NPDES General Permit WAG130000

Federal Aquaculture Facilities and Aquaculture Facilities Located in Indian Country within the Boundaries of Washington State

Biological Evaluation

For

Endangered Species Act Section 7 Consultation

with the

National Marine Fisheries Service and the U.S. Fish and Wildlife Service

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1 INTRODUCTION

The United States Environmental Protection Agency (EPA) EPA, Region 10 is proposing to reissue the National Pollutant Discharge Elimination System (NPDES) General Permit (GP) for federal aquaculture facilities and aquaculture facilities located in Indian Country within the boundaries of the State of Washington (WAG130000). The permit will authorize discharge from approximately 25 facilities throughout the state (see Figure 1 for a map for facility locations; see Table 8 for a list of covered facilities and their locations).

The current permit became effective August 1, 2009 and expired July 31, 2014. Since the permit was not reissued by the expiration date, the conditions of the General Permit will continue in force and effect until a new general permit is issued.

The Endangered Species Act (ESA) requires federal agencies to consult with the U. S. Fish and Wildlife Service (USFWS) and the National Marine Fisheries Service (NMFS) if the federal agency's actions could beneficially or adversely affect any threatened and endangered species or their designated critical habitat. In this case, the federal agency is EPA, and the discretionary action is the issuance of a NPDES general permit (GP) for federal aquaculture facilities and aquaculture facilities located in Indian Country within the boundaries of the State of Washington.

2 THE ACTION: REISSUE NPDES GENERAL PERMIT WAG130000

EPA proposes to reissue the NPDES general permit to establish conditions for the discharge of pollutants in wastewaters from federal fish hatcheries and from aquaculture facilities in Indian Country, as defined in 18 USC §1151, to waters of the United States within the boundaries of the State of Washington. Receiving waters for permittees under this general permit are waters of the U.S. located in Indian Country and waters of the State of Washington (which are also waters of the U.S.) where federal facilities discharge directly to state waters. Surface waters include lakes, rivers, ponds, streams, inland waters, marine waters, and all other surface waters and water courses (for the purposes of this permit, surface waters do not include hatchery ponds, raceways, pollution abatement ponds, settling basins, or wetlands constructed solely for wastewater treatment.

Concentrated Aquatic Animal Production Facilities

At 40 CFR 122.24, EPA defines a concentrated aquatic animal production (CAAP) facility as a point source subject to the NPDES permit program. A hatchery, fish farm, or other facility is a CAAP facility if it grows, contains, or holds, aquatic animals in either of two categories: cold water species or warm water species. The cold water species category includes facilities where animals are produced in ponds, raceways, or other similar structures that discharge at least 30 days per year but does not include facilities that produce less than approximately 9,090 harvest weight kg (approximately 20,000 lbs) of aquatic animals per year. It also does not include facilities that feed less than 2,272 kg (approximately 5,000 lbs) of food during the calendar month of maximum feeding.

Cold water aquatic animals include, but are not limited to, the Salmonidae family of fish, such as trout and salmon; and warm water aquatic animals include, but are not limited to, catfish, sunfish, and minnows. Hatcheries use several production systems, including ponds, flow through systems, recirculating systems, and open water systems.

2.1 THIS GENERAL PERMIT: SCOPE AND ELIGIBILITY

Aquaculture facilities will be eligible for coverage under the General Permit regardless of the type of cold water species being reared, type of production system, or whether discharges are to fresh or marine waters provided that the facility operates for at least 30 days per year, holds at least 20,000 pounds of fish at their maximum, and feeds at least 5,000 pounds of feed in the maximum month of feeding. Acclimation ponds need permit coverage if they meet or exceed these thresholds.

Facilities that the EPA has designated as significant contributors of pollution will also be authorized to discharge under this General Permit. This General Permit applies only to cold water facilities.

Facilities and Discharges Excluded from Coverage

A facility with any of the following types of discharges cannot be covered under this permit and must apply for an individual NPDES permit:

- Discharges from aquaculture facilities that hold less than 20,000 pounds of fish at their maximum or whose month of maximum feeding is less than 5,000 pounds, unless they are designated significant contributors of pollution by the EPA.
- Discharges that do not consist solely of effluent from aquaculture facilities. If a discharge from an aquaculture facility mixes with other wastewater (e.g., domestic wastewater) prior to being discharged, the combined discharge is not covered;
- Discharges from facilities where an NPDES permit has been terminated or denied until the EPA expressly issues an authorization to discharge;
- Discharges that contribute to, or may reasonably be expected to contribute to, a violation of an applicable water quality standard;
- Discharges to impaired waters, designated as such pursuant to Section 303(d) of the CWA, which are water-quality limited for a pollutant of concern evaluated in the development of this permit (BOD5, total suspended solids, settleable solids, nutrients, ammonia, and chlorine), unless a wasteload allocation (WLA) has been given to the facility in a TMDL and is applied in this permit. If a waterbody to which an existing Permittee discharges becomes impaired during the next permit cycle, the Permittee may submit information to the EPA that demonstrates that the discharge is not expected to cause or contribute to an exceedance of water quality standards. Then, the EPA will determine 1) whether the discharge would cause or contribute to an exceedance or impairment, and 2) whether the facility may remain covered under this General Permit in future permit cycles or if an individual permit is needed. New dischargers to impaired waterbodies are not eligible under this General Permit, and must seek permit coverage under an individual permit.
- Discharges from processes not associated with fish hatcheries or farms;
- Discharges from fish hatchery or farm processes where the General Permit does not adequately address the environmental concerns associated with the discharge, as determined by the EPA at the time a discharger seeks coverage under the General Permit;
- Discharges to land or to publicly owned treatment works;
- Discharges to waters that constitute an outstanding national resource, such as waters of national and state parks and wildlife refuges and waters of exceptional recreational or ecological significance;
- Discharges to waters that constitute special resource waters in Indian Country -- waters that comprise a special and/or a unique resource to the Reservation.

Receiving Waters

Receiving waters for Permittees under this General Permit are Waters of the United States located in Indian Country and waters of the State of Washington (which are also Waters of the U.S.) where federal facilities discharge directly to state waters.

States, including eligible Indian Tribes, establish water quality standards for receiving waters within their jurisdictions. Water quality standards are composed of designated beneficial water uses to be achieved and protected, as well as water quality criteria necessary to protect designated uses. Under the provisions

of 40 CFR §131.10, the EPA requires states and eligible Indian Tribes to specify appropriate water uses to be achieved and protected. In designating uses of a water body and the appropriate criteria for those uses, states and eligible Indian Tribes must take into consideration the water quality standards of downstream waters and must ensure that its water quality standards provide for attainment and maintenance of the water quality standards of downstream waters.

Tribal Water Quality Standards

A number of tribes within the State of Washington have developed water quality standards. The EPA has approved water quality standards for the Chehalis, Kalispel, Lummi, Makah, Port Gamble S'Klallam, Puyallup, and Spokane Tribes. The EPA has also promulgated standards for the Confederated Tribes of the Colville Reservation. These standards, applicable to waters within the respective reservations, describe use classifications and the applicable water quality criteria. In addition, the EPA has authorized the Swinomish Indians and the Tulalip Tribes to administer their own water quality standards program, though the EPA has not yet approved water quality standards for these tribes. The EPA has reviewed all of the EPA-approved tribal water quality standards within Washington State and believes that this General Permit will be protective of tribal waters. The EPA has also reviewed the Yakama Nation and Tulalip Tribes' tribally approved water quality standards and believes that the General Permit will also be protective of these waters. For the parameters that are pertinent to this General Permit, tribal water quality standards are either identical or very similar to those of Washington State, and do not require modification of permit conditions.

Washington State Water Quality Standards

In developing the GP, the EPA has also given consideration to water quality standards of the State of Washington, Chapter 173-201A of the Washington Administrative Code, because these standards are applicable to the receiving waters for most of the federal facilities or to waters downstream from many of the aquaculture facilities authorized to discharge under the General Permit.

Washington State standards at Washington Administrative Code (WAC) 173-201A-200 (fresh water) and WAC 173-201A-210 (marine water) establish aquatic life, recreation, water supply, shellfish harvesting, and miscellaneous uses, and those at WAC 173-201A-600 (fresh water) and WAC 173-201A-610 (marine water) designate uses for specific waters in the State. In accordance with WAC 173-201A-600, all fresh waters without specific use designations are to be protected for the designated uses of: salmonid spawning, rearing, and migration; primary contact recreation; domestic, industrial, and agricultural water supply; stock watering; wildlife habitat; harvesting; commerce and navigation; boating; and aesthetic values. The EPA has written this General Permit to be protective of these uses.

2.2 Effluent Limitations and Monitoring Requirements

Effluent Limitations

Prohibited Discharges

*The Permittee must **not** discharge to waters of the U.S. from the hatchery complex:*

- Atlantic salmon (Salmo salar).
- Solids, including sludge and grit that accumulate in raceways or ponds, in off-line or full-flow settling basins, or in other components of the production facility in excess of the applicable limits in this permit.
- Hazardous substances, unless authorized by this permit.
- Untreated cleaning wastewater (e.g., obtained from a vacuum or standpipe bottom drain system or rearing/holding unit disinfection).
- Visible foam or floating, suspended or submerged matter, including fish mortalities, kill spawning, processing wastes, and leachate from these materials, in amounts causing, or contributing to, a nuisance or objectionable condition in the receiving water or that may impair designated beneficial uses in the receiving water.
- Disease control chemicals and drugs except those approved by the Food and Drug Administration and/or the EPA for hatchery use or those reported to the EPA in accordance with the aquaculture specific reporting requirements in the General Permit.
- Toxic substances, including drugs, pesticides, or other chemicals, in toxic amounts that may impair designated uses or violate water quality standards of the receiving water.

Prohibited Practices

The Permittee is prohibited from engaging in any of the following practices or otherwise facilitating prohibited discharges described above:

- Practices that allow accumulated solids in excess of the limits to be discharged to waters of the United States from the permitted facility (e.g., the removal of dam boards in raceways or ponds, the cleaning of settling basins, etc.);
- Sweeping, raking, or otherwise intentionally discharging accumulated solids from raceways, ponds, or settling basins to waters of the United States; and/or
- Containing, growing or holding fish within an off-line or in-line settling basin.

Discharge Limits

Permitted Discharges. During the effective period of the Permittee's authorization to discharge, the Permittee is authorized to discharge pollutants from the outfall(s) specified in its NOI within the limits and subject to the conditions set forth in this permit. This permit authorizes the discharge of only those pollutants resulting from facility processes, waste streams, and operations that have been clearly identified in the NOI, including non-production facilities, such as incubators, laboratories, tagging operations, etc. It does not authorize the discharge of any waste streams, including spills and other unintentional or non-routine discharges of pollutants, that are not part of the normal operation of the facility as disclosed in the Permittee's NOI nor does it authorize the discharge of any pollutants that are not ordinarily present in such waste streams.

Discharge Limits. The Permittee must limit discharges from all outfalls authorized under this permit as specified in Tables 1 and 2, below, as applicable. The limits in Table 1 apply to all hatchery discharges

except those from separate off-line settling basin outfalls and rearing pond discharges during drawdown, limits for which are listed in Table 2. All limits represent maximum effluent limits, unless otherwise indicated. The Permittee must comply with the applicable effluent limits in the tables at all times, unless otherwise indicated, regardless of the frequency of monitoring or reporting.

The proposed effluent limitations are identical to those of the previous General Permit, except for additional clarification about total residual chlorine limits (see the General Permit and Fact Sheet for details). Chlorine limits only apply when chlorine or Chloramine-T is being used.

Table 1

Effluent Limitations for Hatchery Discharges (see GP for details)

Pollutant	Average Monthly Limit	Maximum Daily Limit	Instantaneous Maximum
Net **Total Suspended Solids**	5 mg/L	---	15 mg/L
Net **Settleable Solids**	0.1 ml/L	---	---
Total Residual Chlorine – into fresh water	9.0 µg/L	18.0 µg/L	---
Total Residual Chlorine – into marine water	6.1 µg/L	12.3 µg/L	---

Discharge Limits for Off-Line Settling Basins (OLSBs) and for Raceways or Rearing Ponds during drawdown for fish release.

These limits apply to any discharge to waters of the U.S. from an OLSB in addition to limitations listed in Table 1, above, for the total hatchery flow. These limits apply to raceways or pond systems during drawdown for fish release in lieu of the TSS and settleable solids limits in Table 1, above. See Table 2, below. The total residual chlorine limits set forth in Table 1, above, still apply to raceways or pond systems during drawdown for fish release.

Table 2 Effluent Limits for Discharges from Off-line Settling Basins and from Raceways or Rearing Ponds during Drawdown for Fish Release (see GP for details)	
Pollutant	**Maximum Daily Limit**
Total Suspended Solids	100 mg/L
Settleable Solids	1.0 ml/L

Rearing Vessel Disinfection Water: When rearing vessels are disinfected with chlorine, the total residual chlorine effluent limits in Table 1, above, apply (unless they are allowed to dry completely).

