after swim-up the fry feed voraciously.
Source of chromium	dichromate added to	Hanford ground water* with >2000	Almost "pure" water versus natural water

	Farag et al. [3]	**Patton et al [2]**	**Issues and Questions**
	deionized water	µg/L of Cr, diluted with Columbia River water	with many other constituents.
Dosages	0, 24, 54 µg/L for 105d then 0, 120 & 266 µg/L for 30 d	11,24,54,120 & 266µg/L for 98 d then kept in River water to 132 d (but did not dose after 98 d)	Farag increased doses on day 105 because of no gross effects at 24 & 54 µg/L.
water hardness	76-86 mg/L as $CaCO_3$	35-87 mg/L as $CaCO_3$	Essentially the same hardness. Cr toxicity is enhanced in softer water, hence could have been worse in Patton study.
water pH	7.6-8.0	7.0-8.0	Similar
Temperature	9.9-11.8C	5.4-5.6 C	Temperature could be a significant variable.
Conductivity Alkalinity Oxygen	166-180 µS/cm 76-89 mg/L as $CaCO_3$ Oxygen not stated	124-211µS/cm 64-80 mg/L Oxygen 9.2-14 mg/L	Similar
Mortality or survival	No change at 105 d Decline at 120 µg/L and significant decline at 266 µg/L	>98% survival for all groups >98% hatch >98% swim-up	significant difference in results for the different life stages
Growth (length,	Slight Decline at 54	slight growth	These results may be

	Farag et al. [3]	**Patton et al [2]**	**Issues and Questions**
weight)	µg/L. Significant at 120 µg/L	reduction at 49,120 & 266 µg/L	consistent. 120 would be a LOAEL
DNA damage	detectable at 24 µg/L		This would be a LOAEL
Lipid peroxidation as evidence of oxidative stress	inconclusive evidence		
Histopathology	interstitial blood forming cells at reduced at 24 µg/L. Renal tubule damage at 120 µg/L		Uncertain significance. 24 could be a LOEL and 120 a LOAEL.
Glycogen utilization	some decrease at 24 µg/L		Uncertain significance. This could be a LOEL
Behavioral toxicity	Not reported	"no observable differences in behavior (e.g., feeding patterns, startle response, schooling behavior, and response to light."	

*Groundwater from Hanford contained chromium at 2037-2980 µg/L

The studies by the Farag and Patton groups were conducted in the late 1990s and reported on shortly thereafter [13,14]. The details examined here are based on the peer-reviewed publications [3] and Patton et al. [2].

The sequence of life stages relevant to these studies is: egg (about 3 months) with eyed egg stage beginning about halfway through incubation), alevin (3-4 weeks), fry (about 10 weeks), and the parr stage. Nomenclature in the literature varies, and the alevin, fry, and parr are collectively referred to as juveniles. The three studies used different approaches, and tested different life stages. The study methods and results for Farag et al [3]. and Patton et al. [2] are

compared in table 1. The Farag fertilization study is not included in the table because it used an acute exposure approach. The target concentrations of 120 µg/L and 266 µg/L and actual concentrations achieved (100-120 and 260-266), differ slightly, hence the range of values referred to in this paper.

Patton et al. [2] reported that there was no effect of growth or survival at the 260-266 µg/L dose for 98 days of dosing and an additional 5 weeks of observation. Thus 266 µg/L may be a NOAEL for growth and survival of the alevin stage. Farag et al. [3] did not find growth or survival effects at 24 and 54 µg/L after 105 days and increased the doses to 120 and 266 µg/L respectively for an additional month. They reported that growth was impaired and survival was somewhat reduced (97% versus 84%) in the 24/120 dosage group. Survival was significantly reduced (97% to 70%) in the 54/266 dosage group. Therefore for the fry-parr stage, LOAEL for growth (and other metabolic changes) is 120 µg/L. The LOAEL for survival may also be 120 µg/L, but this was not statistically significant. The 266 µg/L is certainly an adverse dose for survival for the parr stage [3], but not for the alevin stage [2].

