Quality Assurance Project Plan

Toxic contaminants in outmigrating juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) from river mouths and nearshore saltwater habitats of Puget Sound

WDFW-Ecology Interagency Agreement # G1200486

July 2013

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Prepared for:

Washington Department of Ecology

Publication Information

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Chemical analysis of the field samples collected by WDFW and NOAA for this project will be conducted under a different agreement between Ecology and NOAA's Northwest Fisheries Science Center (#C1300124, attached).

The contents of neither of these documents do not necessarily reflect the views and policies of the EPA, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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1.0 Quality Assurance Project Plan

Toxic contaminants in outmigrating juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) from river mouths and nearshore saltwater habitats of Puget Sound

July 2013

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2.0 Abstract

Juvenile Chinook salmon (*Oncorhynchus tshawytscha)* can encounter a wide range of water quality conditions, from relatively clean to highly contaminated, as they migrate from freshwater to saltwater in Puget Sound. During this life stage they are particularly sensitive to stressors such as toxic contaminants as they transition from fresh to saltwater. Currently, contaminant monitoring in juvenile salmon is not funded; however, it is a central metric of the Puget Sound Partnership's Toxics in Fish Vital Sign, adopted in 2011 by the Leadership Council. In this study we will measure juvenile Chinook salmon exposure to known chemicals of concern entering Puget Sound via stormwater, wastewater treatment facilities, atmospheric deposition to marine waters and groundwater. Fish will be sampled from four Puget Sound embayments in 2013. For each embayment, sampling sites include one location in the lower river and two locations along adjacent marine shorelines. This sampling augments previous sampling initiated as early as 1998, and will be used to establish a solid time series of contaminant conditions in juvenile Chinook salmon that can be used to fulfill the Toxics in Fish goal of tracking time trends of salmon health. The objectives are to (1) measure the magnitude of exposure, (2) compare exposure in outmigrants across four major embayments and between fresh- and saltwater, and (3) evaluate potential effects on marine survival. Additionally, results from this work will be used to provide a measure of the effectiveness of current toxic reduction strategies and actions, inform future pollution reduction efforts, and enhance recovery of Chinook salmon. This project is linked to an agreement between Ecology and NOAA's Northwest Fisheries Science Center (#C1300124, attached), who will conduct chemical analyses on field samples collected by WDFW and NOAA.

Upon completion of the study, WDFW will produce a final report detailing the findings. The final report will be published and data will be submitted for uploading into Ecology's Environmental Information Management database.

3.0 Background

This report details specific procedures and quality assurance guidelines proposed by the Washington Department of Fish and Wildlife *Toxics in Biota* staff to implement the following project: *Toxic Contaminants in outmigrating juvenile Chinook salmon (Oncorhynchus tshawytscha) from river mouths and nearshore saltwater habitats of Puget Sound*

As a member of the Puget Sound Ecosystem Monitoring Program (PSEMP), the Washington Department of Fish and Wildlife (WDFW) assesses status of and trends in the health of Puget Sound fishes and macro-invertebrates related to their exposure to toxic contaminants. This *Toxics in Biota* effort is one component of PSEMP, a multi-agency effort designed to monitor the health of the Puget Sound ecosystem. PSEMP tracks a broad range of status indicators, including submerged aquatic vegetation, sediment health, fecal contamination in shellfish, water quality and several others. WDFW's *Toxics in Biota* component of PSEMP (a) monitors the status and trends of chemical contamination in Puget Sound biota, (b) evaluates the effects of contamination on the health of these resources and (c) provides information to public health officials for assessing if Puget Sound seafood is safe to eat.

3.1 Study Area

This project is focused on the health of outmigrating juvenile Chinook salmon from four major watersheds within Puget Sound: 1) Skagit River and estuary, 2) Snohomish River and estuary, 3) Green/Duwamish River and Elliott Bay, and 4) Nisqually River and estuary. These river systems provide the majority of wild Chinook salmon entering Puget Sound on their migration and the Pacific Ocean (Rice et al. 2011).

3.2 Logistical Problems

All studies of this type are subject to the normal rigors of conducting sampling in the field. Difficult weather conditions can compromise sample quality or necessitate schedule changes. Although juvenile salmon migrate through the area of interest on a fairly predictable schedule, collections can be compromised if timing is unusual. Beach seining from shore with a boat in can be compromised if anthropogenic or other debris is encountered.

Ideally samples across river systems will be taken synoptically. However some salmon stocks characteristically migrate at slightly different times, which will make simultaneous sampling difficult. We plan to sample the peak of the run, as best judged by the regional salmon biologists working in these rivers. A number of samples for this study will be donated by these biologists, who are conducting regular salmon sampling efforts in our target areas. Coordinating with a number of field biologists simultaneously will require a great deal of planning and careful follow-up.

3.3 History of the Study Area

The study area comprises four large river mouths and their associated estuaries in Puget Sound; Skagit, Snohomish, Duwamish/Green, and Nisqually. These are some of the major rivers draining into Puget Sound, and each system is characterized by unique hydrology, hydro-geography, contaminant loading,

and Chinook spawning and rearing habitat. Collectively, these watersheds encompass a range of land-use practices from relatively undisturbed areas such as the Nisqually, to agricultural regions such as the Skagit, to heavily urbanized areas such as the Green/Duwamish/Elliott Bay.

3.4 Contaminants of Concern

The primary contaminants of concern for this study are well-known and abundant toxic chemicals typically found in the lower rivers and estuaries of Puget Sound that juvenile Chinook salmon may be exposed to as they migrate from fresh water habitats to marine water of Puget Sound and the coastal Pacific Ocean. These contaminants include persistent organic pollutants (POPs), polycyclic aromatic hydrocarbons (PAHs), and four metals (copper, zinc, nickel and cadmium). Although polychlorinated dibenzodioxins (PCDD) and polychlorinated dibenzofurans (PCDF) are of concern because of their high toxicity, they are not included in this study because analytical costs are prohibitive. Extra tissues from this study will be archived and made available for these analyses if funding becomes available.

POPs are fat-soluble, persist in the environment, accumulate in animals with age, and biomagnify through the food chain so they can concentrate in fishes, including salmon. POPs are not easily metabolized and therefore fishes and other animals *carry the risks* from these contaminants with them through their entire life cycle. POPs are subject to global transport and they continue to cycle in the environment decades after their peak use. Three groups of POPs will be assessed in this study, polychlorinated biphenyls (PCBs) – an industrial contaminant, polybrominated diphenylethers (PBDEs) commonly used as flame retardants, and chlorinated pesticides including DDT and its metabolites. Environmental concentrations of PCBs and organochlorine pesticides peaked in the 1960s - 1970s, and then declined rapidly from the late 1970s through to the mid-1980s because of regulations at the national and international level. However, they have shown little decline since then, and are still at concentrations that cause adverse effects in aquatic resources. PBDEs are not regulated by EPA, although certain forms of PBDEs have been banned in Washington (RCW 70.76) and Oregon ORS 453.005-135). PBDEs increased exponentially from the 1970s but currently appear to be declining in some fish species in the Salish Sea (West et al. 2011).

PAHs are toxic and carcinogenic chemicals that occur naturally in coal, crude oil and gasoline and in products made from fossil fuels, such as coal-tar pitch, creosote and asphalt. PAHs enter streams, rivers, and estuaries through industrial discharges, stormwater runoff from highways and other paved surfaces, and atmospheric deposition. PAHs are metabolized by salmon and other fish Varanasi et al., 1989), so they do not accumulate in fish tissues, but nonetheless can be highly toxic to fish. Sediment cores from the Puget Sound region reveal that maximum PAH concentrations occurred between 1945 and 1960, and then decreased for the next 20 to 30 years (MacDonald and Crecelius, 1994). However, a recent study by Washington State Department of Ecology comparing surface sediment collected in 2000 to results from 1989 through 1996 at 10 long-term Puget Sound sites showed that PAH levels were significantly higher in samples collected in 2000 than they were historically at 5 of the 10 sampling (PSAT, 2004). Early declines in PAH concentrations can be attributed to the switch from coal to oil and natural gas for home heating, improvements in industrial emissions controls, and increases in the efficiency of power plants (Gschwend and Hites, 1981). More recent, PAH increases have been linked to

increasing urban sprawl and vehicle traffic in urban and suburban areas (Lefkovitz et al. 1997; Van Metre et al. 2000).

Copper, one the four metals to be analyzed, is widely used in building materials (e.g., copper roofs and treated lumber), automobile parts (e.g., brake pads), and pesticides (Davis et al., 2001). Consequently, copper is often a pervasive contaminant in urban and agricultural watersheds where juvenile salmon rear prior to oceanic migration. It can enter aquatic environments in urban stormwater and agricultural runoff.

3.5 Results of Previous Studies

Chemicals released into the aquatic system from human activities and developments may reduce the health and productivity of fish and wildlife and their food supply**.** Because of, their anadromous life-history, salmon and steelhead (henceforth, for simplicity, "salmon") may be exposed to contaminants in freshwater, estuarine and marine waters. While transitioning from freshwater to saltwater, outmigrant juvenile salmon are also particularly sensitive to stressors such as toxic contaminants. Impairment of water quality in these highly productive estuarine and nearshore habitats represents a significant threat to salmon populations.

Juvenile salmon migrating from their natal streams to Puget Sound integrate contaminant conditions from across the freshwater/saltwater interface, the primary receiving waters for stormwater and many wastewater treatment plants, and can encounter a wide range of water quality conditions. Ocean-type Chinook salmon, the predominant life-history type in Puget Sound, and chum salmon spend considerably more time in estuaries than other salmon species, and thus are more susceptible to contaminant exposure during their out-migrant phase. However, Chinook salmon may accumulate higher persistent bioaccumulative toxics (PBTs) contaminant burdens than other salmon because of their higher trophic status. Once in the saltwater, they may be continually exposed to contaminants that accumulate in urbanized bays and in the coastal water of the North Pacific adjacent to developed and urbanized landscapes.