Monitoring Requirements

Effluent Monitoring

In addition to the monitoring requirements in the previous General Permit, the EPA proposes to require two years of continuous temperature monitoring for all facilities covered by this General Permit that discharge to water bodies impaired for temperature. This will ensure that the Permittee is collecting adequate data to assess compliance with the temperature water quality standards. Facilities that discharge to waters impaired for temperature will be required to monitor the effluent, as well as immediately upstream of the facility. The data collected via continuous temperature monitoring may also be used for development of WLAs in an applicable TMDL, or for ESA consultation.

Monitoring in Table 3, below, must be performed before the effluent is discharged to the receiving water. The EPA proposes the following monitoring requirements in this General Permit:

Table 3 Hatchery Effluent Monitoring Requirements (see GP for details)				
Parameter	**Units**	**Sample Type**	**Sample Frequency**	**Sample Location**
Effluent Flow	Gallons per day	Flow meter, calibrated weir, or other approved method	Monthly	Effluent

Table 3 Hatchery Effluent Monitoring Requirements (see GP for details)				
Parameter	**Units**	**Sample Type**	**Sample Frequency**	**Sample Location**
Net Total Suspended Solids	mg/L	Composite	Monthly	Influent & Effluent
Net Settleable Solid	ml/L	Grab	Monthly	Influent & Effluent
Total Residual Chlorine (including when Chloramine-T is in use)	µg/L	Grab	Monthly	Effluent
Temperature (facilities that discharge to waters impaired for temperature)	ºC	Meter	Continuous (2 years)	Upstream & Effluent

Off-line Settling Basin Effluent Monitoring

Discharges to waters of the U.S. from OLSBs must be monitored as required in Table 4, below.

Table 4 Off-Line Settling Basin Effluent Monitoring Requirements (see GP for details)				
Parameter	**Units**	**Sample Type**	**Sample Frequency**	**Sample Location**
Effluent Flow	Gallons per day	Flow meter, calibrated weir, or other approved method	Monthly	Effluent
Total Suspended Solids	mg/L	Grab	Monthly	Effluent
Settleable Solids	ml/L	Grab	Monthly	Effluent
Ammonia	mg/L	Grab	Quarterly	Effluent

Table 4 Off-Line Settling Basin Effluent Monitoring Requirements (see GP for details)				
Parameter	**Units**	**Sample Type**	**Sample Frequency**	**Sample Location**
Temperature	º C.	Meter	Weekly when OLSB is discharging	Effluent
pH	Standard Units	Meter	Quarterly	Effluent

Monitoring Discharges of Rearing Pond and Raceway Drawdowns for Fish Release

Samples for rearing pond and raceway drawdowns for fish release must be collected regardless of amount of fish in the facility. See Table 5, below.

Table 5 Monitoring Requirements for Discharges from Rearing Pond or Raceway Drawdowns for Fish Release (see GP for details)			
Parameter	**Sample Point**	**Sampling Frequency**	**Type of Sample**
Settleable Solids (mL/L)	Effluent	1/Drawdown	Grab
Total Suspended Solids (mg/L)	Effluent	1/Drawdown	Grab

Monitoring Discharges of Rearing Vessel Disinfection Water

Rearing vessel disinfection water that has been treated with chlorine must be tested before it is allowed to be discharged to waters of the United States; see Table 6, below.

Table 6 Monitoring Requirement for Discharges of Rearing Vessel Disinfection Water			
Parameter	**Sample Point**	**Sampling Frequency**	**Type of Sample**
Total Residual Chlorine (mg/L)	Effluent	1/Discharge	Grab

Surface Water Monitoring

Ammonia, Temperature, and pH Monitoring. All Permittees that have off-line settling basins that discharge directly to surface waters must conduct surface water monitoring quarterly for ammonia, pH, and temperature immediately upstream, outside the influence of the discharge.

Sample Collection. All surface water samples must be grab samples and must be collected at approximately the same time as the effluent samples.

Minimum Levels. All samples must be analyzed for the parameters listed in Table 7 to achieve minimum levels (MLs) that are equivalent to or less than those listed in Table 8. The Permittee may request different MLs if its results have consistently been above the required MLs. Such a request must be in writing and must be approved by the EPA before the Permittee may use the revised MLs.

Reporting Surface Water Monitoring Results. All surface water monitoring results must be submitted to the EPA with the DMRs for the month when the monitoring is conducted. The report must include all information required below, and a summary and evaluation of the analytical results.

Table 7 Surface Water Monitoring Requirements	
Parameter	**Units**
Ammonia Nitrogen as N	mg/L
pH	standard units
Temperature	^{o}C

PCB Monitoring for Facilities in the Spokane Watershed

All facilities that discharge to waters in WRIA 54 (Lower Spokane) and WRIA 57 (Middle Spokane) must monitor their effluent for PCB congeners. As of the date of permit issuance, these permit provision applies to two facilities that discharge within these WRIAs: Ford State Fish Hatchery and Spokane Tribal Hatchery.

The EPA is requiring the use of EPA Method 1668C. Permittees must report the total concentration of "dioxin-like" PCB congeners (see Table 8). A complete congener analysis must also be submitted as an attachment to the DMR. PCB monitoring must take place annually, during the calendar quarter of maximum feeding. For any analysis of PCB congeners using EPA Method 1668, the permittee must target MDLs no greater than the MDLs listed in Table 2 of EPA Method 1668 Revision C (EPA-820-R-10-005) and must analyze for each of the 209 individual congeners.

Permittees must follow the Spokane River Regional Toxics Task Force Quality Assurance Project Plan with respect to data validation and blank censoring. The Task Force QAPP addresses this issue in Section 4.2.2, on Pages 40 and 41. Analytes found in samples at concentrations less than 3 times the associated blank concentration will be flagged with a "B" qualifier. The Task Force QAPP states that "all qualified data will be reported with validation qualifiers, however B flagged data will not be used in congener summations for total PCB" (Page 41). See http://srrttf.org/wp-content/uploads/2013/05/QAPP_FINAL_081114.pdf.

Table 8. Dioxin-Like PCB Congeners

Dioxin-Like PCBs IUPAC #	Homolog Group	Substitution Group	IUPAC Name
non-*ortho* substituted PCBs			
77	tetra-CB	non-ortho	3,3',4,4'-tetra-CB
81	tetra-CB	non-ortho	3,4,4',5-tetra-CB
126	penta-CB	non-ortho	3,3',4,4',5-penta-CB
169	hexa-CB	non-ortho	3,3',4,4',5,5'-hexa-CB
mono-*ortho* substituted PCBs			
105	penta-CB	mono-ortho	2,3,3',4,4'-penta-CB
114	penta-CB	mono-ortho	2,3,4,4',5-penta-CB
118	penta-CB	mono-ortho	2,3',4,4',5-penta-CB
123	penta-CB	mono-ortho	2,3',4,4',5-penta-CB
156	hexa-CB	mono-ortho	2,3,3',4,4',5-hexa-CB
157	hexa-CB	mono-ortho	2,3,3',4,4',5'-hexa-CB

167	hexa-CB	mono-ortho	2,3',4,4',5,5'-hexa-CB
189	hepta-CB	mono-ortho	2,3,3',4,4',5,5'-hepta-

In addition to the BMP requirements at section IV.C.5.e.(12) of the General Permit, Permittees in WRIAs 54 and 57 must use any available product testing data to preferentially purchase paint and caulk with the lowest practicable total PCB concentrations.

2.3 Quality Assurance and Best Management Practices Plans

Quality Assurance Plan Development.

The Permittee must develop a quality assurance plan (QA Plan) for all monitoring required by this permit to assist in planning for the collection and analysis of effluent and receiving water samples in support of the permit and in explaining data anomalies when they occur. The plan must be developed and implemented within 60 days after receiving authorization to discharge under this permit. Any existing QA Plans may be modified to meet this requirement.

Existing Permittees must review and update their QA Plans within 60 days of the reissuance of this General Permit.

Plan contents

At a minimum, the QA Plan must include the following:

- Details on the number of samples, type of sample containers, preservation of samples, holding times, analytical methods, analytical detection and quantification limits for each parameter, type and number of quality assurance field samples, precision and accuracy requirements, sample preparation requirements, and sample shipping methods.
- Description of flow measuring devices used to measure influent and/or effluent flow at each point, calibration procedures, and calculations used to convert to flow units. Facilities with multiple effluent discharge points and/or influent points must describe their method of compositing samples from all points proportionally to their respective flows;
- Maps indicating the location of each sampling point;
- Qualification and training of personnel; and
- Name, address and telephone number of the laboratory used by or proposed to be used by the Permittee.

The Permittee must amend the QA Plan whenever there is a modification in sample collection, sample analysis, or other procedure addressed by the QA Plan and must update it whenever there is a change in ownership or operator.

Copies of the QA Plan must be kept on site and made available to the EPA or applicable tribes upon request. If lack of suitable storage area makes on-site storage impossible, the QA Plan must be in the possession of staff whenever they are working on-site.

Best Management Practices Plan

Through implementation of the best management practices (BMP) plan, the Permittee must prevent or minimize the generation and discharge of wastes and pollutants from the facility to waters of the United States to meet water quality standards and permit requirements; the Permittee must also ensure that disposal or land application of wastes is carried out in such a way as to minimize negative environmental impact and, if applicable, to comply with Washington State solid waste disposal regulations.

The Permittee must develop and implement a BMP Plan that meets the specific requirements listed below. An existing BMP Plan may be modified for use under this section. The Permittee must implement the provisions of the BMP Plan as conditions of this permit within 90 days of receiving authorization to discharge under this permit. Existing Permittees must review and update their BMP Plans within 90 days of the reissuance of this General Permit.

Requirements of the BMP Plan

The BMP Plan must include, at a minimum, the following BMPs. Where a particular practice below is infeasible, the Permittee will substitute another practice to achieve the same end.

Materials Storage

- Ensure proper storage of drugs and other chemicals to prevent spills that may result in the discharge to waters of the United States.
- Implement procedures for properly containing, cleaning, and disposing of any spilled materials.

Structural Maintenance

- Routinely inspect rearing and holding units and waste collection and containment systems to identify and promptly repair damage.
- Regularly conduct maintenance of rearing and holding units and waste collection and containment systems to ensure their proper function.

Record keeping

- Document feed amounts and numbers and weights of aquatic animals to calculate feed conversion ratios.
- Document the frequency of cleanings, inspections, maintenance, and repairs.
- Maintain records of all medicinal and therapeutic chemical usage for each treatment at the facility. Include the information required in the Chemical Log Sheet in Appendix D and in the Annual Reports in Appendix E of the General Permit.
- A copy of the label (with treatment application requirements) and the Material Safety Data Sheet (MSDS) must be maintained in the facility's records for each drug or chemical used at the facility.
- In order to show how the maximum concentrations of chlorine and/or Chloramine-T were derived (see Table 3 for monitoring requirements), facilities must maintain records by chemical and by outfall of the approach/analyses used to determine the elapsed time from its application to its

maximum (peak) effluent concentration, giving consideration to retention times within the facility.

- Permittees must keep the records necessary to provide the water-borne treatment/calculations information required on page 7 of the revised Annual Report (see Appendix E of the General Permit).

Training Requirements

- Train all relevant personnel in spill prevention and how to respond in the event of a spill to ensure proper clean-up and disposal of spilled materials.
- Train personnel on proper structural inspection and maintenance of rearing and holding units and waste collection and containment systems.

Operational Requirements

- Raceways and ponds must be cleaned at such a frequency and in such a manner that minimizes accumulated solids discharged to waters of the U.S.
- Fish feeding must be conducted in such a manner as to minimize the discharge of unconsumed food.
- Fish grading, harvesting, egg taking, and other activities within ponds or raceways must be conducted in such a way as to minimize the discharge of accumulated solids and blood wastes.
- Animal mortalities must be removed and disposed of on a regular basis to the greatest extent feasible.
- Water used in the rearing and holding units or hauling trucks that is disinfected with chlorine or other chemicals must be treated before it is discharged to waters of the U.S.
- Treatment equipment used to control the discharge of floating, suspended or submerged matter must be cleaned and maintained at a frequency sufficient to minimize overflow or bypass of the treatment unit by floating, suspended, or submerged matter; turbulent flow must be minimized to avoid entrainment of solids.
- Procedures must be implemented to prevent fish from entering quiescent zones, full-flow, and off-line settling basins. Fish that have entered quiescent zones or basins must be removed as soon as practicable.
- Procedures must be implemented to minimize the release of diseased fish from the facility.
- All drugs and pesticides must be used in accordance with applicable label directions (FIFRA or FDA), except under the certain conditions (see permit for more detail).
- Participation in Investigational New Animal Drug (INAD) studies, using established protocols; or
- Extralabel drug use, as prescribed by a veterinarian.
- Procedures must be identified and implemented to collect, store, and dispose of wastes, such as biological wastes. Such wastes include fish mortalities and other processing solid wastes from aquaculture operations.
- Facilities must dispose of excess/unused disinfectants in a way that does not allow them to enter waters of the U.S.