A limited number of pore water samples were obtained along the Hanford Reach in 1995 and in 2009. Samples were taken about 30-45 cm (12-18") down in the gravel riverbed. The data indicate that Cr-VI levels declined between 1995 (mean 20 µg/L) and 2009 (mean 15 µg/L). Overall for 284 samples, 40% were below the practical limit of quantification of 3.7 µg/L. Only 3 samples exceeded 266 ppb, the NOAEL for alevin and LOAEL for parr survival. The percentage exceeding the LOAEL of 120 ppb and the LOEL of 24 ppb were 2.5% and 25%, respectively. These exceedances are exaggerated by the higher levels in the 1995 samples. Among the 2009 pore water samples, only 1 exceeded 120 µg/L [15].

DISCUSSION

Acute lethal concentrations for 50% of test organisms (LC-50) are typically measured at 96 hours. LC-50 values for various fish species are above 100,000 µg/L of chromium for most species tested [16]. Toxicity is lower (higher LC-50) in hard and more alkaline waters. LC-50 values for juvenile salmon measured at 96 hours (96 hr LC50) are greater than 100,000 µg/L, with smaller fry (9-13 wks; mean weight 0.5 g) about 20% more susceptible than advanced fry or parr (18-21 wks; mean weight 2.6 g) [17]. Chromium had the lowest toxicity (highest LC-50) of the nine metals tested. By comparison the 96 hour LC-50 for copper, cadmium and mercury were below 100 µg/L [17]. Acute toxicity is of limited value in considering the impact of chronic or recurrent toxic exposures to individuals or populations, hence the need for studies involving weeks or months of exposure covering the full scope of possibly exposed life stages. Velma and Tchounwou [18] tested Goldfish (*Carassius auratus*) at 5% of the LC-50 (4300 µg/L) for one month, finding oxidative and genotoxic damage in both liver and kidney.

Farag et al. [1] exposed gametes and fertilized eggs to Cr-VI for a period of hours, and followed the eggs to hatching. Patton et al [2] dosed from the eyed egg stage through the alevin stage to the mean day of swim-up (day 98), and then observed the fry for an additional five weeks. Farag et al. [3] began dosing parr at 60 days post swim-up. Thus there is essentially no overlap in the timing of the two studies, that would allow comparison of their different methods.

Taken together, the Farag and Patton studies are substantial and cover the spectrum of egg, alevin, and to parr development. In both cases, minimal effects were seen at low doses (24 µg/L), and both conclude that the clean-up target level of 10 µg/Lwould indeed be protective of all life stages. There are substantial methodologic differences between the studies, in both the life stages used and the evidence of effect. Moreover, the finding of greater effects with longer duration, indicates a need for carrying out such studies for additional months.

There seems to have been remarkably little published discussion of the methods and results other than [12]. Patton et al. [2] notes that "Farag et al. [3] also reported impaired growth and survival for chronic exposure (134 days) to hexavalent chromium for juvenile Chinook salmon at exposures ≥120 µg/l." Patton et al. [2] suggest that "juvenile salmon" (a term which would encompass alevin, fry, and parr stages), may be more sensitive after the swim-up stage, when they begin feeding and experience increased metabolism and rapid growth. The differing effect levels of the two studies relate primarily to the different life stages, doses, and endpoints. The underlying methodological differences include different source populations for salmon eggs (Hanford Reach versus remote) and different water sources (natural, multi-contaminant water versus chromium only). The experimental conditions, particularly hardness, aimed to be similar to conditions found along the Reach. Follow-up studies would be needed to examine the impact of these variables.

The apparent resistance of the alevin, reverses the general trend for susceptibility to toxics to decrease as organisms mature and complete development of vulnerable organ systems. Indeed younger fry are more sensitive than older fry in the acute toxicity paradigm [14]. Buhl and Hamilton [19] had shown for three fish species, including Coho (=Silver) Salmon (*Oncorhynchus kisutch*), that the free-swimming juvenile fish were more susceptible to several metals including hexavalent chromium (Cr-VI), than the alevin stage.