Systematic, comprehensive sampling of outmigrant juvenile Chinook salmon in Puget Sound has not occurred, although data from several previous unrelated studies indicate that Chinook salmon from urban rivers and estuaries are exposed to PCBs, chlorinated pesticides, PAHs and PBDEs (Stehr et al.2000, Johnson et al. 2007, Olson et al. 2008, Meador et al. 2010, Sloan et al. 2010). Outmigrating juvenile Chinook salmon may also be exposed to less bioaccumulative contaminants including metals such as copper and zinc, typically present in surface runoff from impervious surfaces.

Salmon exposure to these contaminants in freshwater may have reduced survival. Pertinent to our study of outmigrant Chinook salmon are sub-lethal contaminant exposures in freshwater that reduce salmon growth and, by extension, subsequent size-dependent survival when they migrate to the ocean. Likewise, sub-lethal contaminant exposure in freshwater that impairs immuno-competence may subsequently reduce marine survival, particularly as they make the parr-smolt transformation and enter

marine waters. Contaminant exposures that disrupt the smoltification process may alter time at entry into saltwater as well subsequent growth and immuno-competence. Once in estuaries and nearshore waters, salmon may continue to be exposed to contaminants that affect their growth, immuno-competence and disease susceptibility and ultimately, their survival. Additionally, throughout freshwater, estuarine and nearshore saltwater habitats of Puget Sound, salmon eggs, alevins, fry, smolt and juveniles may be exposed to endocrine disrupting compounds that alter their reproductive health. Below we summarize the potential adverse effects of contaminant exposure on growth, immnuno-competence and disease resistance, and reproductive development of outmigrant Chinook salmon from Puget Sound rivers and the nearshore.

Effects of Contaminant Exposure on Salmon Growth

Adequate energy reserves and normal growth are vital to juvenile fish survival, and also strongly influence reproductive potential of adult fish. Various studies (reviewed by Johnson et al. 2014) documented that exposure to POPs can alter growth rates and condition in fish, particularly fish exposed to high levels (> 1-2 ug/L) of PCBs, PCDDs and chlorinated pesticides. However, Johnson et al. (2007) conclude that the effects of exposure to lower POPs concentrations, which are more representative of environmentally relevant concentrations, are less consistent, with some studies reporting enhanced fish growth at low POP exposures. In general, exposures to environmentally relevant levels of POPs were more likely to affect fish growth if exposures occur during early development. Exposure to low concentrations of POPs may also have neurological effects that impair foraging ability, reduce lipid content and alter energy metabolism, leading to reduced growth (see review by Johnson et al. 2007, and references therein). Petroleum-derived compounds (PAHs) also depress growth rate of juvenile salmon, which can affect their survival (Meador et al. 2006).

Short-term-exposure to low levels of copper reduces the olfactory capacity of salmon and, therefore, their ability to detect important olfactory cues from nearby prey and predators (Baldwin et al. 2003; Sandahl et al. 2007, McIntyre et al. 2008). Copper disrupts olfaction and olfactory-mediated behaviors in Chinook, coho and chum salmon, steelhead, Atlantic salmon, and rainbow trout (reviewed by Tierney et al. 2010, see also Baldwin et al. 2011). These findings support extrapolation of copper toxicity data across species and are relevant to both hatchery and wild fish. In addition to these behavioral effects, modeling by Mebane and Arthaud (2010) suggested that body size reductions due to chronic early life stage exposure to sublethal copper concentrations could reduce juvenile salmon survival and population recovery trajectories.

Several studies in Puget Sound document that growth is impaired for out-migrant juvenile Chinook salmon while migrating through urban estuaries and bays of Puget Sound (Casillas et al. 1995 a,b, 1998; Varanasi et al. 1993). The growth rates of juvenile Chinook salmon collected from urban estuaries (e.g., Hylebos and Duwamish Waterways) and held in the laboratory for 90 days were lower than those for fish from the corresponding hatcheries or from nonurban estuaries. Furthermore, concentrations of plasma hormones involved in the regulation of growth in fish, such as thyroxine (T4), triiodothyronine (T3), and insulin- like growth factor (IGF), were altered in salmon from urban estuaries in comparison with hormone levels in hatchery or non-urban fish (Casillas et al., unpublished data). Thus exposure to

contaminants may interfere with the endocrine modulation of growth in juvenile salmon, reducing overall growth.

Additionally, laboratory exposure experiments using sediment extracts from contaminated Puget Sound sites and model toxic compounds indicated that exposure to toxic contaminants may suppress growth or alter the metabolism of juvenile Chinook salmon (Varanasi et al. 1993, Casillas et al., 1998, Meador et al. 2006). In studies by Casillas et al. (1998), there was some uncertainty regarding the concentrations of PAHs required to suppress growth of juvenile salmon because fish exposed to PAHs alone at concentrations comparable to those present in the Hylebos Waterway did not exhibit consistent reductions in growth in all treatment groups, although growth was reduced consistently in fish exposed to sediment extracts containing PAHs in combination with PCBs and other contaminants. Meador et al. (2006) dosed juvenile Chinook salmon with PAHs at 5 different concentrations in feed encompassing PAH concentrations measured in stomach contents of juvenile salmon from Pacific Northwest estuaries. Significant differences in mean fish weight, and whole body lipids were detected at the two highest doses. At the lowest doses, variability in fish weights increased significantly. Additionally some significant alterations in plasma chemistry enzymes were observed at the second lowest and higher doses. These studies indicate effects of PAHs on fish growth and energy balance but also suggest that other compounds present in contaminated Puget Sound estuaries, such PCBs, are contributing significantly to growth reductions that have been observed in field collected fish; however, more work is needed to determine the relative importance of various compounds in generating this effect.

Effects of Contaminant Exposure on Immuno-competence and Disease Susceptibility

A properly functioning immune system is an important fitness trait that is vital for both individual survival and population productivity (Segner et al., 2012). Contaminant exposure can alter the immune system, either alone (Arkoosh et al., 2010; Arkoosh et al., 2000; Arkoosh et al., 2001) or in conjunction with other stressors (Jacobson et al., 2003), resulting in an increase in susceptibility to naturally occurring pathogens that cause lethal diseases, potentially leading to population level effects (Arkoosh et al., 1998; Loge et al., 2005; Spromberg and Meador, 2005).

Exposure to environmentally relevant concentrations of petroleum-derived compounds such as PAHs, industrial contaminants such as PCBs and flame retardants such as PBDEs suppress the immune system, rendering juvenile Chinook salmon more vulnerable to naturally occurring pathogens (Arkoosh and Collier, 2002; Arkoosh et al. 1994, 1998, 2001, 2010). Arkoosh et al. (1998) demonstrated that Chinook salmon from an urban estuary were more susceptible to bacteria-induced mortality from naturally occurring marine pathogens than were fish from the corresponding hatchery upstream from the urban-estuary, and fish from a nonurban estuary and its corresponding hatchery (Figure 1).

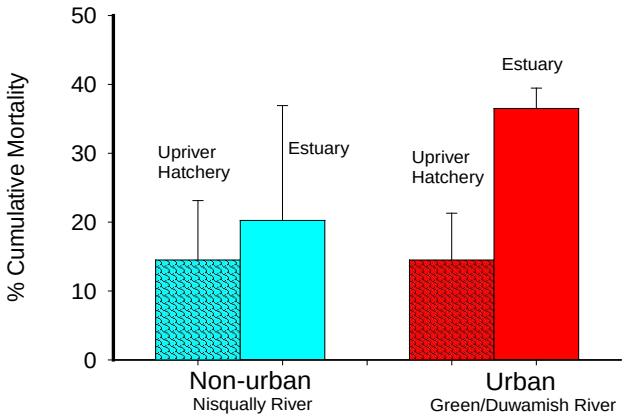

Figure 1. Cumulative mortality of juvenile Chinook salmon from an urban and nonurban estuary and their corresponding hatcheries four days after exposure to the marine pathogen Vibro anguillarum.

Adapted from Arkoosh, M.R. et al., Trans. Am. Fish. Soc., 127, 360–374, 1998; results for 2 -6 X 10-5 dilution of V. anguillarum.

Follow-up laboratory exposure studies with sediment extracts and contaminant model mixtures determined that contaminants such as PCBs and PAHs, apart from other estuarine variables specifically associated with the Duwamish and Hylebos Waterways, could independently suppress immune function and increase disease susceptibility in juvenile Chinook salmon (Arkoosh et al., 1994, 2001). More recently, studies have documented that exposure to PBDEs also influence disease resistance (Arkoosh et al. 2010). Though an adverse health effects threshold for PBDEs has yet to be determined, Arkoosh et al. (2010) demonstrated that juvenile salmon fed an environmentally relevant concentration of PBDE congeners were more susceptible to the marine pathogen *Listonella anguillarum*.

Effects of contaminant exposure on reproductive development

Several environmental contaminants, especially chemicals specifically produced to mimic hormones (e.g., ethynylestradiol in birth control pills), are known to disrupt the endocrine system and affect the reproduction, development and other hormonal functions of fish and wildlife. However, chemicals with relatively low hormonal activity can also pose a health risk because they persist in the aquatic environment (e.g., PAHs and PCBs) or are more persistent in tissues (e.g., PCBs.)

There is evidence that juvenile Chinook salmon are exposed to estrogenic contaminants in estuarine and nearshore waters that can affect their reproductive development. Peck et al. (2011) documented higher plasma levels of estrogen-inducible yolk protein, vitellogenin (VTG), in field caught Chinook salmon at sites such as Elliott Bay and the mouth of the Snohomish River than non-exposed hatchery control fish. Juvenile Chinook salmon with elevated VTG during a sensitive early life stage could experience delayed reproductive effects such as those observed in independent studies on flounder or rainbow trout (Hashimoto et al. 2000 and Bennetau-Pelissero et al. 2001)

3.6 Regulatory Criteria

Although there are no criteria regulating the exposure of juvenile salmon to the contaminants of concern in this study, contaminant burdens will be compared with available health-effects thresholds to evaluate potential health risks to juveniles from exposure to contaminants.