- Facilities must implement procedures to eliminate the release of Polychlorinated Biphenyls (PCBs) from any known sources in the facility- including paint, caulk, or feed. If removing paint or caulk that was applied prior to 1980, refer to the EPA guidance (abatement steps 1-4) at http://www.epa.gov/epawaste/hazard/tsd/pcbs/pubs/caulk/guide/guide-sect4a.htm. Any future application of paint or caulk must be below the allowable TSCA level of 50 ppm. Facilities must implement purchasing procedures that give preference for fish food that contains the lowest amount of PCBs that is economically and practically feasible.

Notice of Intent and Annual Reports

THE EPA has revised the Notice of Intent and Annual Report for this General Permit. They now require significantly more information from Permittees, especially regarding the use of disease treatment chemicals and water-borne treatments. This additional information will be available for future ESA Section 7 consultations. See Appendices A and E of the General Permit.

Scope of the Action

This action is limited to the NPDES general permit, under EPA's Clean Water Act Section 402 authorities. Under this NPDES permit, the EPA has authority over wastewater discharges from permitted facilities. This NPDES permit does not have jurisdiction over issues related to in-stream flow, fish passage, or water withdrawal.

The federal action under ESA consultation is the reissuance of this NPDES general permit, not all activities at the hatchery. The effects evaluated in this BE are limited to the scope of the permit action.

Interrelated and Interdependent Effects

No interrelated or interdependent activities are anticipated as a result of the proposed action.

3 THE ACTION AREA: WASHINGTON STATE

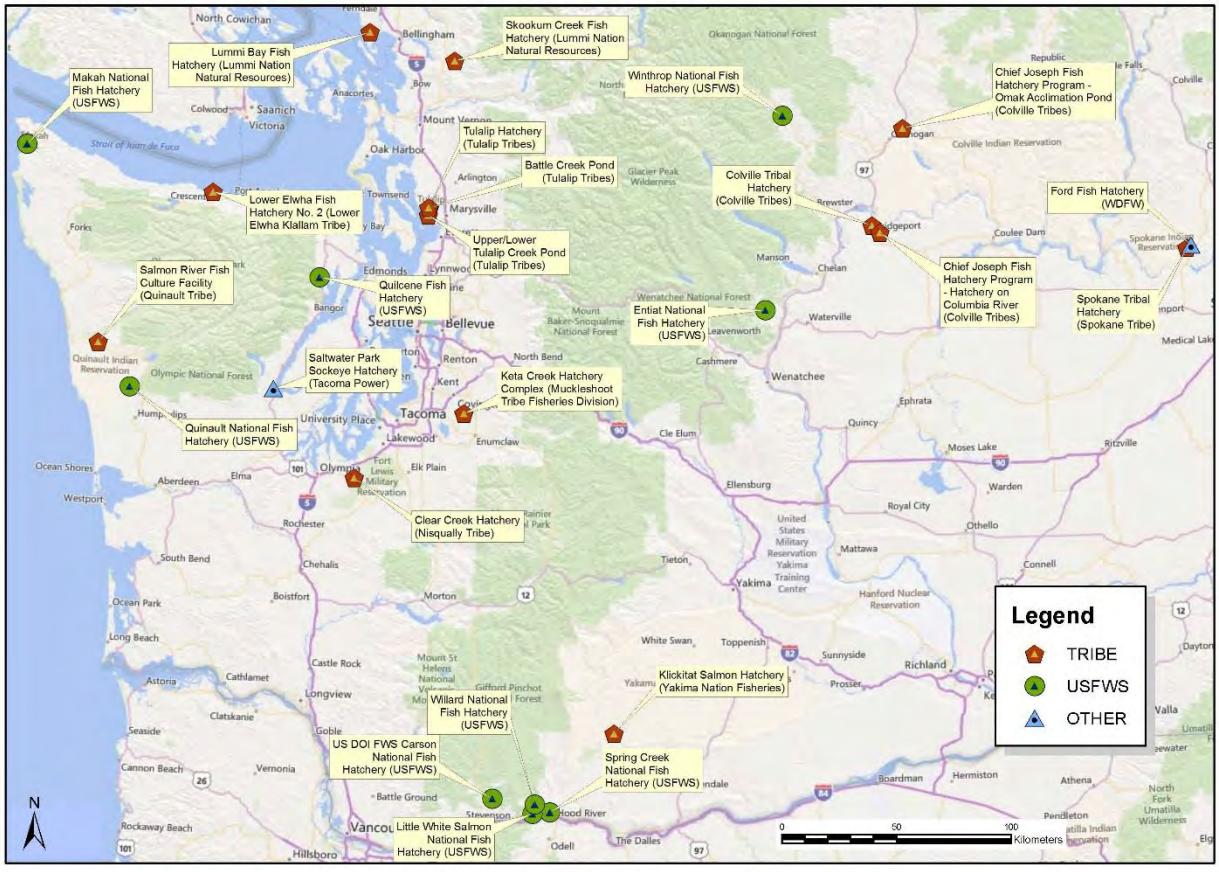
For the purposes of this Biological Evaluation, the EPA is focusing on the receiving water bodies located downstream of the permitted facilities under the NPDES General Permit WAG130000. These receiving water bodies are provided in Table 8 (below) for each of the facilities likely to be covered under this permit. Figure 1 provides a map depicting the locations of facilities currently covered under the GP.

Table 8: Facilities currently covered by WAG130000.

Hatchery Name	Operator	City/Location	Receiving Water
Carson National Fish Hatchery	USFWS	Carson	Wind River
Entiat National Fish Hatchery	USFWS	Entiat	Entiat River
Little White Salmon National Fish Hatchery	USFWS	Cook	Little White Salmon River
Makah National Fish Hatchery	USFWS	Neah Bay	Sooes River
Quinault National Fish Hatchery	USFWS	Humptulips	Cook Creek
Spring Creek National Fish Hatchery	USFWS	Underwood	Columbia River
Willard National Fish Hatchery	USFWS	Cook	Little White Salmon River
Winthrop National Fish Hatchery	USFWS	Winthrop	Methow River
Ford State Fish Hatchery	WDFW	Wellpinit	Chamokane Creek
Salmon River Fish Culture Facility	Quinault Department of Fisheries	Taholah	Salmon River
Tulalip Hatchery	Tulalip Tribes	Tulalip	Tulalip Creek
Upper & Lower Tulalip Creek Ponds	Tulalip Tribes	Tulalip	Tulalip Bay
Battle Creek Pond	Tulalip Tribes	Tulalip	Tulalip Creek, Tulalip Bay
Clear Creek Hatchery	Nisqually Indian Tribe	Olympia	Nisqually River
Colville Tribal Hatchery	Confederated Tribes of the Colville Reservation	Bridgeport	Columbia River
Skookum Creek Fish Hatchery	Lummi Nation Natural Resources	Acme	South Fork Nooksack River
Lummi Bay Fish Hatchery	Lummi Nation Natural Resources	Bellingham	Lummi Bay
Spokane Tribal Hatchery; Permittee is Bonneville Power Association	Spokane Tribe of Indians	Ford	Metamootles/Galbraith Springs
Keta Creek Hatchery Complex	Muckleshoot Indian Tribe - Fisheries Division	Auburn	Crisp Creek
Kickitat Salmon Hatchery; Yakima Nation Fisheries	Yakama Nation Fisheries	Glenwood	Klickitat River

Quilcene National Fish Hatchery	USFWS	Quilcene	Big Quilcene River
House of Salmon; Lower Elwha Fish Hatchery No. 2	Lower Elwha Klallam Tribe	Port Angeles	Elwha River
Chief Joseph Fish Hatchery Program - Omak Acclimation Pond	Confederated Tribes of the Colville Reservation	Omak	Okanogan River
Chief Joseph Fish Hatchery Program - Hatchery on Columbia River	Confederated Tribes of the Colville Reservation	Bridgeport	Columbia River
Saltwater Park Sockeye Hatchery	Tacoma Power	(Yet to be constructed)	So. Hood Canal

Figure 1. Facilities covered by WAG130000.



4 THREATENED AND ENDANGERED SPECIES

As decided in the August 7, 2014 meeting and subsequent coordination between EPA, NMFS, and USFWS, EPA evaluated evaluate the following species and critical habitats. EPA evaluated risks to species, not ESU's of individual species.

ESA Listed Species

1. Spring Chinook Salmon Upper Columbia
2. Chinook Salmon Lower Columbia
3. Chinook Salmon Puget Sound
4. Chum Salmon Columbia River
5. Summer Chum Salmon Hood Canal
6. Coho Salmon Lower Columbia
7. Coho Salmon Columbia River
8. Steelhead Upper Columbia
9. Steelhead Middle Columbia
10. Steelhead Lower Columbia
11. Steelhead Puget Sound
12. Bull Trout
13. Snake River Sockeye
14. Snake River Fall Chinook
15. Snake River Spring/Summer Chinook
16. Snake River Steelhead
17. Southern Resident Killer Whales
18. Puget Sound/Georgia Basin Bocaccio
19. Puget Sound/Georgia Basin Canary Rockfish
20. Puget Sound/Georgia Basin Yelloweye Rockfish
21. Pacific Eulachon
22. Oregon Spotted Frog
23. Marbled Murrelet

Critical Habitat

1. Bull Trout
2. Spring Chinook Salmon Upper Columbia
3. Chinook Salmon Lower Columbia
4. Chinook Salmon Puget Sound
5. Chum Salmon Columbia
6. Summer Chum Salmon Hood Canal
7. Eulachon
8. Steelhead Upper Columbia
9. Steelhead Middle Columbia
10. Steelhead Lower Columbia
11. Steelhead Snake River
12. Snake River Sockeye
13. Snake River Fall Chinook

14. Snake River Spring/Summer Chinook
15. Southern Resident Killer Whales
16. Proposed for critical habitat: Lower Columbia River Coho

As decided in the August 7, 2014 meeting and subsequent coordination *between EPA, NMFS, and USFWS, EPA did **not** evaluate the following species:*

- Green sturgeon
- Short-tailed albatross
- Western snowy plover
- Blue whale
- Fin whale
- Humpback whale
- Sei whale
- Sperm whale
- Green sea turtle
- Leatherback turtle
- Loggerhead turtle

4.1 Species Descriptions

Chinook Salmon *(Oncorhynchus tshawytscha)*

Chinook salmon are easily the largest of any salmon, with adults often exceeding 40 pounds (18 kg); individuals over 120 pounds (55 kg) have been reported. Chinook mature at about 36 inches and 30 pounds. Chinook salmon are very similar to coho salmon in appearance while at sea (blue-green back with silver flanks), except for their large size, small black spots on both lobes of the tail, and black pigment along the base of the teeth.

Adults migrate from a marine environment into the freshwater streams and rivers of their birth in order to mate (called anadromy). They spawn only once and then die (called semelparity). They feed on terrestrial and aquatic insects, amphipods, and other crustaceans while young, and primarily on other fishes when older.

Populations exhibit considerable variability in size and age of maturation, and at least some portion of this variation is genetically determined. There is a relationship between small size and long distance of migration that may also reflect the earlier timing of river entry and the cessation of feeding for Chinook salmon stocks that migrate to the upper reaches of river systems. Body size, which is related to age, may be an important factor in migration and spawning bed, or redd, construction success.

Juvenile Chinook may spend from 3 months to 2 years in freshwater before migrating to estuarine areas as smolts and then into the ocean to feed and mature. Chinook salmon remain at sea for 1 to 6 years

(more commonly 2 to 4 years), with the exception of a small proportion of yearling males (called jack salmon) which mature in freshwater or return after 2 or 3 months in salt water.

There are different seasonal (i.e., spring, summer, fall, or winter) "runs" in the migration of Chinook salmon from the ocean to freshwater, even within a single river system. These runs have been identified on the basis of when adult Chinook salmon enter freshwater to begin their spawning migration. However, distinct runs also differ in the degree of maturation at the time of river entry, the temperature and flow characteristics of their spawning site, and their actual time of spawning. Freshwater entry and spawning timing are believed to be related to local temperature and water flow regimes.