The alevin derive energy by absorbing the yolk sac, and they do not eat organisms that live in the pore water. Fry and parr eat voraciously and grow quickly. This could account for both different exposures and different vulnerability. Patton et al. [2] terminated the chromium exposure when the fish were no longer in the gravel stage. They followed the free-swimming fish for an additional month, but did not find increased mortality. They terminated observations at an earlier stage than that at which Farag et al. [3] began dosing parr in their second study.

In addition to the non-overlap in the dosing chronology, two other design features emphasized by the authors, could explain the apparent discrepancy in results. The Farag et al. and Patton et al. took care to explain and justify why they made different decisions about the source of the fish and the source of the water.

a) *Source of the fish.* Patton's breeding stock were from the Priest Rapids Hatchery, hence of Columbia River origin. This is a population that over a half century may have been naturally selected for resistance to Cr-VI and other contaminants. The South Dakota hatchery population from which Farag et al. [1] obtained eggs, may have had its own history of exposure, probably to agricultural pesticides, but would probably not been exposed to Cr-VI. Farag et al. [1] state "The Chinook salmon from the McNenny Fish Hatchery … should have no history of pre-exposure to contaminants in the Hanford Reach of the Columbia River."

b) *Source of the water.* Natural groundwater, as simulated by Patton et al. [2] may have many other constituents which could protect (or enhance) any effect of chromium. A protective substance, possibly the strong antioxidant effect of selenium, might confer protection in natural water, but not in deionized water. Thus Patton et al. [2] strove to emulate the natural exposure to chromium, while Farag et al. [1,3] sought to isolate the toxicity of chromium.

In both cases, the strengths of the two studies offer avenues for future research to clarify some basic issues in aquatic toxicology of chromium. Studies comparing the two paradigms on the same life stages would address this question. We conclude, based on these studies that detectable effects of unknown significance on juvenile salmon occur with chronic exposure to as low as 24 µg/L, but this is a LOEL rather than a LOAEL. For free-swimming fish, significant toxicity (LOAEL) which may impair survival occurs around 120 µg/L and possibly as low as 50 µg/L. However, these values are not necessarily relevant to Hanford and Columbia River salmon. If the non-feeding, pre-swim-up, alevin stage which stays in the gravel for about two weeks, is not susceptible to chromium, then the elevated concentrations of hexavalent chromium in the pore water would have little impact on survival. This is demonstrated in the Patton et al. [2] paper. Conversely, since the chromium concentrations in the river itself are very low [20] , free swimming fish are unlikely to encounter chromium at levels above the LOAEL of 120 µg/L or even 50 µg/L. Tiller et al. [20] found no detectable chromium (i.e. less than 3.7 µg/L) in Columbia River water, and no difference in the chromium content of juvenile Chinook Salmon collected upriver from Hanford and along the Hanford Reach. Thus the relative susceptibility of free-swimming juvenile salmon to chromium is not relevant in a water body with negligible amounts of chromium.

At the highest doses used (266 µg/L), there were no effects on fertilization [1], or on alevin survival [2], but significant reduction of parr survival [2]. Although a small percent of pore water samples had chromium levels above 266 µg, only one recent (2009) sample exceeded this

level. Therefore, there is little likelihood that Cr-VI reaching the Columbia River is impacting salmon populations. Moreover, although 10 µg/L in pore water would indeed be protective, there is no evidence that levels as high as 50 µg/L would jeopardize the fish. Li et al. [21] found no effect on markers of oxidative stress in juvenile (25 g) Rainbow Trout (*Oncorhynchus mykiss*) exposed to Cr-VI for 7 days at up to 200 µg/L.