4.0 Project Description

This project is designed to evaluate the extent and magnitude of exposure of juvenile Chinook salmon to well-known and abundant toxic chemicals in their environment, as the fish migrate from their juvenile fresh water habitats to marine systems. The project will estimate exposure of salmon to these chemicals in four of the largest river mouths and associated marine shorelines in Puget Sound. These river mouths represent a wide range of potential contaminant conditions from highly urbanized (Duwamish/Green River), to moderately urbanized (Snohomish River), to primarily agricultural (Skagit River) and mostly rural, undeveloped (Nisqually River) watersheds.

The basic intent of this study is to establish a baseline status condition of toxic contaminants in juvenile Chinook salmon that can be used as a starting point for long-term trend monitoring. In addition it will provide an initial assessment of potential health effects from exposure to the measured chemicals, by consulting published health-effects exposure thresholds.

4.1 Project Goal

The goals of this study are threefold: (a) estimate the extent and magnitude of exposure of outmigrating juvenile Chinook salmon to three classes of toxic contaminants, (b) compare exposure among four major river mouth systems, and (c) compare exposure of salmon while they are still in fresh water, with exposure of salmon that have moved to nearby marine waters.

4.2 Project Objectives

The objectives of this study are to (a) collect juvenile Chinook salmon, (b) create composite samples of whole bodies, stomach contents and gill tissue, (c) analyze whole bodies for persistent bioaccumulative and toxic contaminants (PBTs), stomach contents for PAHs, and gills for metals.

4.3 Information Needed and Sources

We will be generating new data on toxic contaminants in juvenile Chinook salmon, presented as wet weight concentration. Pre-existing PSEMP and NOAA contaminant data on this species and life stage will be incorporated when pertinent, for context. See Table 7, Section 7.7 for a summary of these data.

4.4 Target Population

The target population for this study is juvenile Chinook salmon from Puget Sound watersheds. The sampling will target unmarked, presumably wild Chinook salmon, but marked hatchery Chinook may also be collected as necessary to obtain sufficient tissue for analyses. In particular, we will target Chinook salmon originating from the Skagit, Snohomish, Green/Duwamish and Nisqually river systems, which provide the majority of wild Chinook salmon entering Puget Sound on their migration and the Pacific Ocean (Rice et al. 2011).

4.5 Study Boundaries

This study will take place in the lowest reaches of each river and in along marine shorelines within three kilometers of river mouths.

4.6 Tasks Required

Tasks involved in this study include:

- Collecting juvenile salmon
- Resecting tissues
- Creating composite samples of homogenized tissue
- Submitting tissue samples to the contract NOAA lab for analysis of chemicals.
- QA/QC review
- Formatting data for relational database
- Analysis of data for PSEMP/DFW report
- Transfer of data to EIM

4.7 Practical Constraints

The most pertinent practical constraints here relate to availability of fish and stomach contents. Although migration timing is well known for this species, abundance varies spatially across a relatively short window of opportunity. Sampling is constrained to this window. Successful PAH analysis will be constrained by collection of a sufficient mass of stomach contents. We may need to take up to 10 individuals with full stomachs to obtain sufficient mass of contents for the PAH analyses.

Chinook salmon are a listed species under the Endangered Species Act, which constrains the number of fish we are allowed to take. The current permit (see Appendix A) under which the authors of this study must act allows for taking 853 juvenile Chinook salmon (178 hatchery and 378 naturally produced fish).

5.0 Organization and Schedule

5.1 Key Individuals and Their Responsibilities

Table 1. Organization of project staff and responsibilities.

Name	Title	Phone #	Email	Responsibilities
Sandra M. O'Neill	Senior Research Scientist	360.902.2666	sandra.oneill@dfw.wa.gov	Principal Investigator and lead author
James E. West	Senior Research Scientist	360.902.2842	james.west@dfw.wa.gov	Co-investigator
Jennifer A. Lanksbury	Fish and Wildlife Biologist 3	360.902.2820	jennifer.lanksbury@dfw.wa.gov	Project support, lab and field
Laurie A. Niewolny	Fish and Wildlife Biologist 2	360.902.2687	laurie.niewolny@dfw.wa.gov	Project support, lab and field
Andrea Carey	Fish and Wildlife Biologist 2	360.902.2849	andrea.carey@dfw.wa.gov	Project support, lab and field
Stefanie Orlaineta	Part-time temporary technician	360.902.2657	stefanie.orlaineta@dfw.wa.gov	Project support, lab and field
Tom Gries,	NEP QA Coordinator	360.407.6327	tgri461@ecy.wa.gov	reviews QAPP and draft report
William Kammin	Ecology QA Officer	360.407.6964	wkam461@ecy.wa.gov	approves QAPP

5.2 Project Schedule

Table 2. Proposed schedule for completing field and laboratory work

Field and laboratory work	Due date	Lead staff
Field work completed	August 15	Sandie O'Neill
Laboratory analyses completed	30 September, 2013	
Quarterly reports		
Author lead	James West	
Schedule QAPP approved – 15 May, 2013 Complete lab analysis – 30 Sept, 2013 Final Report -- 31 Aug, 2014		
1st quarterly report	Short progress report with invoice	
2nd quarterly report	Short progress report with invoice	
3rd quarterly report	Short progress report with invoice	
4th quarterly report	Short progress report with invoice	
Final report		
Author lead and support staff	Sandra O'Neill, James West, Jennifer Lanksbury, Laurie Niewolny, and Andrea Carey	
Schedule		
Draft due to peer reviewers and NEP staff	30 June, 2014	
Final report due	31 August, 2014	

5.3 Budget and Funding

This project is supported by an Interagency agreement with Ecology with funding from Toxics and Nutrients Prevention, Reduction, and Control. This overall effort is funded by EPA's National Estuary Program (NEP). Match for this study is provided by WDFW in the form of staff time, vessel use, and laboratory supplies.

Table 3. Proposed WDFW budget for 2013/14 juvenile Chinook salmon contamination study.

Task Timeline and Cost (Field Activities Only)		
Project Task	**Cost**	**Completion Date**
Task 1-Project Administration/Management	$7,283	August 31, 2014
Task 2-Quality Assurance Project Plan Development (QAPP)	$7,283	April 30, 2013
Task 3-Salmon Collections and Sample Processing	$11,518	September 30, 2013
Total:	$26,084	August 31, 2014

6.0 Quality Objectives

6.1 Measurement Quality Objectives

The objective for analytical chemistry is to employ methods sufficient to evaluate the target analytes, with limits of detection sufficient to identify and measure the analytes, at a cost that meets the needs for geographic coverage of this study. The general quality objective of this study is to collect a minimum of three and up to five composite tissue samples from three tissue matrices. Based on previous studies described in Section 7.7 and Table 7, this sample size should be sufficient for a statistically rigorous comparison across river systems and between fresh and salt water. Table four summarizes the maximum number of samples that may be analyzed. In addition, samples will be collected using operating procedures (described herein) consistent with previous work, including field sampling devices and techniques, laboratory resection of fish tissues, and analytical chemistry for target analytes. This will ensure consistency and comparability of existing data.

Table 4. Maximum number of juvenile Chinook tissue composites to be collected and analyzed for chemical contaminants during this study.

Watershed Mouth	Habitat Type	Whole body	Stomach contents	Gills
Skagit	Freshwater	5	5	5
Skagit	Saltwater (2 locations)	10	10	10
Snohomish	Freshwater	5	5	5
Snohomish	Saltwater (2 locations)	10	10	10
Duwamish/Green	Freshwater	5	5	5
Duwamish/Green	Saltwater (2 locations)	10	10	10
Nisqually	Freshwater	5	5	5
Nisqually	Saltwater (2 locations)	10	10	10
Total[a]		60	60	60

[a] Two QA samples will be run per batch of 12 field samples for organic chemistry.

Minimum QA criteria for PCBs, PBDEs, and organochlorine pesticides analyzed in salmon whole bodies and for PAHs analyzed in salmon stomach contents for this study are summarized in the following Table 5 (taken from Sloan et al., 2006, Table 8). In this table PAHs are synonymous with Polycyclic Aromatic Compounds (PACs).

Table 5. Minimum analytical quality assurance criteria reproduced Table 8 in from Sloan et al. 2006.

Table 8. Minimum analytical quality assurance criteria: Polycyclic aromatic compounds (PACs) and persistent organic pollutants (POPs) by gas chromatography/mass spectrometry (GC/MS).

Quality assurance element	Minimum frequency	Acceptance criteria
Instrument calibration	Once every batch of samples or once every two batches in one continuous analytical sequence	Analyte concentrations are to be calculated using point-to-point calibration with at least four concentration levels of calibration standards.
Continuing calibration	At start and end of every analytical sequence and every 10 or fewer field samples	The RSD of the analyte responses relative to the internal standard is to be ≤ 15% for the repetitions.
Reference materials: Sediment: NIST SRM 1944, NIST SRM 1941b Mussel tissue: NIST SRM 1974b Blubber: NIST SRM 1945 Fish tissue: NIST SRM 1946, NIST SRM 1947	One with every batch of 20 or fewer field samples	Concentrations of ≥ 70% of individual analytes are to be within 30% of either end of the 95% confidence interval of the reference values. These criteria do not apply to analytes with concentrations below their lower LOQ when the lower LOQ is within or greater than the 95% confidence interval, nor to those analytes known to have coeluting compounds.
Method blank	One with every batch of 20 or fewer field samples	No more than 5 analytes in a method blank are to exceed 2 × lower LOQ. Samples are not corrected for analytes found in the blank.
Sample replicates (i.e., duplicates or triplicates)	One with every 20 or fewer field samples	RSDs are to be ≤ 15% (equivalent to relative percent difference ≤ 30% for duplicates) for ≥ 90% of the analytes that have concentrations ≥ 1 ng/g.
Internal standards/surrogates	At least one internal standard/ surrogate is added to every sample	The recoveries are to be 60–130%.
Interlaboratory comparisons	At least one per year	In conjunction with the NIST or the IAEA.

Minimum QA criteria for zinc, nickel, copper, and cadmium by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in salmon gill tissue are summarized in the following Table 6.

Table 6. Required batch quality control measures and quality assurance criteria for the ICP-MS metals Cu, As, Cd, and Pb. Reproduced from KCEL SOP 624v2.