Adult female Chinook will prepare a redd (or nest) in a stream area with suitable gravel type composition, water depth and velocity. The adult female Chinook may deposit eggs in 4 to 5 "nesting pockets" within a single redd. Spawning sites have larger gravel and more water flow up through the gravel than the sites used by other Pacific salmon. After laying eggs in a redd, adult Chinook will guard the redd from just a few days to nearly a month before dying. Chinook salmon eggs will hatch, depending upon water temperatures, 3 to 5 months after deposition. Eggs are deposited at a time to ensure that young salmon fry emerge during the following spring when the river or estuary productivity is sufficient for juvenile survival and growth.

As the time for migration to the sea approaches, juveniles lose their parr marks, the pattern of vertical bars and spots useful for camouflage. They then gain the dark back and light belly coloration used by fish living in open water. Chinook salmon seek deeper water, avoid light, and their gills and kidneys begin to change so that they can process salt water.

Salmonid species on the west coast of the U.S. have experienced dramatic declines in abundance during the past several decades as a result of various human-induced and natural factors. There is no single factor solely responsible for this decline, given the complexity of the salmon species life history and the ecosystem in which they reside.[1]

Chum Salmon *(Oncorhynchus keta)*

Second only to Chinook salmon in adult size, chum salmon individuals have been reported up to 3.6 feet (1.1 m) and 45 pounds (20 kg). However, average weight is around 8 to 15 pounds (3.6 to 6.8 kg). Chum salmon are best known for the enormous canine-like fangs and striking body color of spawning males (a calico pattern, with the front two-thirds of the flank marked by a bold, jagged, reddish line and the posterior third by a jagged black line). Females are less flamboyantly colored and lack the extreme dentition of the males. Ocean stage chum salmon are metallic greenish-blue along the back with black speckles.

In order to mate, chum salmon adults migrate from a marine environment into the freshwater streams and rivers of their birth. They spawn only once and then die. Unlike most species that rear extensively in fresh water, chum salmon form schools, presumably to reduce predation. Chum salmon feed on insects

[1] http://www.nmfs.noaa.gov/pr/species/fish/chinooksalmon.htm

and marine invertebrates while in rivers. As adults, their diet consists of "copepods", fishes, "mollusks", squid, and "tunicates." Chum salmon spawn in the lowermost reaches of rivers and streams, typically within 62 miles (100 km) of the ocean. Spawning sites are often near springs. They migrate almost immediately after hatching to estuarine and ocean waters, in contrast to other Pacific salmonids, which migrate to sea after months or even years in fresh water. This means that survival and growth in juvenile chum salmon depend less on freshwater conditions than on favorable estuarine and marine conditions.

Critical habitat was designated on September 2, 2005, for the threatened Columbia River ESU and Hood Canal Summer-run ESU. The species has the widest natural geographic and spawning distribution of any Pacific salmonid, primarily because its range extends farther along the shores of the Arctic Ocean than that of the other salmonids. Spawning populations are known from Korea and Japan and into the far north of Russia. Historically, in North America, chum salmon were distributed throughout the coastal regions of western Canada and the United States, as far south as Monterey, California. Presently, major spawning populations are found only as far south as Tillamook Bay on the northern Oregon coast. Chum salmon may historically have been the most abundant of all Pacific salmonids. Seven of 16 historical spawning populations in the Hood Canal Summer-run ESU are extinct. Recently some of these populations have shown encouraging increases in numbers, but the 2005 status review report [pdf] [6.3 MB] shows that the population trend overall is a 6% decline per year. In the Columbia River, historical populations reached hundreds of thousands to a million adults each year. In the past 50 years, the average has been a few thousand a year. Currently, it is thought that 14 of the 16 spawning populations in the Columbia River ESU are extinct. About 500 spawners occur in the ESU presently, and the long-term trend is flat. Salmonid species on the west coast of the United States have experienced dramatic declines in abundance during the past several decades as a result of various human-induced and natural factors. There is no single factor solely responsible for this decline, given the complexity of the salmon species life history and the ecosystem in which they reside.[2]

Coho Salmon *(Oncorhynchus kisutch)*

The size of an adult coho may measure more than 2 feet (60 cm) in length and can weigh up to 35 pounds (16 kg). However, the average weight of adult coho is 8 pounds (3.6 kg). Coho salmon have dark metallic blue or greenish backs with silver sides and a light belly and there are small black spots on the back and upper lobe of the tail while in the ocean. The gumline in the lower jaw has lighter pigment than does the Chinook salmon. Spawning fish in inland rivers are dark with reddish-maroon coloration on the sides.

Coho salmon adults migrate from a marine environment into freshwater streams and rivers of their birth in order to mate. They spawn only once and then die. Adults return to their stream of origin to spawn and die, usually at around three years old. Some precocious males known as "jacks" return as two-year-old spawners. Spawning males develop a strongly hooked snout and large teeth. Females prepare several redds (nests) where the eggs will remain for 6-7 weeks until they hatch. As the time for migration to the sea approaches, juvenile coho salmon lose their parr marks, a pattern of vertical bars and spots useful for camouflage, and gain the dark back and light belly coloration used by fish living in

[2] http://www.nmfs.noaa.gov/pr/species/fish/chumsalmon.htm

open water. Their gills and kidneys also begin to change at this time so that they can process salt water. In their freshwater stages, coho feed on plankton and insects, and switch to a diet of small fishes as adults in the ocean. Coho spend approximately the first half of their life cycle rearing and feeding in streams and small freshwater tributaries. Spawning habitat is small streams with stable gravel substrates. The remainder of the life cycle is spent foraging in estuarine and marine waters of the Pacific Ocean. Critical habitat was designated on May 5, 1999 for the Central California Coast and Southern Oregon/ Northern California Coast coho salmon.

The species was historically distributed throughout the North Pacific Ocean from central California to Point Hope, Alaska, through the Aleutian Islands, and from the Anadyr River, Russia, south to Hokkaido, Japan. Coho probably inhabited most coastal streams in Washington, Oregon, and central and northern California. Some populations, now considered extinct, are believed to have migrated hundreds of miles inland to spawn in tributaries of the upper Columbia River in Washington, and the Snake River in Idaho. Coho still occur in Alaska as well. The long term trend for the listed populations is still downward, though there was one recent good year with an increasing trend in 2001. Salmonid species on the west coast of the United States have experienced dramatic declines in abundance during the past several decades as a result of various human-induced and natural factors. There is no single factor solely responsible for this decline, given the complexity of the salmon species life history and the ecosystem in which they reside.[3]

Steelhead Trout *(Oncorhynchus mykiss)*

Steelhead trout can reach up to 55 pounds (25 kg) in weight and 45 inches (120 cm) in length, though average size is much smaller. They are usually dark-olive in color, shading to silvery-white on the underside with a heavily speckled body and a pink to red stripe running along their sides. They are a unique species; individuals develop differently depending on their environment. While all *O. mykiss* hatch in gravel-bottomed, fast-flowing, well-oxygenated rivers and streams, some stay in fresh water all their lives. These fish are called rainbow trout. The steelhead that migrate to the ocean develop a slimmer profile, become more silvery in color, and typically grow much larger than the rainbow trout that remain in fresh water.

Adults migrate from a marine environment into the freshwater streams and rivers of their birth in order to mate. Unlike other Pacific salmonids, they can spawn more than one time (called iteroparity). Migrations can be hundreds of miles. Young animals feed primarily on zooplankton. Adults feed on aquatic and terrestrial insects, mollusks, crustaceans, fish eggs, minnows, and other small fishes (including other trout).

Maximum age is about 11 years. Males mature generally at 2 years and females at 3 years. Juvenile steelhead may spend up to 7 years in freshwater before migrating to estuarine areas as smolts and then into the ocean to feed and mature. They can then remain at sea for up to 3 years before returning to freshwater to spawn. Some populations actually return to freshwater after their first season in the

[3] http://www.nmfs.noaa.gov/pr/species/fish/cohosalmon.htm

ocean, but do not spawn, and then return to the sea after one winter season in freshwater. Timing of return to the ocean can vary, and even within a stream system there can be different seasonal runs.

Steelhead can be divided into two basic reproductive types, based on the state of sexual maturity at the time of river entry and duration of spawning migration: stream-maturing and ocean-maturing. The stream-maturing type (summer-run steelhead in the Pacific Northwest and northern California) enters freshwater in a sexually immature condition between May and October and requires several months to mature and spawn. The ocean-maturing type (winter-run steelhead in the Pacific Northwest and northern California) enters freshwater between November and April, with well-developed gonads, and spawns shortly thereafter. Coastal streams are dominated by winter-run steelhead, whereas inland steelhead of the Columbia River basin are almost exclusively summer-run steelhead.

Adult female steelhead will prepare a redd (or nest) in a stream area with suitable gravel type composition, water depth, and velocity. The adult female may deposit eggs in 4 to 5 "nesting pockets" within a single redd. The eggs hatch in 3 to 4 weeks. Steelhead are capable of surviving in a wide range of temperature conditions. They do best where dissolved oxygen concentration is at least 7 parts per million. In streams, deep low-velocity pools are important wintering habitats. Spawning habitat consists of gravel substrates free of excessive silt. Critical habitat for 10 west coast steelhead DPSs was designated on September 2, 2005.

In the United States, steelhead trout are found along the entire Pacific Coast. Worldwide, steelhead are naturally found in the Western Pacific south through the Kamchatka peninsula. They have been introduced worldwide. In recent years, some populations have shown encouraging increases in population size while others have not. Salmonid species on the west coast of the United States have experienced dramatic declines in abundance during the past several decades as a result of various human-induced and natural factors. However, given the complexity of the salmon species life history and the ecosystem in which they reside, there is no single factor solely responsible for this decline.

Bull Trout *(Salvelinus confluentus)*

Bull trout (Salvelinus confluentus) are members of the family Salmonidae and are char native Washington, Oregon, Idaho, Nevada, Montana and western Canada. Compared to other salmonids, bull trout have more specific habitat requirements that appear to influence their distribution and abundance. They need cold water to survive, so they are seldom found in waters where temperatures exceed 59 to 64 degrees (F). They also require stable stream channels, clean spawning and rearing gravel, complex and diverse cover, and unblocked migratory corridors. Bull trout may be distinguished from brook trout (Salvelinus fontinalis) by several characteristics: spots never appear on the dorsal (back) fin, and the spots that rest on the fish's olive green to bronze back are pale yellow, orange or salmon-colored. The bull trout's tail is not deeply forked as is the case with lake trout (Salvelinus namaycush).

Bull trout exhibit two forms: resident and migratory. Resident bull trout spend their entire lives in the same stream/creek. Migratory bull trout move to larger bodies of water to overwinter and then migrate back to smaller waters to reproduce. An anadromous form of bull trout also exists in the Coastal-Puget Sound population, which spawns in rivers and streams but rears young in the ocean. Resident and

juvenile bull trout prey on invertebrates and small fish. Adult migratory bull trout primarily eat fish. Resident bull trout range up to 10 inches long and migratory forms may range up to 35 inches and up to 32 pounds. Bull trout are currently listed coterminously as a threatened species.[4]

Sockeye Salmon *(Oncorhynchus nerka)*

The size of an adult returning to spawn may measure up to 2.8 feet (86 cm) in length and weigh an average of 8 pounds (3.6 kg). The adult spawners are unique in appearance. They typically turn bright red, with a green head; hence they are commonly called "red" salmon in Alaska. During the ocean and adult migratory phase, sockeye often have a bluish back and silver sides, giving rise to another common name, "bluebacks."

Adults migrate from a marine environment into freshwater streams and rivers or lakes of their birth in order to mate. They spawn only once and then die. Sockeye salmon exhibit a wide variety of life history patterns that reflect varying dependency on the freshwater environment. With the exception of certain river-type and sea-type populations, the vast majority of sockeye salmon spawn in or near lakes, where the juveniles rear for 1 to 3 years prior to migrating to sea. For this reason, the major distribution and abundance of large sockeye salmon stocks are closely related to the location of rivers that have accessible lakes in their watersheds for juvenile rearing. Females spawn in 3 to 5 redds (nests) over a couple of days. Hatching usually occurs after 6 to 9 weeks. Most sockeye fry then rear in lakes where they feed on aquatic insects and "plankton".

As the time for migration to the sea approaches for the anadromous forms, the juvenile loses its parr marks, which are a pattern of vertical bars and spots useful for camouflage. They then gain the dark back and light belly coloration used by fish living in open water. During this time their gills and kidneys begin to change so that they can process salt water. These "smolts", as they are called, initially stay close to the shore and feed on insects and plankton. Once they move offshore, their diet turns mainly to "amphipods", "copepods", squid, and some fishes.

Most sockeye salmon stay at sea for 2 years, returning to spawn at about age 4, but some may be 5-6 years old when they spawn.

There are some sockeye that are non-anadromous, meaning that they spend their entire lives in freshwater. Non-anadromous *Oncorhynchus nerka* in the Pacific Northwest are known as "kokanee." Occasionally, a proportion of the juveniles in an anadromous sockeye salmon population will remain in their rearing lake environment throughout life and will be observed on the spawning grounds together with their anadromous siblings. Taxonomically, the kokanee and sockeye salmon do not differ.