We note that Farag and Patton studies were conducted in the 1990s, and that new biomarkers, particularly for molecular, genetic, cellular and immunologic damage, could be studied today. These might alter the determinations of NOAELs and LOAELs, and identify additional endpoints, but would probably not alter the conclusion about survival of juvenile Chinook Salmon. Uncontrollable factors such as global climate and local weather, and controllable factors such as water management and fishing, have much greater impacts on salmon survival and spawning recruitment (number of adults returning) [7] and are likely to mask any toxic impacts directly on salmon. Moreover, salmon in the Columbia are part of a complex ecosystem, the integrity of which is essential to support the salmon.

The best studies of Cr-VI toxicity to fertilization, hatching, growth and survival of early life stages of salmon employed different methodologies and test conditions, but complement each other with respect to the life stages tested. Hexavalent chromium travelling through groundwater plumes reaches the Columbia River, upwells through the gravel as pore water, and is then diluted quickly by the rapidly flowing river. Early life stages (eggs and alevin) of Chinook Salmon mature in the gravel redds where a few redds might encounter high levels of Cr-VI in pore water. The alevin do not feed until they become fry and swim up out of the gravel into the river, where they feed voraciously. The alevin stage is less vulnerable to Cr-VI than the free swimming stages which, however, are rarely or never exposed to Cr-VI concentrations above 10 ug/L. Further research balancing the two designs across all the life stages, and extending the duration of dosing and observation, would clarify our understanding of Cr-VI dynamics in the Chinook Salmon population. The very low frequency of pore water samples with Cr-VI levels exceeding 100 µg/L, makes it very unlikely that Cr-VI is affecting individual salmon or salmon populations.

ACKNOWLEDGMENTS

We thank the many people who have discussed these topics with us, or who have helped in the research, including K. Brown, J. Clarke, L. Bliss,, M. Gilbertson, C. Jeitner, D. Kosson, T. Pittfield and C.W. Powers. Brown and Clarke compiled the pore water data. This project was mainly funded by the Consortium for Risk Evaluation with Stakeholder Participation (Department of Energy, DE-FC01-86EW07053), with additional funding from NIEHS (P30ES005022) and

Rutgers University. The views and opinions expressed in this paper are those of the authors, and do not represent the funding agencies.

REFERENCES

[1] A.M. FARAG, D.D. HARPER, L. CLEVELAND, W.G BRUMBAUGH, and EE LITTLE, *"The potential for chromium to affect the fertilization process of Chinook Salmon (Oncorhynchus tshawytscha) in the Hanford Reach of the Columbia River*," Archives of Environmental Contamination and Toxicology, **50**, 575-579 (2006a).

[2] G.W. PATTON, D.D. DAUBLE, and C.A. MCKINSTRY, "*Evaluation of early life stage fall Chinook Salmon exposed to hexavalent chromium from a contaminated ground water source*," Environmental Monitoring and Assessment, **133**, 285-294 (2007).

[3]A.M. FARAG , T. MAY, G.D. MARTY, M. EASTON, D.D. HAPER, and E.E LITTLE, D, Little E.E., "*The effect of chronic chromium exposure on the health of Chinook Salmon (Oncorhynchus tshawytscha),"* Aquatic Toxicology, **76,** 246-257 (2006b).

[4] NATIONAL FISHERIES INSTITUTE (NFI), " Top Ten List of Seafoods Consumed." (2013) Accessed 6 Nov 2013 http://www.aboutseafood.com/press/press-releases/nfi-top-ten-list-familiar-school-fish

[5] V.L. BUTLER and J.E. O'CONNOR, " *9000 years of salmon fishing on the Columbia River, North America*," Quaternary Research, **62**, 1-8 (2004).

[6]D.D DAUBLE and D.G. WATSON, "Status of fall Chinook Salmon populations in the mid-Columbia River, 1948-1992," North American Journal of Fisheries Management, **17**, 283-300 (1997).

[7] W.E. MCCONNAHA, and J.A. LICHATOWICH, *The problem of the Columbia River salmon*, *in Return to the River ,* pp 1-28, R.N. WILLIAMS , Ed., Elsevier-Academic Press, New York, New York (2006).