Quality Control Element	Description of Element	Frequency of Implementation	Control Limit
			Liquid
Method Blank (MB)	Interference-free matrix to assess overall method contamination	1 per QC batch	< MDL & > -MDL [b]
Spike Blank (SB)	Interference-free matrix containing all target analytes	1 per QC batch	85% - 115%
Matrix Spike (MS)	Sample matrix spiked with all/subset of target analytes prior to digestion	1 per QC batch	75% -125%
Matrix Spike (MS) and Matrix Spike Duplicate (MSD)	Sample matrix spiked with all/subset of target analytes prior to digestion	1 per QC batch or (LD) – Ultra Low level analysis only.	75% -125% %Recovery 20% RPD
Lab Duplicate (LD) [a]	Self explanatory	1 per QC batch or MSD – Routine level analysis only.	≤ 20% RPD, when at least one value is > RDL
Filtration Blanks (Routine)	Method blank for the filtration process if samples filtered in the lab	2 per QC batch	< MDL & > -MDL
Filtration Blank (Ultra-low)	Method blank for the filtration process	1 per QC batch	< MDL & > -MDL

[a] No calculation performed when both sample and duplicate values < RDL
[b] Method blank result must be <MDL but also be above the equivalent negative MDL value (e.g., if MDL = 1.0, then the methods blank result must <1 but > -1). A method blank result of -2 would indicate possible problems with the method blank or with calibration.

Measurement quality objectives for bias associated with measurement of % lipids are that each NIST SRM result should be within its control limits (Sloan et al, 2006):

- Upper control limit = [1.35 × (certified concentration + uncertainty value for 95% confidence)]
- Lower control limit = [0.65 × (certified concentration – uncertainty value for 95% confidence)]

The measurement quality objective for % solids is drying samples to a constant weight.

6.2 Precision

Precision is monitored and controlled within batches using laboratory replicates of field samples and across batches by analyzing Standard Reference Materials (SRM) of applicable matrix i.e., tissue. For this study NIST SRM 1974b will be used for all organics[1]. Applicable SRMs or Certified Reference Materials (CRM) will be used for metals analysis. Cross-batch precision is expressed as the relative standard deviation (RSD) for repeated measurements. The RSD of analyte responses relative to the internal standard must be ≤ 15% for the repetitions (see Table 5 for POPs and Table 6 for metals).

6.3 Bias

Bias or accuracy of samples is evaluated by comparing measured SRM values with National Institute of Standards and Technology (NIST) certified values. In addition for POPs, concentrations of ≥70% of individual analytes are to be within 30% of either end of the 95% confidence interval of the reference values.

6.4 Sensitivity

The Lower Limit of Quantitation (LOQ, Table 5) for all organic chemicals in this study is "the concentration that would be calculated if that analyte had a GC/MS response area equal to its area in the lowest level calibration standard used in that calibration. When an analyte is not detected in a sample or it has a response area that is smaller than its area in the lowest level calibration standard used, the concentration of the analyte in that sample is reported to be less than the value of its lower LOQ." (Sloan et al. 2006). Typically LOQ values for PAHs that have been reported to PSEMP by this method are in the range of 0.2 to 0.8 ng/g wet weight. In this study, the PAHs' LOQs are given as a range because tissue sample LOQs are affected by the field sample mass used. The LOQ is the lowest concentration at which a PAH's sample result will be reported.

A Method Detection Limit (Table 6) for all metals samples will be calculated for each analytical batch according to KCEL's SOP 604v6 for CVAA (King County Environmental Laboratory, 2012) and 624v2 for ICP-MS (King County Environmental Laboratory, 2009).

6.5 Comparability

The SOPs described in this document (Sloan et al., 2004; Sloan et al., 2006; KCEL 2009; and KCEL 2012) are consistent with other concurrent and future sampling efforts that could be used as comparison for juvenile salmon tissues collected in this study.

6.6 Representativeness

The sampling design in this study is aimed at representing contaminant conditions as tissue residues in juvenile salmon across a wide range of potential contaminant conditions. The study design optimizes spatial coverage to represent conditions from rivers draining lightly developed, rural watersheds to highly urbanized or agricultural watersheds. In addition sample sizes and locations are selected to

[1] SRM 1974b is no longer available from NIST. The NOAA lab has enough matrix on hand for this study, however, a suitable alternative may be substituted, at the chemist's discretion.

maximize power for representing contaminant condition of salmon in both freshwater and saltwater, around the time they are smolting (out-migrating from freshwater to saltwater).

Because each Puget Sound watershed and river is unique, the degree to which results from this study can be used to represent other non-sampled systems is uncertain. However, we expect this study will yield some basic tenets regarding contaminant exposure of a sensitive life stage of Chinook salmon in Puget Sound, relative to watershed land-use characteristics.

6.7 Completeness

This study will be considered complete when the minimum number of samples for all proposed tissue has been collected. This consists of three composite samples per location/tissue, and three fish per tissue composite.

7.0 Overall Study Design

The study is designed to address the question "what is the exposure of juvenile Chinook salmon to a commonly observed suite of toxic contaminants, as they migrate from fresh- to saltwater in Puget Sound "? This question generates the null hypothesis; there is no difference between tissue residue of contaminants in salmon across four sampling locations (river mouths) and between fresh- and saltwater habitats at four river mouths. The sampling will target unmarked, presumably wild Chinook salmon, but marked hatchery Chinook may also be collected as necessary to obtain sufficient tissue for analyses.

Tissue residues will also be compared with published effects thresholds to evaluate the potential health effects on juvenile salmon from exposure to contaminants.

At each sampling site, we will collect unmarked, presumably wild juvenile Chinook salmon, to create composite samples of whole fish (less stomachs), stomach contents, and gill tissue as described in Varanasi et al. (1993), Stein et al. (1995) and Stehr et al. (2000). Each composite sample will comprise no more than 10 fish (to minimize the number of fish killed for the study) and no fewer than three fish (to maximize the number of fish represented in the samples. Although ten is the desired number of fish per composite, sufficient numbers may not be available at each site.

In addition to chemical contaminants we will measure covarying and potentially explanatory chemicals such as stable isotopes of carbon (^{13}**C** and nitrogen (^{15}N). These analytes help to distinguish fish tissue as being derived from terrestrial versus marine carbon sources (^{13}**C**), and to estimate the relative trophic level of fish sampl ed (^{15}N).

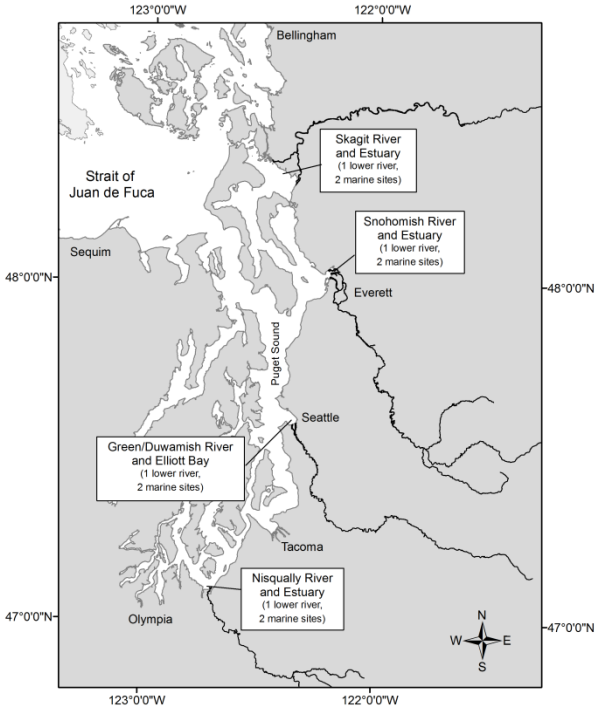

Figure 2. Location of four major river mouths where juvenile salmon will be sampled

7.1 Sampling location and frequency

All samples will be collected in the 2013 spring/summer outmigration period for Chinook salmon (see Table 4). We anticipate sampling to commence mid-May and continue until as late as mid-July.

Specific sampling areas will be selected based on (a) presence of fish, (b) accessibility and suitability of the shoreline for beach seining or fyke netting, and (c) availability of opportunistic samples from existing salmon monitoring programs. Samples will be collected by WDFW and NOAA staff, as well as field biologists from other Agencies or groups (e.g., tribes) who will be in the field sampling juvenile Chinook salmon for a variety of purposes. Some sampling locations will be selected not only to take advantage of such existing work, but also to minimize sampling effort and the number of fish taken from each system, to meet ESA permit requirements.

All juvenile Chinook salmon sampled for this study by all field biologists will be taken following the standard operating procedures outlined in this document.

Samples will be timed to match the peak outmigration run, as best judged by the area biologists working in these systems. We plan to sample each location once.

7.2 Map of study area

Figure 2 shows the four river mouths selected for this study.

7.3 Parameters to be determined

Parameters to be determined in this study include:

- Sampling information
  - Location
  - Date/time
  - Sampling method
- Biological metrics
  - Fish fork length (mm)
  - Total body mass (g)
  - Fish sex
- Tissue chemistry
  - Persistent bioaccumulative toxics in whole body (minus stomachs)
  - PAHs in stomach contents
  - Metals in fish gills
  - Stable isotopes of carbon and nitrogen

7.4 Field measurements

Field measurements related to capturing salmon include date, time, location (latitude/longitude sampling device). The hand-held GPS units (Garmin, GPSmap 76C, and GPSmap 176) available to PSAMP staff report coordinates to the nearest 0.00001 decimal degrees (1.11 m/3.64 ft).

7.5 Assumptions underlying design

- Sampling across the peak of the outmigration run of salmon is sufficient to represent conditions in the system from which they were sampled.
- Tissue residues of contaminants are correlated with exposure to contaminants, so that tissue residues are a reasonable proxy for contaminant conditions in fish prey and their environment
- Tissue residues of contaminants are a suitable proxy for evaluating health risks from exposure to contaminants.
- Removing a gill arch for metals analysis has a negligible effect on POPs results for whole bodies
- Removing stomach contents for PAH analyses has a negligible effect on POPs results for whole bodies

7.6 Relation to objectives and site characteristics

The locations selected for this study are ideal because they (a) represent the largest watersheds and river output in Puget Sound, (b) support the most abundant outmigration runs of juvenile Chinook salmon, and (3) represent a wide range of watershed land uses and contaminant regimes.