Sockeye spend approximately the first half of their life cycle rearing in lakes. The remainder of the life cycle is spent foraging in estuarine and marine waters of the Pacific Ocean. Critical habitat was

[4] http://ecos.fws.gov/speciesProfile/profile/speciesProfile.action?spcode=E065

designated for the Snake River ESU on December 28, 1993 and for the Ozette Lake ESU on September 2, 2005.

On the Pacific coast, sockeye salmon inhabit riverine, marine, and lake environments from the Klamath River and its tributaries north and west to the Kuskokwim River in western Alaska. As they generally require lakes for part of their life cycle, their distribution in river systems depends on the presence of usable lakes in the system, and thus can be more intermittent than for other Pacific salmon.

They are the third most abundant of the seven species of Pacific salmon, after pink salmon and chum salmon. However, the Snake River ESU has remained at very low levels of only a few hundred fish, though there have been recent increases in the number of hatchery reared fish returning to spawn. Salmonid species on the west coast of the United States have experienced dramatic declines in abundance during the past several decades as a result of various human-induced and natural factors. There is no single factor solely responsible for this decline, given the complexity of the salmon species life history and the ecosystem in which they reside.[5]

Southern Resident Killer Whale *(Orcinus orca)*

Killer whales most widely distributed marine mammals, found in all parts of the oceans; most abundant in colder waters, including Antarctica, the North Atlantic and Pacific Oceans. They also occur, though at lower densities, in tropical, subtropical, and offshore waters. Killer whales are generally considered monotypic (belonging to one species). However, genetic studies and morphological evidence have led many cetacean biologists to now consider the existence of multiple species or subspecies of killer whales worldwide.

The species shows considerable size "dimorphism". Adult males develop larger pectoral flippers, dorsal fins, tail flukes, and girths than females. Female killer whales reach sexual maturity when they grow to about 15-18 feet (4.6 m-5.4 m) long, depending on geographic region. The gestation period for killer whales varies from 15-18 months. Birth may take place in any month--there is no distinct calving season. Calves are nursed for at least 1 year, and may be weaned between 1-2 years old. The birth rate for killer whales is not well understood, but, in some populations, is estimated as every 5 years for an average period of 25 years.

Killer whales are highly social animals that occur primarily in relatively stable social groups that often range in size from 2 to 15 animals. Larger groups (rarely as large as several hundred individuals) occasionally form, but are usually considered temporary groupings of smaller social units that probably congregate for seasonal concentrations of prey, social interaction, or mating. Single whales, usually adult males, also occur in Bigg's killer whale populations (as discussed below). Differences in spatial distribution, abundance, behavior, and availability of food resources probably account for much of the variation in group size among killer whale populations.

[5] http://www.nmfs.noaa.gov/pr/species/fish/sockeyesalmon.htm

Scientific studies have revealed many different populations--or even potentially different species or subspecies--of killer whales worldwide. These different populations of killer whales may exhibit different dietary needs, behavior patterns, social structures, and habitat preferences. Therefore, interbreeding is not expected to occur between different populations, in spite of the overlap between home ranges.

The most well-studied killer whale populations occur in the eastern North Pacific Ocean. Three distinct forms, or ecotypes, of killer whales are recognized: Resident, Transient (or Bigg's), and Offshore. The three types differ in morphology, ecology, behavior, and genetics. A recent genetic study suggests the transient type has been separated from all other killer whales for approximately 750,000 years and might represent a separate species or subspecies, known among researchers as Bigg's killer whales. All three types of killer whales share at least part of a home range, yet they are not known to intermix with one another. The resident and transient types both have multiple populations within their range.

Killer whales are widely distributed around the world, but the Southern Residents population is listed as "endangered" under the ESA. Resident killer whale populations in the eastern North Pacific mainly feed on salmonids, showing a strong preference for Chinook salmon. Southern Resident killer whales are the only known resident population to occur in the U.S. Southern residents are comprised of three pods: J, K, and L pods. The Southern Residents are considered one "stock" under the Marine Mammal Protection Act (MMPA) and one "distinct population segment" (therefore, "species") under the Endangered Species Act (ESA).

The Southern Resident Killer Whale population is currently estimated at about 80 whales, a decline from its estimated historical level of about 200 during the late 1800s. Beginning in the late 1960s, the live-capture fishery for oceanarium display removed an estimated 47 whales and caused an immediate decline in Southern Resident numbers. The population fell an estimated 30% to about 67 whales by 1971. By 2003, the population increased to 83 whales. Due to its small population size, NMFS listed this segment of the population as endangered under the Endangered Species Act (ESA) in 2005 and designated critical habitat in 2006.

Their range during the spring, summer, and fall includes the inland waterways of Washington state and the transboundary waters between the United States and Canada. Relatively little is known about the winter movements and range of the Southern Resident stock. However, in recent years, they have been regularly spotted as far south as central California during the winter months.[6]

Bocaccio *(Sebastes paucispinis)*

Bocaccio are large Pacific coast rockfish that reach up to 3 feet (1 m) in length. They have a distinctively long jaw extending to at least the eye socket. Their body ranges in color from olive to burnt orange or brown as adults. Young bocaccio are light bronze in color and have small brown spots on their sides. Rockfishes are unusual among the bony fishes in that fertilization and embryo development is internal, and female rockfish give birth to live larval young. Larvae are found in surface waters, and may be distributed over a wide area extending several hundred miles offshore. "Fecundity" in female bocaccio ranges from 20,000 to over 2 million eggs, considerably more than many other rockfish species. Larvae

[6] http://www.nmfs.noaa.gov/pr/species/mammals/cetaceans/killerwhale.htm

and small juvenile rockfish may remain in open waters for several months, being passively dispersed by ocean currents.

Larval rockfish feed on diatoms, dinoflagellates, tintinnids, and cladocerans, and juveniles consume copepods and euphausiids of all life stages. Adults eat demersal invertebrates and small fishes, including other species of rockfish, associated with kelp beds, rocky reefs, pinnacles, and sharp dropoffs. Approximately 50 percent of adult bocaccio mature in 4 to 6 years. Bocaccio are difficult to age but are suspected to live as long as 50 years.

Bocaccio are most common between 160 and 820 feet (50-250 m) depth, but may be found as deep as 1,560 feet (475m). Adults generally move into deeper water as they increase in size and age but usually exhibit strong site fidelity to rocky bottoms and outcrops. Juveniles and subadults may be more common than adults in shallower water, and are associated with rocky reefs, kelp canopies, and artificial structures, such as piers and oil platforms.

NMFS proposed designation of critical habitat for yelloweye rockfish, canary rockfish, and bocaccio of the Puget Sound/ Georgia Basin in August 2013. Bocaccio range from Punta Blanca, Baja California, to the Gulf of Alaska off Krozoff and Kodiak Islands. They are most common between Oregon and northern Baja California. In Puget Sound, most bocaccio are found south of Tacoma Narrows.

Recreational catch and effort data spanning 12 years from the mid-1970s to mid-1990s suggests possible declines in abundance in Washington. Additional data over this period show the number of angler trips increased substantially and the average number of rockfish caught per trip declined. Taken together, these data suggest declines in the population over time. Currently there are no survey data being taken for this species, but few of these fish are caught by fishermen and none have been caught by Washington state biological surveys in 20 years, suggesting a very low population abundance. They are thought to be at an abundance that is less than 10% of their unfished abundance. A 2005 stock assessment by NOAA Fisheries suggests bocaccio there have higher populations than was thought to be the case.

Bocaccio are fished directly and are often caught as bycatch in other fisheries, including those for salmon. Adverse environmental factors led to recruitment failures in the early- to mid-1990s. Various state restrictions on fishing have been put in place over the years. Current regulations in the state of Washington, where the species is most at risk, limit the daily rockfish catch to three rockfish total (of any species). Because this species is so slow-growing, late to mature, and long-lived, recovery from the above threats will take many years, even if the threats are no longer affecting the species. In April 2010, NMFS listed the Puget Sound/ Georgia Basin DPS as Endangered.[7]

Canary Rockfish *(Sebastes pinniger)*

Canary rockfish are large rockfish that reach up to 2.5 feet (77 cm) in length and 10 pounds (4 kg) in weight. Adults have bright yellow to orange mottling over gray, 3 orange stripes across the head, and orange fins. Animals less than 14 inches long have dark markings on the posterior part of the spiny dorsal fin and gray along the lateral line.

[7] http://www.nmfs.noaa.gov/pr/species/fish/bocaccio.htm

Rockfishes are unusual among the bony fishes in that fertilization and embryo development is internal and female rockfish give birth to live larval young. Larvae are found in surface waters and may be distributed over a wide area extending several hundred miles offshore. "Fecundity" in female canary rockfish ranges from 260,000 to 1.9 million eggs, considerably more than many other rockfish species. Larvae and small juvenile rockfish may remain in open waters for several months, being passively dispersed by ocean currents. Larval rockfish feed on diatoms, dinoflagellates, tintinnids, and cladocerans, and juveniles consume copepods and euphausiids of all life stages. Adults eat demersal invertebrates and small fishes, including other species of rockfish, associated with kelp beds, rocky reefs, pinnacles, and sharp dropoffs. Approximately 50 percent of adult canary rockfish are mature at 14 inches (36 cm) total length (about 5 to 6 years of age). Canary rockfish can live to be 75 years old. Canary rockfish primarily inhabit waters 160 to 820 feet (50 to 250 m) deep but may be found to 1400 feet (425 m). Juveniles and subadults tend to be more common than adults in shallow water and are associated with rocky reefs, kelp canopies, and artificial structures, such as piers and oil platforms. Adults generally move into deeper water as they increase in size and age but usually exhibit strong site fidelity to rocky bottoms and outcrops where they hover in loose groups just above the bottom.

NMFS proposed designation of critical habitat for yelloweye rockfish, canary rockfish, and bocaccio of the Puget Sound/ Georgia Basin in August 2013. Canary rockfish range between Punta Colnett, Baja California, and the Western Gulf of Alaska. Within this range, canary rockfish are most common off the coast of central Oregon.

Recreational catch and effort data spanning 12 years from the mid-1970s to mid-1990s suggests possible declines in abundance. While catch data are generally constant over this time period, the number of angler trips increased substantially, and the average number of canary rockfish caught per trip declined. Taken together, these data suggest declines in the population over time. Currently there are no survey data being taken for this species, but few of these fish are currently caught by fishermen, suggesting a low population abundance. Canary rockfish used to be one of the three principal species caught in Puget Sound in the 1960s.

Canary rockfish are fished directly and are often caught as bycatch in other fisheries, including those for salmon. Adverse environmental factors led to recruitment failures in the early- to mid-1990s. Various state restrictions on fishing have been put in place over the years, including banning retention of canary rockfish in Washington in 2003. Because this species is slow growing, late to mature, and long-lived, recovery from these threats will take many years, even if the threats are no longer affecting the species. In April 2010, NMFS listed the Puget Sound/ Georgia Basin DPS as Threatened.[8]

Yelloweye Rockfish *(Sebastes ruberrimus)*

Yelloweye rockfish are very large rockfish that reach up to 3.5 feet (~1 m) in length and 39 pounds (18 kg) in weight. They are orange-red to orange-yellow in color and may have black on their fin tips. Their

[8] http://www.nmfs.noaa.gov/pr/species/fish/canaryrockfish.htm

eyes are bright yellow. Adults usually have a light to white stripe on the lateral line; juveniles have 2 light stripes, one on the lateral line and a shorter one below the lateral line.

Rockfishes are unusual among the bony fishes in that fertilization and embryo development is internal and female rockfish give birth to live larval young. Larvae are found in surface waters and may be distributed over a wide area extending several hundred miles offshore. "Fecundity" in female yelloweye rockfish ranges from 1.2 to 2.7 million eggs, considerably more than many other rockfish species. Larvae and small juvenile rockfish may remain in open waters for several months being passively dispersed by ocean currents. Larval rockfish feed on diatoms, dinoflagellates, tintinnids, and cladocerans, and juveniles consume copepods and euphausiids of all life stages. Adults eat demersal invertebrates and small fishes, including other species of rockfish, associated with kelp beds, rocky reefs, pinnacles, and sharp dropoffs. Approximately 50 percent of adult yelloweye rockfish are mature by 16 inches (41 cm) total length (about 6 years of age). Yelloweye rockfish are among the longest lived of rockfishes, living up to 118 years old.