[8] R.R Whitney, C.C Coutant, and P.R.Mundy, "Mitigation of salmon losses due to hydroelectric development, in Return to the River, pp. 325-416, R.N. WILLIAMS, Eds., Elsevier-Academic Press, New York, New York, (2006).

[9] M.C. HEALEY, *Life history of Chinook Salmon (Oncorhynchus tshawytscha*), pp. 311-394, L. MARGOLIS and C. GROOT, Eds., University of British Columbia Press, Vancouver. Pacific Salmon Life Histories (1991).

[10] EPA 2013. Chromium in drinking water. U.S. Environmental Protection Agency. http://water.epa.gov/drink/info/chromium/#three [accessed Nov 6, 2013]

[11] Washington 2013. Columbia River. Washington State Department of Ecology. http://www.ecy.wa.gov/programs/nwp/Columbia_River.htm [accessed Nov 6, 2013]

[12] D.D DAUBLE, T.M. POSTON, G.W.PATTON, R.E. PETERSON, "Evaluation *of the Effects of Chromium on Fall Chinook Salmon in the Hanford Reach of the Columbia River,"* Pacific Northwest Laboratory, PNL-14008, U.S. Department of Energy DE-AC06-76RL01830, (2003).

[13] A.M. FARAG, A.J. DELONAY, W.G.BRUMBAUGH, E.E.LITTLE, L. CLEVELAND, and D.F. WOODWARD, "*The Potential for Chromium to Adversely affect Chinook Salmon (Onchrhynchus tshawytscha) in the Hanford Reach of the Columbia River,"* U.S. Fish & Wildlife Service, Upper Columbia River Basin Field Office, Spokane, Washington, (2000).

[14] G.W. PATTON, D.D. DAUBLE, M.A. CHAMNESS, C.S. ABERNETHY, and C.A. MCKINSTRY, *"Chromium Toxicity Test for Fall Chinook Salmon (Oncorhynchus tshawytscha) Using Hanford Site Grounwater: Onsite Early Life Stage Toxicity Evaluation. PNNL-13471*," Pacific Northwest National Laboratory, Richland Washington, (2001).

[15] WASHINGTON CLOSURE HANFORD (WCH), "*Field Summary Report for Remedial Investigation of Hanford Site Releases to the Columbia River. Collection of surface water, river sediment, and island soils. Appendix E. Cr+6 Concentrations in Pore Water*.WCH-352. Department of Energy, Washington, D.C. (2009).

[16] R. EISLER, "*Chromium hazards to fish, wildlife, and invertebrates: a synoptic review*" United States Fish and Wildlife Service, Patuxent, Maryland Biological Report **85** (1996).

[17]S.J. HAMILTON and K.J. BUHL, "*Safety assessment of selected inorganic elements to fry of Chinook Salmon (Oncorhynchus tshawytscha),"* Ecotoxology and Environmental Safety **20**,307-324 (1990).

[18] V. VELMA and P.B TCHOUNWOU, "Oxidative Stress and DNA Damage Induced by Chromium in Liver and Kidney of Goldfish, *Carassius auratus*," Biomarker Insights. 2013 8:43-51.

[19] K.J. BUHL and S.J. HAMILTON, "Relative sensitivity of early life stages of Artic grayling, coho salmon, and rainbow trout to nine inorganics," *Ecotoxicological Environment,* **22,** 184-197 (1991).

[20] B.L. TILLER, D.D. DAUBLE, G.W. PATTON, and T.M. POSTON*, "Monitoring tissue concentrations of chromium and fish condition in juvenile fall Chinook Salmon from the Hanford Reach of the Columbia River PNNL-1447,"* Pacific Northwest National Laboratory, Richland Washington (2004).

[21] Z.H. LI, P. LI, and T. RANDAK, '*Evaluating the toxicity of environmental concentrations of waterborne chromium (VI) to a model teleost, Oncorhynchus mykiss : a comparative study of in vivo and in vitro.'* Comparative. Biochemical Physiology Part C, **153**, 400-407 (2011).