7.7 Characteristics of existing data

Systematic, comprehensive monitoring of juvenile salmon for contaminant exposure has not occurred in Puget Sound; however, sampling conducted by WDFW and NWFSC indicates that many juvenile Chinook salmon from Puget Sound urban populations are exposed to several PBT and non-persistent contaminants. Exposure to PBTs such as PCB and PBDEs are often above estimated effects thresholds or at concentrations at which know effects occur (Table 8). More limited PBT exposure assessments have been completed for chum, coho and pink salmon. Generally, concentrations of PBTs in coho and pink salmon are lower than those observed for Chinook salmon from the same locations, whereas concentrations in Chinook and chum salmon are similar (Stehr et al., 2000; Olson 2008). Such differences are likely related to habitat use, diet and metabolism. Assuming the estuary is an important source of contaminants for out-migrant salmon, higher contaminant exposures in Chinook and chum salmon are consistent with the more prolonged period of estuarine exposure in these species (Quinn 2005).

Table 7. Concentrations of PCBs and PBDEs in whole body samples of juvenile Chinook salmon from Puget Sound estuaries, and percentages of samples exceeding health effects thresholds for PCBs and PBDEs. NA = no data available. See review in section 3.5

Site	N	Mean ± SD concentration of PCBs (ng/g lipid)	Mean ± SD concentration of PBDEs (ng/g lipid)	% of samples with PCBs > 2400 ng/g lipid	% of samples with PBDEs > 1400 ng/g lipid
Skagit	12	2000 ± 2000 [2]	1300 ± 3500 [1]	23%	7.70%
Snohomish	6	4000 ± 1700 [2]	2400 ± 1100 [1]	85%	86%
Elliott Bay	6	14000 ± 13000 [2]	560 ± 390 [1]	100%	0%
Duwamish	13	4800 ± 2200 [2,3]	560 ± 770[1]	86%	17%
Commencement Bay	21	1700 ± 1100 [4]	NA	24%	NA
Nisqually	1	1500 [4]	NA	0%	NA
Squaxin Pass	6	5200 ± 270 [5]	570 ± 330 [5]	100%	100%
Skokomish	1	980 [3]	NA	0%	NA

[1]Sloan et al. 2010; [2]unpublished NWFSC data; [3]Johnson et al. 2007; [4]Olson et al. 2008; and [5]WDFW unpublished data

Additionally, exposure to non-persistent PAHs has been examined in juvenile Chinook, coho, and chum salmon from 5 hatcheries and their respective estuaries of five river systems of Puget Sound: the Green-Duwamish, the Puyallup-Hylebos/Commencement Bay, the Nisqually, the Snohomish, and the Skokomish (McCain et al. 1990; Olson et al., 2007; Stehr et al., 2000; Stein et al., 1995). Salmon collected from the Duwamish and Commencement Bay/Hylebos Waterway estuaries adjacent to Seattle and Tacoma showed elevated levels of PAH metabolites in bile in comparison to fish from hatcheries or from the less-urbanized Nisqually and Skokomish systems.

Recent data on concentrations of copper, zinc, nickel and cadmium and other metals are lacking for juvenile Chinook salmon. Additionally, information is lacking on the extent to which juvenile salmon are exposed to chemical of emerging concerns, including xenoestrogens, pharmaceuticals, personal care products, and newer use pesticides like pyrethroids. These emerging contaminants have been detected in freshwater streams in Puget Sound and in discharge from waste water treatment plants. It is not yet known the extent to which juvenile salmon are exposed to these chemicals in freshwater habitats, and what effects such exposure might have on long-term survival. Chemicals of emerging concern are not covered by this proposal.

8.0 Sampling Procedures – Field and Lab

8.1 Field Measurements and Field Sampling Standard Operating Procedures

The SOP outlined below describes the gear and procedures to be employed to catch juvenile Chinook salmon for this study, handling of fish between the field and the lab, and creation of composite samples in the in the lab in preparation for chemical analyses.

8.1.1 Collecting juvenile Chinook salmon

Two basic methods will be used to collect juvenile salmon (1) beach seines, and (2) fyke nets, as described herein.

8.1.1.1 Beach Seine

Beach seines used for this study will be of varying length and width, but each will share common construction characteristics:

- Knotless woven nylon mesh with mesh opening ranging from ¼" to ½"
- Rectangular panel construction with a floating head rope and a sinking footrope
- Panel size approximately 100 ft long x 8 ft high.

We will generally follow seining protocols described in (Puget Sound Estuary Program, 1990), Varanasi et al. (1993) and Roegner et al. (2009); in brief, the net will be paid out via boat from shore, turning from perpendicular to parallel to shore as the net is paid out. The boat will drag the net in a semicircle parallel to shore and bring the end of the net back to shore encompassing the target shoreline. The net will be anchored at its trailing edge on shore, and pulled in by staff on the beach from both ends, once the leading edge has reached the shore.

8.1.1.2 Fyke Net

Fyke nets consist of a tapering net funnel typically set fixed in shallow water. Panels of netting are set at the mouth of the net to herd fish into the funnel at the fish are swimming along shore.

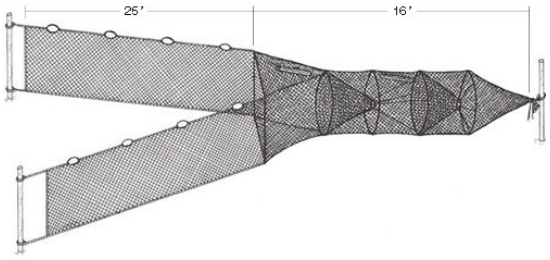

Figure 3. Example of a fixed fyke net. Image copied from Les Industries Fipec Inc.

8.1.2 Sample identification

Fish will be removed from nets or traps, placed in a pre-labeled plastic Ziploc bag, and the bag placed on ice. Field staff will wear nitrile gloves where feasible to minimize potential contamination of whole

bodies. Staff will remove stomach contents as soon as possible, either in the field or the lab, depending on weather and the feasibility of dissecting fish in the field (see section 8.2.3).

Individual salmon will be identified with pre-selected PSEMP fish identification numbers (FishIDs) and placed in individual plastic Whirlpacs as they are processed. Iced fish will be moved to a -20 deg. C. freezer as soon as possible, within 6 hours of capture.

8.1.3 Field log

The lead scientist for each field survey will maintain a bound Rite-in-the-Rain field log with detailed notes for each day's activities. Entries are made in the daily log either in permanent ink or pencil. Minimum information recorded is:

- Name and location of project
- Field personnel
- Sequence of events
- Gear used and description of fishing activity
- Any changes to plan
- Weather conditions
- Date, time, location name and/or coordinates,
- ID and description of each sample
- Water depth, temperature and salinity
- Unusual circumstances that may affect interpretation of results

8.2 Lab Measurements and Standard Operating Procedures

8.2.1 Equipment, reagents and supplies for analytical chemistry

The following inventory will be confirmed prior to all fish-processing activities:

- Terg-A-Zyme® for cleaning lab surfaces and instruments
- Isopropyl Alcohol - B&J Brand® Multipurpose ACS, HPLC
- Tap water
- Teflon Squeeze bottles
- Heavy duty aluminum foil – Reynolds 627 (60.96 cm wide x 0.94 mm thick)
- Scissors - stainless steel
- Forceps - stainless steel
- Spatula – stainless steel, flat blade/round blade
- Mixing spoon – stainless steel
- Measuring tape – cloth
- Stainless Steel mixing bowl
- Sample jars – clear, short, wide mouth 8 oz jars, I-CHEM Certified 200-0250 series, Type III glass with Teflon-lined polypropylene lid (Figure 5)
- Bench scales– such as A&D EK-6000H (6,000 x 0.1 grams) (Figure 6)

- Sample jar labels – cryogenic, laser printer ready, Diversified Biotech LCRY-2380 0.94in. x 0.50in and LCRY-1258 2.625in x 1.0in.
- Lab coat/apron
- Nitrile exam gloves – talc-free
- Eye protection
- Freezers – walk-in freezer at -20°C, chest freezer at -15°C

Figure 4. Pre-cleaned Series 200 I-Chem jar

8.2.2 Lab setup and preparation for tissue chemistry

8.2.2.1 Preparation of Lab Record forms

Specimen forms will be created for this study that will identify samples using nomenclature described below. A daily log of operations is kept in the lab. A series of codes are assigned and printed on all lab forms; identification code for the survey (SurveyID), station StationID, specimen (FishID) and sample (SampleID).


Figure 5. Bench scale

8.2.2.2 Use and creation of sampling codes

SurveyID: Each survey carried out by the PSEMP unit is assigned a SurveyID to differentiate it from surveys of the past and future. The PSEMP database manager creates a unique alpha numeric code that identifies the survey type and the year.

StationID: Each station sampled by PSEMP is assigned a StationID code to help differentiate it from other locations sampled in the past, present and future. The database manager compares the latitude/longitude information for the sampling location in question against those of StationIDs listed in the database to determine if the location has been sampled in the past. A new location is assigned a descriptive name that is unique from all other StationIDs (using all capital letters for the text in the code) and a location which has been sampled in the past is assigned the same SampleID as the past sampling effort(s).

For specimens acquired from a source outside PSEMP (e.g. WDFW test fishery, WDFW survey, Tribal test fishery), if derived from a fixed[2] site, PSEMP uses the sources assigned name as the StationID; however, if the fixed site corresponds to an establish PSEMP station, the PSEMP StationID is used.

SampleID: All samples created by PSEMP are assigned a unique SampleID code that differentiates each sample from similar samples collected in the past, present or future. A SampleID is a unique alpha-numeric code that is assigned to an analytical sample; either a sample taken from an individual or a

[2] fixed site – a specific location that is returned to repeatedly over time.

composite of individual tissues. Each id consists of six parts, a two-character year code, a two or more character site code, a dash, a two-character species code, a one or two-character matrix code and either a two-digit (composite sample) or 4-digit (individual FishID) sample number.