Juveniles and subadults tend to be more common than adults in shallower water, and are associated with rocky reefs, kelp canopies, and artificial structures such as piers and oil platforms. Adults generally move into deeper water as they increase in size and age, but usually exhibit strong site fidelity to rocky bottoms and outcrops. Yelloweye rockfish occur in waters 80 to 1560 feet (25 to 475 m) deep, but are most commonly found between 300 to 590 feet (91 to 180 m). NMFS proposed designation of critical habitat for yelloweye rockfish, canary rockfish, and bocaccio of the Puget Sound/ Georgia Basin in August 2013. Yelloweye rockfish range from northern Baja California to the Aleutian Islands, Alaska, but are most common from central California northward to the Gulf of Alaska.

Recreational catch and effort data spanning 12 years from the mid-1970s to mid-1990s suggests possible declines in abundance. While catch data are generally constant over time, the number of angler trips increased substantially, and there was a decline in the average number of rockfish caught per trip. Taken together, these data suggest declines in the population over time. Currently there are no survey data being taken for this species, but few of these fish are caught by fishermen, suggesting a low population abundance. Yelloweye rockfish are fished directly and are often caught as bycatch in other fisheries, including those for salmon. Adverse environmental factors led to recruitment failures in the early- to mid-1990s. Various state restrictions on fishing have been put in place over the years leading to the current ban on retention of yelloweye rockfish in Washington in 2003. Because this species is slow growing, late to mature, and long-lived, recovery from these threats will take many years, even if the threats are no longer affecting the species. In April 2010, NMFS listed the Puget Sound/ Georgia Basin DPS as Threatened.[9]

Eulachon *(Thaleichthys pacificus)*

Eulachon (commonly called smelt, candlefish, or hooligan) are a small, anadromous fish from the eastern Pacific Ocean. They are distinguished by large canine teeth on the bone in the roof of the mouth ("vomer") and 18 to 23 rays in their anal fin. Like Pacific salmon they have an "adipose fin"; it is sickle-

[9] http://www.nmfs.noaa.gov/pr/species/fish/yelloweyerockfish.htm

shaped. The paired fins are longer in males than in females. All fins have well-developed breeding tubercles (raised tissue "bumps") in ripe males, but these are poorly developed or absent in females. As adults, they are brown to blue on their backs and on top of their heads, lighter to silvery white on the sides, and white on the ventral surface. Their backs may have fine, sparse speckling. They feed on plankton but only while at sea.

Eulachon typically spend 3 to 5 years in saltwater before returning to freshwater to spawn from late winter through mid spring. During spawning, males have a distinctly raised ridge along the middle of their bodies. Eggs are fertilized in the water column. After fertilization, the eggs sink and adhere to the river bottom, typically in areas of gravel and coarse sand. Most eulachon adults die after spawning. Eulachon eggs hatch in 20 to 40 days. The larvae are then carried downstream and are dispersed by estuarine and ocean currents shortly after hatching. Juvenile eulachon move from shallow nearshore areas to mid-depth areas. Within the Columbia River Basin, the major and most consistent spawning runs occur in the mainstem of the Columbia River as far upstream as the Bonneville Dam, and in the Cowlitz River. Eulachon occur in nearshore ocean waters and to 1,000 feet (300 m) in depth, except for the brief spawning runs into their natal (birth) streams. Spawning grounds are typically in the lower reaches of larger snowmelt-fed rivers with water temperatures ranging from 39 to 50°F (4 to 10°C). Spawning occurs over sand or coarse gravel substrates.

Oregon Spotted Frog *(Rana pretiosa)*

Oregon spotted frog is an amphibian species from British Columbia, Washington, Oregon, and California. The Oregon spotted frog is named for the characteristic black spots covering the head, back, sides, and legs… [USFWS has] determined that the Oregon spotted frog is impacted by one or more of the following factors:

- Habitat necessary to support all life stages continues to be impacted or destroyed by human activities that result in the loss of wetlands to land conversions; hydrologic changes resulting from operation of existing water diversions/manipulation structures, new and existing residential and road developments, drought, and removal of beavers; changes in water temperature and vegetation structure resulting from reed canary grass invasions, plant succession, and restoration plantings; and increased sedimentation, increased water temperatures, reduced water quality, and vegetation changes resulting from the timing and intensity of livestock grazing (or in some instances, removal of livestock grazing at locations where it maintains early seral stage habitat essential for breeding).
- Predation by nonnative species, including nonnative trout and bullfrogs.
- Inadequate existing regulatory mechanisms that result in significant negative impacts, such as habitat loss and modification.
- Other natural or manmade factors including small and isolated breeding locations, low connectivity, low genetic diversity within occupied sub-basins, and genetic differentiation between subbasins.

Watson *et al.* (2003, p. 298) summarized the conditions required for completion of the Oregon spotted frog's life cycle as shallow water areas for egg and tadpole survival; perennially deep, moderately vegetated pools for adult and juvenile survival in the dry season; and perennial water for protecting all

age classes during cold wet weather. The Oregon spotted frog inhabits emergent wetland habitats in forested landscapes, although it is not typically found under forest canopy.[10]

Marbled Murrelet *(Brachyramphus marmoratus)*
The marbled murrelet is a small, chubby seabird that has a very short neck. During the breeding season it has dark brown to blackish upperparts and a white belly and throat that are greatly mottled. During the winter the upperparts become grey, dark marks form on the sides of the breast and a white ring develops around the eye. Males and females are similar in appearance and size. Juveniles are similar to the adult winter plumage, but with dusky mottling on the underparts. Vocalisations include a sharp keer' or low kee'.[11] The primary cause of marbled murrelet population decline is the historic and ongoing loss and modification of nesting habitat through commercial timber harvests, human-induced fires, and land conversions, and to a lesser degree, through natural causes such as wild fires and wind storms. Additional causes of decline include oil spills, gill-net fishing, marine pollution, and predation.[12]

Oregon Spotted Frog (Rana pretiosa)

On August 29, 2014, the USFWS determined threated status under the ESA for Oregon spotted frog (http://www.gpo.gov/fdsys/pkg/FR-2014-08-29/pdf/2014-20059.pdf)

According to the Federal Register listing (FR 79 168 51658),

"Results of a habitat utilization and movement study at Dempsey Creek in Washington indicate that adult frogs made infrequent movements between widely separated pools and more frequent movements between pools in closer proximity (Watson et al. 2003, p. 294), but remained within the study area throughout the year. Home ranges averaged 5.4 ac (2.2 ha), and daily movement was 16–23 ft (5–7 m) throughout the year (Watson et al. 2003, p. 295). During the breeding season (February–May), frogs used about half the area used during the rest of the year. … Recaptures of Oregon spotted frogs at breeding locations in the Buck Lake population in Oregon indicated that adults often move less than 300 ft (100 m) between years (Hayes 1998a, p. 9)."

On September 15, 2014, the USFWS provided the EPA with a GIS geodatabase of the 2013 proposed critical habitat for the Oregon spotted frog. The EPA overlaid the critical habitat layers with WAG130000 facility location data (see Figure 2). The Yakama Nation's Klickitat Salmon Hatchery is the closest WAG130000 facility to Oregon spotted frog critical habitat. The hatchery is 3,988 meters from the frog's critical habitat (see Figure 3). Since the published home range of Oregon spotted frog (Watson et al. 2003. J. Herpetology 37:292-300) is 2.2 hectares (0.022 square kilometers), there is no overlap of habitat with the any facilities covered by the General Permit.

Because of the external toxic mode of action of the chemicals discharged by WAG130000 facilities, combined with their short persistence in the environment, dietary ingestion and food web transfer of these chemicals is unlikely.

[10] Excerpts from http://www.gpo.gov/fdsys/pkg/FR-2014-08-29/pdf/2014-20059.pdf.
[11] http://ecos.fws.gov/speciesProfile/profile/speciesProfile.action?spcode=B08C#status
[12] http://ecos.fws.gov/docs/action_plans/doc3159.pdf

Thus, Oregon spotted frog will not be exposed to the effects of any chemicals or WAG130000 facility operations, and this draft General Permit will have no effect on the frog.

Figure 2. Oregon Spotted Frog and Marbled Murrelet Critical Habitat Imposed Over Facility Locations

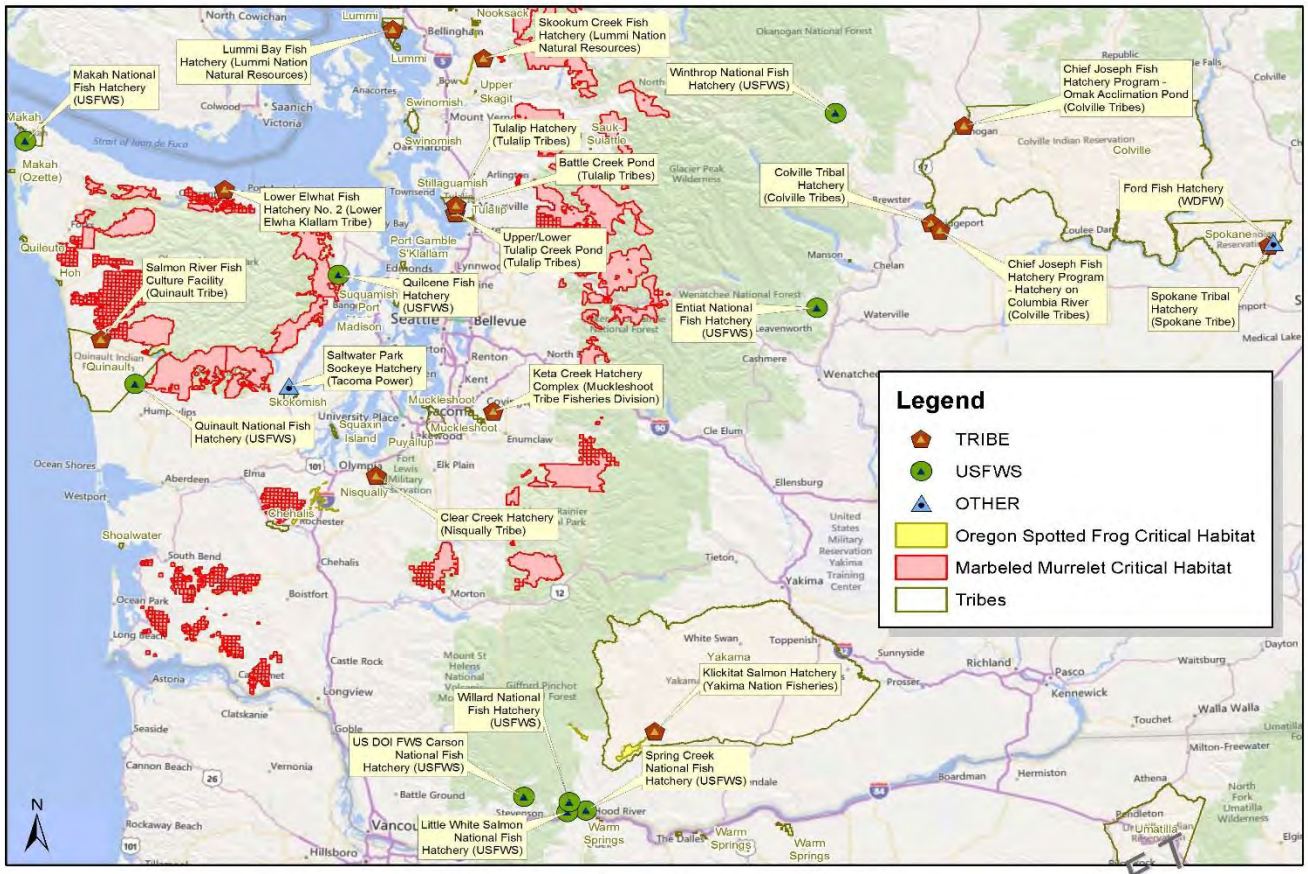
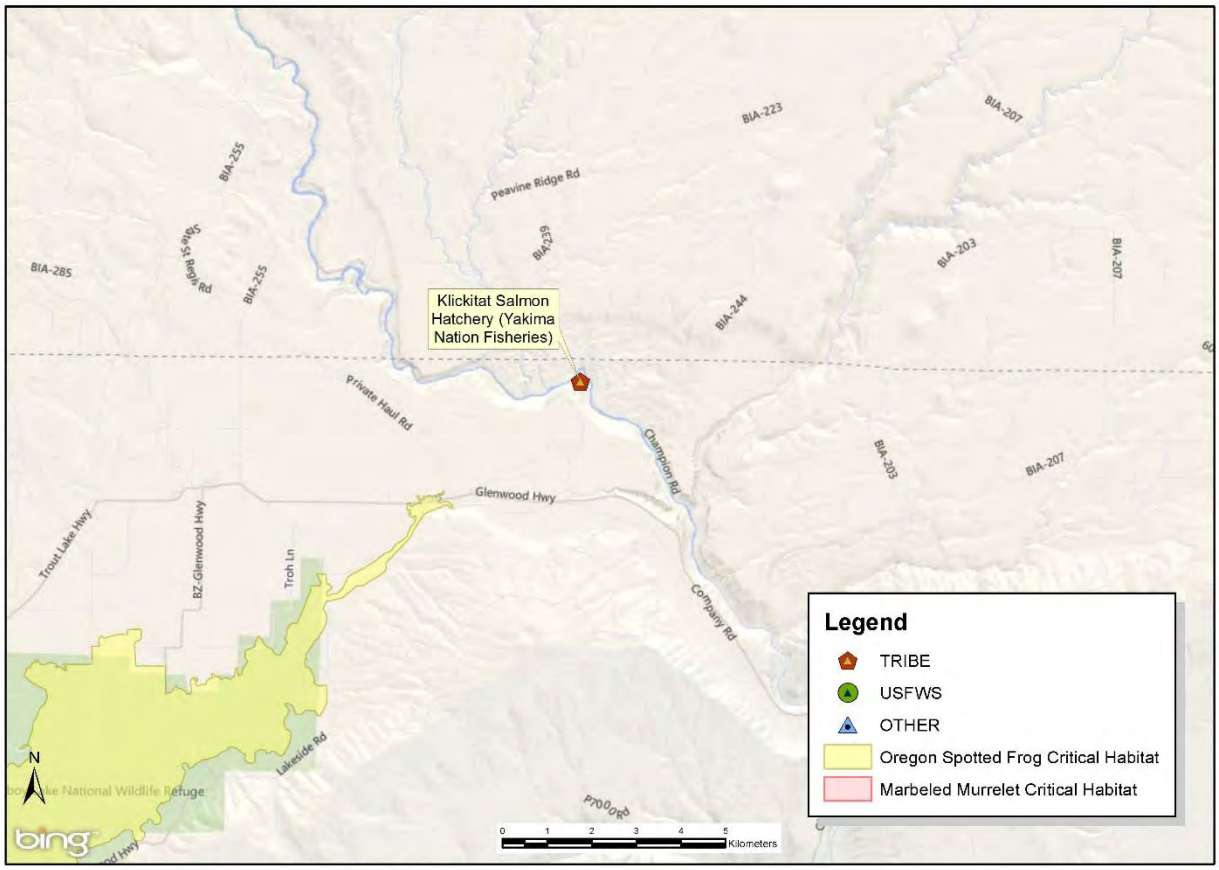