Unique SampleIDs are assigned by concatenating numbers of label acronyms as follows:

- Two digit year,
- Two or three (typically) digit station identifier
- A dash "-"
- Two digit species
- Single digit matrix
- A sequential number

For example : **13QB-PHSE01**, from 20**13**, **Q**uilcene **B**ay, **P**acific **H**erring, **S**pawned **E**ggs, 01.

8.2.4.3 Use and creation of forms

Once the database manager has determined the sampling codes, he/she then prepares a Specimen Form for use in the lab. The forms are printed on waterproof paper to facilitate use in the lab environment. The following information is captured on a Specimen Form:

1. Station Information
    a. SurveyID – database manager provides, preprinted on form
    b. StationID – database manager provides, preprinted on form
    c. Collection Date – preprinted on form and Time?
2. Specimen Information
    a. Species – preprinted on form
    b. Effort – Enter the EffortID if one has been assigned or a general description of the effort (e.g. Tow-1, Tow-2, Set-1, Set-2, etc.)
    c. FishID code
    d. SampleID – database manager provides, preprinted on the form.
3. Observations

8.2.4.4 Labeling sample jars

To facilitate identification of composite samples compiled in glass jars, corresponding labels are attached to both the lid and the jar. Both labels are printed on cryogenic, laser printer ready labels produced by Diversified Biotech. The lid label has the SampleID printed on it and the jar label has the Year, Station, Species, Matrix, SampleID, Date (capture), jar Weight (empty weight with lid on) and tissue weight.

8.2.4.5 Chain of Custody

A Chain of Custody/Task Order form will be initiated when sample jars are created, to track location, disposition, and entity responsible for each jar. COC forms will be signed and dated each time sample jars change hands, most importantly when they are delivered from WDFW to the analytical laboratory.

8.2.4.5 Equipment cleaning procedure

When processing specimens for contaminant analysis, anything (work-surfaces, instruments, etc.) that may contact those portions of a specimen that are subject to contaminant analysis must be cleaned before use.

A “clean” work-surface, means a surface (lab counter, cutting board, sorting tray, etc.) covered by aluminum foil fresh off the roll. The work surface is covered with at least one layer of aluminum foil and the foil must be changed between composites.

"Clean" instruments means stainless steel dissection tools and grinding apparatus (hand grinder and cutting blades) that have been washed in warm soapy water (Terg-A-Zyme®), thoroughly rinsed three times under warm running tap water, followed by a rinse with deionized water (held in teflon squeeze bottle), solvent rinsed using isopropyl alcohol (held in a teflon squeeze bottle) and then placed on aluminum foil for air drying.

The same clean instruments/surface can be used repeatedly, without re-cleaning, on specimens contributing to the same composite. They must be subjected to the complete cleaning procedure between composites. Lab personnel must change nitrile gloves between composites.

8.2.3 Biometrics and Preparation of Tissue Composites

Thawed fish samples will be resected to remove gills and stomach contents. Individual tissue samples for each composite will be combined into a stainless steel mixing vessel for grinding and homogenization. Stomachs will be accessed by opening the fish with a pair of fine scissors, cutting the fish along its ventral median from vent to gills. Once the stomach is exposed, a second pair of pre-cleaned “inside” scissors is used to cut open the stomach. Contents will be scooped out using a pre-cleaned stainless steel spatula. The remainder of the fish, including the emptied stomach, will be used for the whole body composite.

Tissue resections will generally follow Washington Department of Ecology’s Standard Operating Procedure for whole bodies and body parts (Ecology, 2010). In brief, whole body, stomach content, and gill composite samples will be created by combining tissues from up to 10 fish each. Tissue or whole bodies (minus gills and stomach contents) will be handled using metals-free tools (e.g., ceramic knives, titanium forceps). Tissues will be subsequently ground and homogenized in a glass I-Chem jar using a titanium grinder or non-metallic tools. All resection instruments will be cleaned between samples with a succession of enzymatic soap, tap water, deionized water, and isopropyl alcohol. Homogenized samples will be placed in pre-cleaned, pre-labeled I-Chem Series 200 jars. The weight of tissue added to the composite jar will be determined by taring the scale to the jar weight prior to adding the tissue. Subsamples from each composite may then be removed and distributed to additional labeled jars or vials for archiving. Samples will be labeled and frozen to -20°C until transfer to the analytical lab. A minimum of 3 grams of tissue will be taken for each sample. When possible, replicate samples will be created for archive.

9.0 Chemical Analyses

9.1 Analytes

Table 9. Organic analytes to be measured in this study.

Persistent organic pollutants:	No. Analytes	Method	Limit of Quantitation - LOQ (wet weight)	Expected Range (wet weight)
Polychlorinated biphenyl (PCB) congeners	40	Sloan et al. 2004 [a]	0.2-0.8 ng/g	LOQ to 20 ng/g
Polybrominated diphenylethers (PBDEs) congeners	11	Sloan et al. 2004	0.2-0.8 ng/g	LOQ to 20 ng/g
Organochlorine pesticides (OCPs)	25	Sloan et al. 2004	0.2-0.8 ng/g	LOQ to 20 ng/g
Polycyclic Aromatic Hydrocarbons (PAHs)	45	Sloan et al. 2004	0.2-0.8 ng/g	LOQ to 20 ng/g

[a]Sloan, C. A., D. W. Brown, et al. (2004). Extraction, cleanup, and gas chromatography/mass spectrometry analysis of sediments and tissues for organic contaminants., U.S. Dept. Commerce. NOAA Tech. Memo. NMFS-NWFSC-59.

Table 10. Metals to be measured in this study.

Metals	No. Analytes	Method	Method Detection Limit (wet weight)	Expected Range (wet weight)
Zinc (Zn)	1	KCEL SOP 624v2 [a]	0.004 µg/g	MDL to 5 µg/g
Nickel (Ni)	1	KCEL SOP 624v2	0.004 µg/g	MDL to 5 µg/g
Copper (Cu)	1	KCEL SOP 624v2	0.004 µg/g	MDL to 5 µg/g
Cadmium (Cd)	1	KCEL SOP 624v2	0.002 µg/g	MDL to 5 µg/g

[a] KCEL SOP 624v2: King County Environmental Laboratory Standard Operating Procedure 624v2 - ICPMS Analysis of Water, Wastes, Sediments and Tissues by the Thermo X Series II CCT (see Appendix E)

Table 11. Conventionals to be measured in this study.

Metals	No. Analytes	Method	Method Detection Limit (wet weight)	Expected Range (wet weight)
Lipid content (% total extractibles)	1	gravimetric	0.1%	0.5 to 3%
Dry Weight (%)	1	gravimetric	0.1%	10-20%
δ15 Nitrogen	1	See section 9.5		
δ13 Carbon	1	See section 9.5		

9.2 Matrix

Three matrices from juvenile Chinook salmon are targeted in this study (1) stomach contents for PAH analysis, (2) whole body (minus stomach contents) for analysis of PBTs, and (3) gill tissue for analysis of metals

9.2.1 Number of samples

The maximum number of samples to be submitted for chemical analysis in this study is expected to be 180, comprising 60 composites each of stomach contents, gill, and whole body (minus stomach contents).

9.2.2 Analytical methods

Analyses for persistent organic pollutants and metals will be conducted by separate labs. All POPs and percent lipids will be analyzed by NOAA Fisheries, Northwest Fisheries Science Center, Seattle WA. Metals analyses will be conducted by one of two laboratories, King County Environmental Lab and Frontier Global Sciences. The selection of metals lab will be based on an evaluation of cost and limits of detection given the amount of gill tissue resected from the salmon. Percent solids will be measured for each sample at each lab.

Persistent Organic Pollutants

All POPs in this study will be analyzed by NOAA Fisheries according to Sloan et al. (2004). This analytical method is consistent with previous WDFW/PSEMP studies. In brief, this method comprises three steps: (a) extraction, (b), cleanup by silica/aluminum columns and size-exclusion high-performance liquid chromatography (SEC HPLC), and (c) quantitation of chlorinated hydrocarbons (CHs) and aromatic hydrocarbons (AHs) using gas chromatography /mass spectrometry (GC/MS) with selected-ion monitoring (SIM). Samples are extracted using accelerated solvent extraction (ASE with methylene chloride), which provides an extract that can be used for AH, CH recovery and gravimetric lipid evaluation. This method also includes alterations to typical GC/MS methods to stabilize the instrument and improve accuracy such as chemical ionization filaments (to increase source temperature), employing a cool on-column injection system in the GC, a guard column before the analytical column, and point-to-point calibration to improve data fit over the full range of GC/MS calibration standards (Sloane et al. 2004).

Metals

Nickel, zinc, cadmium, and copper will be analyzed by the King County Environmental Laboratory (KCEL) via Thermo Elemental X Series II CCT (Collision Cell Technology) Inductively Coupled Plasma Mass

Spectrometer (ICP-MS) following KCEL SOP 624v2. This SOP incorporates elements of EPA 200.8 revision 5.4, SW-846 6020A February 2007, ILM05.3 Exhibit D part B, and PSEP 1997. Total solids will be analyzed via KCEL SOP 307v3 to facilitate reporting metals data in both dry and wet weight concentrations.

Stable Isotopes

Stable isotopes of carbon (^{13}C) and nitrogen (^{15}N) will be measured by Mass Spectrometry (following Herman et al. 2005) after preparation as follows:

1. Homogenized tissue samples freeze-dried overnight
2. Freeze-dried tissue pulverized in a micro-ball mill
3. 0.4 to 0.6 mg powder of each sample placed into separate tin cups, in triplicate
4. Combusting samples in a Costech elemental analyzer attached to a Thermo-Finnegan Delta Plus Isotope Ratio Mass Spectrometer

Values are calibrated with internal standards every ten samples. Unenriched histidine is used as a control material to evaluate set-to-set reproducibility, analyzed after every 25 samples. Stable isotope results are expressed in "delta" (δ) notation in ‰:

$$\delta Z = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad (1),$$

where Z is ^{15}N or ^{13}C,
R_{sample} is the ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$ for the tissue sample, and
$R_{standard}$ is the ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$ of standards (atmospheric air for nitrogen and Pee Dee Belemite limestone for carbon.