Figure 3. Klickitat Salmon Hatchery Distance to Oregon Spotted Frog Critical Habitat (3,988 meters)



Marbled Murrelet *(Brachyramphus marmoratus)*

The marbled murrelet is federally listed under the ESA as a threatened species in Washington, Oregon and California, and State-listed as endangered in California and as threatened in Oregon and Washington. According to the USFWS (http://www.fws.gov/arcata/es/birds/mm/m_murrelet.html),

"Marbled murrelets are long-lived seabirds that spend most of their life in the marine environment, but use old-growth forests for nesting. Courtship, foraging, loafing, molting, and preening occur in near-shore marine waters. Throughout their range, marbled murrelets are opportunistic feeders and utilize prey of diverse sizes and species. They feed primarily on fish and invertebrates in near-shore marine waters although they have also been detected on rivers and inland lakes.

Threats include loss of habitat, predation, gill-net fishing operations, oil spills, marine pollution, and disease. Recent reviews have concluded that the risk of predation is currently a larger threat than previously considered."

On September 15, 2014, the USFWS provided the EPA with a GIS geodatabase of the marbled murrelet critical habitat. The EPA overlaid the critical habitat layers with WAG130000 facility location data (see Figure 2). No facilities are located within the marbled murrelet's critical habitat, but a handful of facilities are within 5 kilometers (see Table 9 for a list of the closest facilities to marbled murrelet critical habitat).

Table 9. Distance to Marbled Murrelet Critical Habitat

Facility	Operator	Distance to Critical Habitat
Quinault National Fish Hatchery	USFWS	502 meters
Quilcene National Fish Hatchery	USFWS	1,687 meters
Salmon River Fish Culture Facility	Quinault Indian Nation	2,367 meters
Lower Elwha House of Salmon	Lower Elwha Klallam Tribe	3,388 meters
Skookum Creek Fish Hatchery	Lummi Nation	5,446 meters
Saltwater Park Sockeye Hatchery	Tacoma Power	8,077 meters

Because of the external toxic mode of action of the chemicals discharged by WAG130000 facilities, and because of their short persistence in the environment, dietary ingestion and food web transfer of these chemicals is unlikely. Since all WAG130000 facilities are located outside marbled murrelet critical habitat, it is also very unlikely that the operations or maintenance required by this NPDES permit could disturb the habitat or the nesting birds (e.g., noise from settling pond dredging).

Thus, marbled murrelets will not be exposed to the effects of any chemicals or WAG130000 facility operations, and this draft General Permit will have no effect on the bird.

5 RISK ASSESSMENT

5.1 ECOLOGICAL RISK ASSESSMENT METHODOLOGY

Introduction

The toxicity assessment approach used for Washington hatchery chemicals within this Biological Evaluation references the ecological risk assessment based analysis and effect determination approaches used in both EPA's Biological Evaluation (Shephard et al. 2008) for Oregon's 2004 aquatic life criteria (the Oregon Toxics Biological Evaluation), and in the most recent EPA aquatic life criteria for individual chemicals (e.g. Aquatic Life Ambient Water Quality Criteria for Carbaryl – 2012 (EPA 2012) and Aquatic Life Ambient Water Quality Criteria for Ammonia – Freshwater 2013 (EPA 2013)). This Biological Evaluation, as well as both the Oregon Toxics Biological Evaluation (Shephard et al. 2008) and current EPA water quality criteria documents (EPA 2013, EPA 2012) perform their evaluations using a standard EPA (1998) ecological risk assessment approach.

However, with the exception of freshwater and marine chlorine, and freshwater chloride ion (part of sodium chloride, although EPA does not have sodium chloride criteria *per se*), none of the chemicals evaluated in this Biological Evaluation have existing EPA aquatic life criteria or Washington water quality standards. The absence of water quality criteria for nearly all chemicals in this Biological Evaluation necessitated several modifications and additions to the Biological Evaluation methodology used the Oregon Toxics Biological Evaluation and current EPA aquatic life criteria derivations.

As this Biological Evaluation evaluates a number of chemicals, much of the information required for an ecological risk-based toxicity assessment of hatchery chemicals, for example the life history and dietary preferences of the threatened and endangered species under evaluation (Section 2), is equally applicable to all chemicals evaluated in the Biological Evaluation. Such information will not be repeated in this section. Instead, reference will be made as needed to appropriate sections of the Biological Evaluation for each individual chemical toxicity assessment.

Ecological Risk Assessment Approach Used in the Biological Evaluation

The EPA (1998) ecological risk assessment approach consists of three main phases:

1.) Problem formulation,
2.) Analysis, and
3.) Risk characterization

Problem formulation is the planning phase of the ecological risk assessment process. Within this Biological Evaluation, problem formulation involves:

- defining the objectives of the evaluation,
- integrating available information on the stressor(s) of interest
- identifying assessment endpoints (explicit expressions of valued environmental features to be protected),

- preparing a conceptual model illustrating the relationships between ecological entities and the stressors to which they are exposed,
- formulating risk hypotheses that describe the assumed relationship between stressors and ecological entities
- developing an analysis plan describing how data are collected and analyzed, and
- providing a description of how risks are to be characterized.

The analysis phase of the ecological risk assessment process follows the analysis plan generated during problem formulation to perform two characterizations: characterization of exposure and characterization of ecological effects (often called the toxicity assessment in ecological risk assessment literature). Exposure and ecological effects characterizations focus on the contaminant sources, exposure pathways, and toxic effects most likely to cause adverse effects on the assessment endpoint, as summarized in the conceptual model.

Assessment endpoints and conceptual models help identify measurable attributes to quantify and predict change. However, assessment endpoints and conceptual models often do not identify specific items that can be measured. As one example, a valued environmental attribute to be protected described as survival provides no detail regarding how survival is to be quantified. Therefore, a major goal of the analysis plan generated in problem formulation is to define measures that can be quantified. To complete the example, the survival attribute is evaluated when possible with empirical LC_{50} data generated from laboratory studies of chemical toxicity to aquatic species. Toxicity data such as LC_{50} values are termed measures of effect in this Biological Evaluation.

EPA (1998) ecological risk assessment guidance identifies three categories of measures that address both sensitivity and likely exposure to stressors:

- **Measures of exposure**: measures of stressor existence and movement in the environment and their contact or co-occurrence with the assessment endpoint.
- **Measures of ecosystem and receptor characteristics:** measures of ecosystem characteristics that influence the behavior and location of entities selected as the assessment endpoint, the distribution of a stressor, and life history characteristics of the assessment endpoint or its surrogate that may affect exposure or response to the stressor.
- **Measures of effect**: measurable changes in an attribute of an assessment endpoint or its surrogate in response to a stressor to which it is exposed.

The analysis plan within the problem formulation phase of ecological risk assessments identifies measures as appropriate for the risk assessment. Detailed descriptions of the information for measures of exposure and measures of effect are presented in the analysis phase of the ecological risk assessment of each hatchery chemical. As the ecosystem and receptor characteristics are the same for each chemical evaluated (e.g. the same threatened and endangered species are evaluated for all hatchery chemicals), the measures of ecosystem and receptor characteristics are described elsewhere in this Biological Evaluation.

The analysis phase of the risk assessment within this Biological Evaluation presents the available information on exposure and chemical effects on threatened and endangered species that are forwarded to the third phase of the ecological risk assessment process: risk characterization.

Tasks performed within the analysis phase include the following:

- Selection of data that will be used in risk characterization on the basis of their utility for evaluating the risk hypotheses
- Analyze exposure by examining the sources of stressors, the distribution of stressors in the environment, and the extent of co-occurrence or contact between the stressors and the ecological entities and receptors under evaluation
- Analyze effects by examining stressor-response relationships, evidence that the stressor causes or is associated with adverse effects on ecological entities
- Evaluate the relationship between measures of effect and assessment endpoints

The risk characterization phase of the ecological risk assessment process integrates the results of the characterization of exposure and the characterization of ecological effects from the analysis phase to evaluate the likelihood of adverse ecological effects on threatened and endangered species associated with exposure to hatchery chemicals in the environment. Uncertainties in the risk characterization are discussed and the chemical effect determinations are made for the ESA listed species.

Problem Formulation

Objective of the Biological Evaluation of the Chemicals Used at Washington Fish Hatcheries

The objective of this section of the Biological Evaluation is:

To determine whether an EPA renewal and approval of a general NPDES permit for federal aquaculture facilities and aquaculture facilities in Indian Country within the State of Washington is protective of federally threatened and endangered fully aquatic and aquatic-dependent species present in Washington.

Many chemicals are used in the course of normal hatchery operations, not all of which are discharged to receiving waters. This means that some hatchery chemicals pose no effect to threatened and endangered species. Threatened and endangered species cannot be adversely affected by chemicals to which they are not exposed.

Not all threatened and endangered species are found statewide. Some species have limited distribution within Washington, and those distributions do not overlap portions of the state where hatchery discharges are present. Threatened and endangered species whose distributions do not overlap areas with hatchery discharges cannot be adversely affected by hatchery releases, again because they are not exposed to hatchery chemicals. Other threatened and endangered species, particularly those living in estuarine or marine systems, may only be exposed to releases from one or a few hatcheries. The combination of which threatened and endangered species are exposed to which hatchery chemicals has been used to define the final subset of all federally listed threatened and endangered species in Washington evaluated within this Biological Evaluation.

Chemicals Used at Washington Hatcheries

One of the primary purposes of any NPDES permit is to regulate the discharge of chemicals from a facility into the aquatic environment. In order to complete an NPDES permit, EPA must know the chemicals used at a facility, specifically those which potentially or actually are released to the environment from facility operations. Chemicals used at hatcheries but not released to receiving waters are not evaluated in this Biological Evaluation. The list of chemicals used and potentially or actually discharged differs among the 25 hatcheries covered by this general NPDES permit.

The list of chemicals evaluated in this Biological Evaluation was developed after conversations with at least one member of the staff, most often the hatchery manager, at each of the 25 hatcheries in Washington covered under this NPDES general permit renewal. These discussions identified not only the chemicals used at hatcheries, but which chemicals were used at each hatchery, which chemicals were released to receiving waters from each hatchery, and which hatchery chemical were not released to receiving waters where threatened and endangered species were present.

Chemicals used but not released to the environment ranged from those used in laboratory testing procedures (e.g. pH buffers, conductivity standards); disinfectants; injectable chemicals, antibiotics such as azithromycin, where injected adult fish were subsequently disposed of in upland facilities, not released back to surface waters or used in nutrient enhancement of streams; medicated feeds, where fish feed is dosed with an antibiotic or parasite control chemical; and anesthetics, chemicals used to calm or immobilize fish. Finally, there is a small group of chemicals used by some hatcheries in Washington for various other purposes, such as a skin protectant for hatchery staff used at one hatchery; a group of four herbicides historically used at one hatchery; and sodium thiosulfate used to neutralize chlorine or iodine.