Percent Lipids

Percent lipids in each sample are represented by total extractables, according to Sloan et al. 2004. Briefly samples from the extraction step of the POP analyses (Section 9.5.1) will be evaporated and compared to the mass of the original, unextracted sample (paraphrasing from Sloan et al. 2004):

> The pan containing the sample for total extractables from Section 3 is placed on a covered rack in the hood and the solvent is allowed to completely evaporate (approximately 1–2 hours). The pan is dried in a 50°C oven for 2 hours, then cooled in a desiccator overnight. The pan is weighed to the nearest 0.0001g and the weight is recorded as the "Pan w/TE" weight. The percent total extractables (% TE) content of the sample is calculated as follows:

> % TE = ((Pan w/TE – Pan) x (ASE Vial w/Extract – ASE Vial) x 100%)/
> ((ASE Vial w/Extract – ASE Vial w/o TE Extract) x Sample Weight).

Percent solids (Dry Weight) Determination

The percent of the sample as dry weight is determined by simple drying of tissues according to Sloan et al. 2004 (paraphrasing):

> Pre-homogenized tissue (1 + 0.5 g) is placed into the pan, and the pan is weighed to the nearest 0.001 g. The weight is recorded as the "Pan w/Wet Sample" weight.

The pan is placed in a drying oven at 120°C for 24 hours then cooled in a desiccator for 30 minutes. The pan is weighed to the nearest 0.001 g, and the weight is recorded as the "Pan w/Dry Sample"weight. The percent dry weight of the sample is determined as follows:

$$\% \text{ Dry Weight} = ((\text{Pan w/Dry Sample} - \text{Pan}) \times 100\%) / (\text{Pan w/Wet Sample} - \text{Pan})).$$

9.2.3 Sensitivity/Method Detection Limit (MDL)

The Lower Limit of Quantitation (LOQ) for all POPs in this study is "the concentration that would be calculated if that analyte had a GC/MS response area equal to its area in the lowest level calibration standard used in that calibration. When an analyte is not detected in a sample or it has a response area that is smaller than its area in the lowest level calibration standard used, the concentration of the analyte in that sample is reported to be less than the value of its lower LOQ." (Sloan et al. 2006). Typically LOQ values for POPs that have been reported to PSEMP by this method are in the range of 0.2 to 0.8 ng/g wet weight.

EPA defines Method Detection Limit (MDL) in Appendix A to 40 CFR Part 136 as the "minimum concentration of a substance that can be measured and reported with 99 percent confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the element". In this study, the metal's MDLs are concentrations that cannot be detected or detected at a concentration less than the associated method detection limit considering tissue sample detection limits are affected by the sample mass used, matrix and polyatomic/isobaric interferences. The MDL is the lowest concentration at which a sample result will be reported. Table 10 lists the respective method detection limits for the four metals of concern (Hg, As, Cu, Zn, Cd, and Pb). They range from 0.002 to 0.005 µg/g wet weight.

9.3 Sample preparation methods

Tissue samples are homogenized per Section 8.2.3. Prior to extraction each homogenized sample will be mixed again thoroughly with a clean spatula.

10.0 Quality Control Procedures

Quality control of all field activities will be supervised by the PI. All personnel will have available to them copies of the QAPP and pertinent SOPs. The PI will review all notes entered into the field log at the end of each activity, and prior to leaving each study site or other significant location. For analytical chemistry, quality control procedures, quality assurance criteria and corrective actions for persistent organic pollutant (POPs) data are detailed in Sloan et al. (2006). Briefly, precision is monitored and controlled within batches using laboratory replicates of field samples (2 replicates run for every batch of 12 samples) and across batches by analyzing Standard Reference Materials (SRMs – one per batch). Cross-batch precision is expressed as the relative standard deviation (RSD) for repeated measurements. The RSD of analyte responses relative to the internal standard must be ≤ 15% for the repetitions.

For POPs analysis, accuracy of samples is evaluated by comparing measured SRM values with National Institute of Standards and Technology (NIST) certified values for 1974b Blue Mussel. Concentrations of ≥70% of individual analytes are to be within 30 % of either end of the 95% confidence interval of the reference values. One method blank is run for every 20 or fewer field samples. No more than 5 analytes in a method blank are to exceed 2x the lower LOQ before corrective action is taken. The corrective action will be to re-extract and re-analyze the affected samples. Data are reported by the analytical lab without blank correction. It is up to the user to decide if and how to correct data with respect to blank contamination, and how or whether such data should be censored with qualifiers. At least one internal standard (surrogate) is added to each sample, with acceptable recoveries ranging from 60 to 130%.

Quality control measures and quality assurance criteria for metals data are detailed in Table 6. Briefly, precision is monitored and controlled within batches using laboratory replicates of field samples and matrix spike duplicates (one per batch). Accuracy of analysis is evaluated by comparing measured standard reference material (SRM) values and a laboratory control sample (LCS) with the respective certified values. A SRM of applicable matrix will be selected to be analyzed i.e., tissue. Method blanks and spikes are evaluated for overall run and process contamination. These are run every batch as is applicable.

11.0 Data Management Procedures

11.1 Data recording/reporting requirements

Data for both field samples and QC samples are received from analytical laboratories in Excel spreadsheets in various formats. PSEMP staff format these data into a structure compatible with the Toxics in Biota (TIB) database. The TIB database is a relational format created in Access, with separate tables for (1) field effort data, (2) biological characteristics of individuals used to create samples, (3) many-to-many cross reference for individuals-to-composites, (4) sample tracking, condition and summary statistics, and (5) chemical analyses. Data are examined visually using Excel filters and sorting procedures to identify formatting or transcription errors. Raw analyte concentrations are compared with expected ranges to identify potential outliers. In addition preliminary summary statistic tables, scatter plots, and time trend plots are created to examine the new data.

11.2 EIM data upload procedures

All data generated by this project will be submitted to Ecology's EIM for later export to EPA's STORET database.

12.0 Audits and Reports

12.1 Frequency of Audits

The NWFSC analytical lab participates in annual NIST or IAEA interlab comparison studies.

12.2 Responsibility for reports

WDFW staff will submit a draft report to peer reviewers and to the NEP QC for comment. The report will include summary statistics of all analytes, a statistical comparison of each analyte (or group total) by study location and site type, with inclusion of covariates if needed. Pattern analysis for selected analytes may be included. Tissue concentrations will be compared with appropriate thresholds as available from the literature.

The final report will address comments received as deemed appropriate. Data packages will be prepared for submittal to EIM and later export to EPA's STORET database, as detailed in the Scope of Work. James E. West is responsible for these products.

13.0 Data Verification and Validation

13.1 Field data verification, requirements, and responsibilities

All sample location data for this study will be verified by comparing GIS-plotted latitude and longitude data with field notes to confirm locations plot correctly. If GPS locations plot incorrectly they will be replotted using narrative documentation of locations from field notes.

13.2 Lab data verification and validation

Data generated by the analytical lab are reviewed for out-of-bounds values, transcription errors and other problems by at least two chemists. Final review is conducted by a lab manager who approves data before they are released to the client. Prior to database entry WDFW staff will compare results with MQOs and review data by comparing results with similar species or matrices in the PSEMP database. Individual data, means, and standard deviations are plotted and putative outliers evaluated for validity. Evaluation of the validity of putative outliers includes reviewing all collection, biological, and analytical data for potential transcription errors, communication with analytical labs to verify reported values are correct, and evaluation of biological covariates that might explain otherwise unanticipated values. PSEMP does not currently conduct data validation by a third party reviewer.

14.0 Data Quality (Usability) Assessment

14.1 Process for determining whether project objectives have been met

The success of meeting data quality objectives is evaluated based on the outcome of quality control procedures during analytical procedures. Typically if QC criteria are not met the problem is identified by staff from the analytical lab, corrected, and sample (or extract) re-run. In cases where QC criteria have not been met and there is not enough tissue to be reanalyzed, the data will be censored with appropriate qualifiers to allow an objective evaluation of the usability of the final record. Rejected data are censored with an "R" or equivalent qualifier. We expect rejected data to be rare based on (1) a long history of employing these methods to measure target analytes in a wide range of Puget Sound biota matrices, (2) the range of data values we expect in this study, and (3) appropriate (tenth-of-ppb) limits of quantitation (with the singular possible exception of potential blank contamination for naphthalene-compounds).

Adequacy of sample number will be evaluated during the statistical analysis of analytes. We have predicted that three to five replicates per class will provide enough power to distinguish spatial trends in most individual PAH analytes, however a final evaluation of sample size adequacy will be made after this analysis.

14.2 Data analysis and presentation methods

Toxics data collected for this study are part of a long-running tissue residue monitoring program. This program has a long history of data analysis and presentation, which will be continued in the present study. Analysis and presentation of contaminant and covariate data will be conducted using programs commonly employed by PSEMP to compare spatial distribution of contaminants. This includes a General Linear Model that compares contaminant concentrations across geographic locations while adjusting for potentially confounding covariates such as animal size. Analyte results may be log-normalized to achieve normality and homoscedasticity. A Tukey's *post hoc* multiple range test will be used to discriminate the significance of observed differences by sample location and fresh- versus saltwater. If normality and homoscedasticity are not achievable with data transformation, non parametric analogs of ANOVA may be used. Similarity matrices of various combinations of individual analytes will be created to perform Multivariate Dimensional Scaling comparisons among sample types, and used to compare contaminant patterns.

14.3 Treatment of non-detects

Non detected analytes are censored with a "less than limit of quantitation" (<LOQ) or "U" qualifier for POPs and "less than the detection limit" for metals . The value reported for non-detected analytes will be the LOQ or MDL. It is the responsibility of users to decide how to use censored data.

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Appendix A. Scientific Research Permit 1566-3R

UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Northwest Region
7600 Sand Point Way NE
Seattle, Washington 98115

March 13, 2012 F/NWR3

Lyndal Johnson
Supervisory Zoologist
NMFS Northwest Fisheries Science Center
2725 Montlake Blvd. E
Seattle, WA 98112

Re: Permit 1566-3R

Dear Ms. Johnson:

Enclosed is Scientific Research Permit 1566-3R issued to the NMFS Northwest Fisheries Science Center (NWFSC) under the authority of Section 10(a)(1)(A) of the Endangered Species Act. The permit authorizes the NWFSC to annually take listed salmonids while conducting research on assessing the exposure of juvenile salmon in Puget Sound, Washington to emerging contaminants.

The National Marine Fisheries Service (NMFS) requires that the individuals acting under the authority of Permit 1566-3R review the permit before engaging in the permitted activities. Please sign and date the last page then fax a copy of it (or mail a photocopy) to our office to the attention of Mitch Dennis. Our fax number is (503) 230-5441. Please note that you are not authorized to conduct activities under Permit 1566-3R until our office receives a signed copy of the signature page.

We direct your attention to Sections A and B, which describe the yearly take limits and the permit conditions. Permit 1566-3R authorizes take at the levels, by the means, in the areas and for the purposes stated in the permit application. Permit 1566-3R is also subject to annual authorization based on your reported annual take and compliance with the authorization requirements. Annual reports are due by January 31 each year. Permit 1566-3R expires on December 31, 2016.

If you have any questions concerning the permit, please contact Mitch Dennis at (360) 753-9580.

Sincerely,

for William W. Stelle, Jr.
Regional Administrator

Enclosure

cc: File copy - [1566-3R], F/EN6 - NMFS Enforcement (Raneses), F/NWC1 - Northwest Fisheries Science Center (Ferguson)

National Marine Fisheries Service (NMFS)
Endangered Species Act (ESA) Section 10(a)(1)(A) Research Permit
(16 U.S.C. §§ 1531-1543) (50 CFR Parts 222-226)

Permit Number:	1566-3R
Permit Type:	Scientific Research
Expiration Date:	December 31, 2016
Reporting Period:	January 1 through December 31
Annual Report Due:	January 31

Permit Holder:	Lyndal Johnson Supervisory Zoologist NMFS Northwest Fisheries Science Center
Address:	2725 Montlake Blvd. E Seattle, WA 98112
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Email:	Kinsey.Frick@noaa.gov
Principal Investigator:	Lyndal Johnson
Co-investigators:	Bernadita Anulacion, Dan Lomax, Kate Macneale, Mark Myers, Sandie O'Neill, O. Paul Olson, Sean Sol, Frank Sommers, Jim West, Maryjean Willis, Gina Ylitalo

Authorization:
The NWFSC is hereby authorized to conduct research activities that will take salmonid species listed under the ESA. The taking is subject to the provisions of section 10(a)(1)(A) of the ESA (16 U.S.C. §§ 1531-1543), NMFS regulations governing permits to take listed species, and the conditions set forth in this permit. The species are:

Puget Sound (PS) Chinook salmon (*Oncorhynchus tshawytscha*)
Hood Canal summer-run (HCS) chum salmon (*O. keta*)
PS steelhead (*O. mykiss*).

Abstract:
The NWFSC is hereby authorized to annually take listed salmonids while conducting research

designed to sample outmigrant juvenile salmon from various embayments in the Puget Sound area and screen them for exposure to estrogenic compounds, PBDEs, pharmaceuticals, and personal care products. Juvenile Chinook salmon are anticipated to be the most affected by these contaminants because of their extended estuarine residence, so the NWFSC has chosen them as the target species for this study. The research would benefit Chinook by identifying areas in Puget Sound where they may be at risk due to contaminant exposure, so appropriate toxics reduction activities can be undertaken. The NWFSC proposes to use beach seines to capture fish every six to eight weeks between May and September at approximately seven locations. Up to 60 juvenile Chinook salmon per site per sampling event would be weighed, measured, and euthanized with MS–222. The NWFSC would take bile, plasma, and stomach contents from the fish and then conduct whole-body analyses on them. Juvenile Chinook and other fish species not needed for sample collection would be counted, identified, and released. Any PS Chinook unintentionally killed during the research would be used in lieu of a fish that would otherwise be sacrificed.

A. Take Descriptions and/or Levels

This permit is for activities to be conducted over an approximately five-year period. Annual take levels (listed below) are subject to NMFS' annual authorization process (see Section B - Conditions). Please note these are total yearly take limits for all projects covered by this permit.

Listed Species	Life Stage	Origin	Take Activity	# of Fish Authorized for Take	Authorized Unintentional Mortality	Research Location	Research Period
PS Chinook	Juvenile	Naturally Produced	Capture, Handle, Release	35	0/35	Hood Canal and Puget Sound	May - September
PS Chinook	Juvenile	Listed Hatchery Intact Adipose	Capture, Handle, Release	19	0/19	Hood Canal and Puget Sound	May - September
PS Chinook	Juvenile	Naturally Produced	Intentional Mortality	378	-	Hood Canal and Puget Sound	May - September
PS Chinook	Juvenile	Listed Hatchery Intact Adipose	Intentional Mortality	178	-	Hood Canal and Puget Sound	May - September
HCS Chum	Juvenile	Naturally Produced	Capture, Handle, Release	90	0/90	Hood Canal and Puget Sound	May - September
HCS Chum	Juvenile	Listed Hatchery Intact Adipose	Capture, Handle, Release	42	0/42	Hood Canal and Puget Sound	May - September
PS Steelhead	Juvenile	Naturally Produced	Capture, Handle, Release	50	0/50	Hood Canal and Puget Sound	May - September

Listed Species	Life Stage	Origin	Take Activity	# of Fish Authorized for Take	Authorized Unintentional Mortality	Research Location	Research Period
PS Steelhead	Juvenile	Listed Hatchery Intact Adipose	Capture, Handle, Release	15	0/15	Hood Canal and Puget Sound	May - September

B. Conditions Common to All Research Permits Issued by NMFS' Northwest Region

Not all of these conditions may apply to the specific actions authorized by this permit. Nonetheless, failure to adhere to any condition that does apply may cause NMFS to revoke the permit.

1. The permit holder must ensure that listed species are taken only at the levels, by the means, in the areas and for the purposes stated in the permit application, and according to the conditions in this permit.

2. The permit holder must not intentionally kill or cause to be killed any listed species unless the permit specifically allows intentional lethal take.

3. The permit holder must handle listed fish with extreme care and keep them in cold water to the maximum extent possible during sampling and processing procedures. When fish are transferred or held, a healthy environment must be provided; e.g., the holding units must contain adequate amounts of well-circulated water. When using gear that captures a mix of species, the permit holder must process listed fish first to minimize handling stress.

4. Each researcher must stop capturing and handling listed fish if the water temperature exceeds 70 degrees Fahrenheit at the capture site. Under these conditions, listed fish may only be identified and counted. Additionally, electrofishing is not permitted if water temperatures exceed 64 degrees Fahrenheit.

5. If the permit holder anesthetizes listed fish to avoid injuring or killing them during handling, the fish must be allowed to recover before being released. Fish that are only counted must remain in water and not be anesthetized.

6. The permit holder must use a sterilized needle for each individual injection when passive integrated transponder tags (PIT-tags) are inserted into listed fish.

7. If the permit holder unintentionally captures any listed adult fish while sampling for juveniles, the adult fish must be released without further handling and such take must be reported.

8. The permit holder must exercise care during spawning ground surveys to avoid disturbing listed adult salmonids when they are spawning. Researchers must avoid walking in salmon streams whenever possible, especially where listed salmonids are likely to spawn. Visual observation must be used instead of intrusive sampling methods, especially when just determining fish presence.

9. The permit holder using backpack electrofishing equipment must comply with NMFS' Backpack Electrofishing Guidelines (June 2000) available at http://www.nwr.noaa.gov/ESA-Salmon-Regulations-Permits/4d-Rules/upload/electro2000.pdf.

10. The permit holder must obtain approval from NMFS before changing sampling locations or research protocols.

11. The permit holder must notify NMFS as soon as possible but no later than two days after any authorized level of take is exceeded or if such an event is likely. The permit holder must submit a written report detailing why the authorized take level was exceeded or is likely to be exceeded.

12. The permit holder is responsible for any biological samples collected from listed species as long as they are used for research purposes. The permit holder may not transfer biological samples to anyone not listed in the application without prior written approval from NMFS.

13. The person(s) actually doing the research must carry a copy of this permit while conducting the authorized activities.

14. The permit holder must allow any NMFS employee or representative to accompany field personnel while they conduct the research activities.

15. The permit holder must allow any NMFS employee or representative to inspect any records or facilities related to the permit activities.

16. The permit holder may not transfer or assign this permit to any other person as defined in Section 3(12) of the ESA. This permit ceases to be in effect if transferred or assigned to any other person without NMFS' authorization.

17. NMFS may amend the provisions of this permit after giving the permit holder reasonable notice of the amendment.

18. The permit holder must obtain all other Federal, state, and local permits/authorizations needed for the research activities.

19. On or before January 31st of every year, the permit holder must submit to NMFS a post-season report in the prescribed form describing the research activities, the number of listed fish taken and the location, the type of take, the number of fish intentionally killed and unintentionally killed, the take dates, and a brief summary of the research results. The report must be submitted electronically on our permit website, and the forms can be found at https://apps.nmfs.noaa.gov/. Falsifying annual reports or permit records is a violation of this permit.

20. If the permit holder violates any permit condition they will be subject to any and all penalties provided by the ESA. NMFS may revoke this permit if the authorized activities are not conducted in compliance with the permit and the requirements of the ESA or if NMFS

determines that its ESA section 10(d) findings are no longer valid.

21. If any listed juvenile fish are unintentionally killed during these activities they must be used in place of intentional mortalities.

22. Listed fish mortalities and tissue samples will be analyzed/archived at the NOAA Fisheries laboratory (2725 Montlake Blvd E.; Seattle, WA).

C. Penalties and Permit Sanctions

1. Any person who violates any provision of this permit is subject to civil and criminal penalties, permit sanctions, and forfeiture as authorized under the ESA and 15 CFR part 904 [Civil Procedures].

2. All permits are subject to suspension, revocation, modification, and denial in accordance with the provisions of subpart D [Permit Sanctions and Denials] of 15 CFR part 904.

for _____
William W. Stelle, Jr.
Regional Administrator

March 13, 2012
Date

_____
Lyndal Johnson
Supervisory Zoologist
NMFS Northwest Fisheries Science Center

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Date