When all chemicals to which fish are exposed during hatchery operations at one or more Washington hatcheries are combined, the following list was obtained (Table Methods-1).

Table Methods-1. List of Chemicals Used at Fish Hatcheries in Washington to Which Fish Are Exposed During Hatchery Operations

Disinfectants	**Anesthetics**	**Injectable Antibiotics**	**Medicated Feeds**	**Miscellaneous Use**
Chloramine-T	MS-222	Azithromycin	Erythromycin	AquaNeat®
Chlorine	Sodium Chloride	Draxxin®	Florfenicol	Escalade®
Formalin		Erythromycin	Oxytetracycline	Landmark®
Hydrogen peroxide		Vibrio vaccine	Romet®	Pendulum®
Potassium permanganate			SLICE® (emamectin benzoate)	PolyAqua®
Povidone-iodine				Sodium thiosulfate
Virkon® Aquatic				

Based on EPA discussions with hatchery personnel, fish pathologists, and fish health experts with the USFWS, Tribes, and the U.S. Food and Drug Administration, the use patterns, use volumes, and disposal practices of a number of hatchery chemicals eliminates or severely limits discharge of some chemicals

into the environment where threatened and endangered species are found. Such chemicals do not have complete and significant exposure pathways to threatened and endangered species, and do not pose ecological risks to threatened and endangered species. Thus, there is no need in this Biological Evaluation to evaluate risks from a number of hatchery chemicals. The clearest examples are that the uses of Virkon Aquatic (a disinfectant that releases free oxygen, similar to the mode of action of hydrogen peroxide) and vibrio vaccine have been discontinued by Washington hatcheries.

The injected drugs listed in Table Methods-1 are not evaluated in this Biological Evaluation for the following reasons, all of which when combined lead to insignificant exposure of threatened and endangered species to chemicals injected into adult fish at hatcheries:

1. Targeted, very small doses are injected into adult fish only, not the younger life stages of fish eventually released from hatcheries.
2. The therapeutic doses of injected chemicals are not toxic to the injected fish, and would therefore not be toxic to threatened and endangered species in the environment after any biotransformation, metabolism, depuration and dilution of the injected chemical before it is discharged to the environment.
3. Injected fish are not released to the environment for nutrient enhancement purposes, nor are they consumed by humans. Instead, the carcasses of injected fish are disposed in landfills after spawning is complete.
4. Injectable drugs are not used at very many Washington hatcheries. Injections are specific chemicals injected into adult fish at low concentrations and volumes to treat one of several specific diseases.
5. Treatment of a large number of adult fish with injectable drugs would be cost prohibitive, limiting the incentive for hatcheries to use large quantities of injectables.
6. Discussions with hatchery personnel and fish health professionals have confirmed to EPA their belief that injected chemicals or their metabolic transformation products are either not released to the environment, or if released at all, are released in negligible quantities.

The chemicals added to medicated feeds listed in Table Methods-1 are not evaluated in this Biological Evaluation for the following reasons, all of which when combined lead to insignificant exposure of threatened and endangered species to chemicals used in medicated feeds:

1. Feed is expensive, so hatchery managers have every incentive to waste as little as possible. Medicated feeds are even more expensive than non-medicated feeds.
2. Hatcheries frequently clean or vacuum their raceways after feeding. The cleaning frequency varies from weekly to daily, depending on the facility and conditions.
3. Settling basins allow uneaten food particles to settle out before hatchery water is discharged.
4. USFWS hatchery settling ponds are of sufficient size that even the fine particles are settled out prior to reaching receiving waters.
5. Medicated feed concentrations used at hatcheries are not toxic to the fish in the hatchery. Medicated feed concentrations in receiving waters would be diluted from what's used in the hatchery.
6. Hatcheries are generally trying to slow fish growth so they do not grow too quickly, and generate less feed waste since they are not trying to maximize fish growth.

7. Hatcheries try to avoid using medicated feed in the first place, since its use is for disease control, and normal hatchery operations attempt to prevent disease, not treat it.
8. During medicated feed treatments occurring during disease outbreaks, hatcheries remove excess feed even more frequently than normal to make the fish-raising environment as clean as possible.

Minimal use patterns and volumes, and lack of appreciable discharge into receiving waters is also the reason EPA has not evaluated several other chemicals used at hatcheries:

1. MS-222. This anesthetic is used at 11 Washington hatcheries, at an exposure concentration of 10 grams per 50 gallon tank (roughly 50 mg/L). These tanks are isolated from hatchery raceways, and their contents are not discharged into receiving waters after use.
2. PolyAqua®. A skin protectant used during fish handling at one hatchery. De minimus use and discharge.
3. Sodium thiosulfate. Used by two hatcheries to neutralize excess chlorine or iodine used during disinfection. As long as sodium thiosulfate is not used in stoichiometric excess, where such excess results in reduced oxygen concentrations in water, the reaction products of sodium thiosulfate with halides are non-toxic halide anions (i.e. chloride, iodide).
4. Herbicides (Colville Tribal Hatchery only). The Colville Tribal Hatchery identified four herbicides historically used around upland portions of the hatchery, not near raceways or other aquatic systems. Based on information from the Colville Tribe, these herbicides are used in a manner that they are not released into either hatchery water or surface receiving waters. Thus, there would be no expected exposure of threatened and endangered species to these herbicides, which are not discussed further in this Biological Evaluation.

The final list of chemicals used at Washington hatcheries that EPA believes have the potential to be released to receiving waters where threatened and endangered species are present are the following:

- Chloramine-T
- Chlorine
- Formalin
- Hydrogen peroxide
- Potassium permanganate
- Povidone-iodine
- Sodium chloride

Of the above seven chemicals, all but sodium chloride (NaCl or common table salt) will be evaluated in detail in this Biological Evaluation.

Sodium Chloride Use at Washington Hatcheries

Sodium chloride receives an abbreviated evaluation in this section, per agreement between EPA and the Services, as its use concentration at hatcheries is within 2 – 3x of its naturally occurring concentration in many freshwaters, and the use volumes are quite small compared to the total volume of water discharged by hatcheries. Three hatcheries in Washington currently report using sodium chloride. Two of the three

Washington hatcheries currently using sodium chloride (Skookum Creek Fish Hatchery, Makah National Fish Hatchery) discharge into estuarine systems.

The common current use of NaCl at hatcheries is to calm fish and reduce stress on them either immediately before handling, or during transport of the fish. When handled or otherwise stressed, freshwater fish tend to increase their respiration rate, resulting in a higher than normal influx of fresh water across the gills and into the body. To counter the increased influx of water, fish pump water back across the gills, a process requiring increased energy expenditure by the fish. By increasing the NaCl content of the transport or holding water, the above process of excess water intake, followed by energy expenditure to eliminate the excess water is inhibited (Francis-Floyd 1995). The salt addition reduces energy expenditure by the fish, thus reducing its stress level. For this purpose, salt is added to water to increase its chloride content to 0.1 to 0.3% (= 1000 to 3000 mg/L chloride). Slowly dissolving salt blocks are often added to transport or handling water to effect the desired increase in salt content. By contrast, full strength seawater has a chloride content of roughly 18,980 mg/L. EPA's acute and chronic chloride freshwater water quality criteria are 860 mg/L and 230 mg/L, respectively. The exposure durations of fish to sodium chloride to reduce stress are short, on the order of minutes to hours.

Historically some hatcheries have used NaCl to manipulate salinity as a treatment for external parasites. The change in salt content simulates a natural behavior exhibited by salmonids affected by parasites, who will migrate between waters of different salinities (usually from fresh to more saline waters) to rid themselves of parasites. This behavioral pattern mimics the smoltification process of juvenile salmonids during their outmigration from freshwater to marine waters. While the salmon are naturally tolerant of these salinity changes, as long as they are not too extreme, the internal osmatic balance of freshwater parasites is disrupted by such behavior, the likely mode of action of NaCl in the treatment of parasites.

Evaluation of Hatchery Chemicals that are Mixtures

The risk assessment of each hatchery chemical will include evaluations of not only the active ingredient of the above chemicals, but other chemicals present in the commercially available mixtures of these chemicals. For example, the stock 37% formalin solution that is diluted for use at hatcheries contains 37% formaldehyde and between 10-15% methanol, with the remainder being water. Formalin as a mixture, formaldehyde and methanol risks will be evaluated separately if the empirical toxicity data are available to do so.

Federally Threatened and Endangered Species in Washington Exposed to Hatchery Discharges

A total of 11 fully aquatic and 13 aquatic-dependent federally threatened and endangered species are present in Washington, and are listed in Table Methods-2. Within this Biological Evaluation, an aquatic-dependent species is a reptile, bird or mammal that preys on one or more fully aquatic species, but which itself does not have gills, and thus cannot remain submerged in water for its entire life span.

Table Methods-2. Complete list of fully aquatic and aquatic-dependent federal threatened and endangered species in Washington.

Fully Aquatic (N = 11)	**Aquatic-Dependent (N = 13)**
Bocaccio (*Sebastes paucispinis*)	Green sea turtle (*Chelonia mydas*)

Bull trout (*Salvelinus confluentus*)	Leatherback turtle (*Dermochelys coriacea*)
Canary rockfish (*Sebastes pinniger*)	Loggerhead turtle (*Caretta caretta*)
Chinook salmon (*Oncorhynchus tshawytscha*)	Marbled murrelet (*Brachyramphus marmoratus*)
Chum salmon (*Oncorhynchus keta*)	Short-tailed albatross (*Phoebastria albatrus*)
Coho salmon (*Oncorhynchus kisutch*)	Western snowy plover (*Charadrius nivosus*)
Eulachon (*Thaleichthys pacificus*)	Steller sea lion (*Eumetopias jubatus*)
Green sturgeon (*Acipenser medirostris*)	Orca (*Orcinus orca*)
Sockeye salmon (*Oncorhynchus nerka*)	Blue whale (*Balaenoptera musculus*)
Steelhead (*Oncorhynchus mykiss*)	Fin whale (*Balaenoptera physalus*)
Yelloweye rockfish (*Sebastes ruberrimus*)	Humpback whale (*Megaptera novaeangliae*)
	Sei whale (*Balaenoptera borealis*)
	Sperm whale (*Physeter macrocephalus*)

Discussions between EPA and the National Marine Fisheries Service (NMFS) led to an agreement between EPA and NMFS that green sturgeon are not present in any of the waters to which hatcheries in Washington discharge, and therefore would not need to be evaluated in this Biological Evaluation. These discussions also led to agreements that eulachon are only exposed to discharges from the Lower Elwha Klallam Tribe Fish Hatchery, and that the three rockfish (*Sebastes*) species are exposed only to discharges from three hatcheries: the Lower Elwha Klallam Tribe Fish Hatchery, and two Lummi Nation hatcheries (Lummi Bay and Skookum Creek Fish Hatcheries).

EPA has determined that this NPDES permit will have no effect on aquatic-dependent species, which are not expected to occur in the project area or be affected by the permit. Based on the known locations of orca in inland marine waters of Washington relative to the locations of hatcheries covered under this permit, dietary ingestion of hatchery chemicals is the only potentially complete exposure pathway of orca to hatchery chemicals. A distinct population segment of Southern Resident killer whales spend part of the year in the following inland marine waters of Washington: Strait of Georgia, Strait of Juan de Fuca, and Puget Sound, where they feed largely but not solely on Chinook salmon (NMFS 2008).

As will be discussed in more detail later in this Biological Evaluation, the chemicals released to surface waters by Washington hatcheries are disinfectants with short residence times in the environment, and which are unlikely to bioaccumulate into aquatic species serving as prey for any avian or mammalian species. The lack of bioaccumulation of hatchery chemicals into orca prey precludes the existence of a complete and significant exposure pathway of discharged hatchery chemicals to orca. EPA therefore is not performing any quantitative evaluations of risks to orca from releases of hatchery chemicals in Washington.

The three sea turtle and five baleen whale species generally reside too far offshore of the Pacific coast of Washington to be exposed to hatchery chemicals, and are not evaluated in this Biological Evaluation. Steller sea lion haul out points are not located near any of the Washington hatcheries covered under this permit, thus, they are not expected to be exposed to chemicals released by hatcheries, and are also not evaluated in this Biological Evaluation.

Of the three threatened and endangered bird species, none are believed to be appreciably exposed to chemicals released by Washington hatcheries. Marbled murrelet feed on small fish species such as sand lance, as well as some invertebrate species. As is the case for orca, the absence of a complete and

significant dietary exposure pathway of hatchery chemicals to marbled murrelets means that there is no potential for their exposure to hatchery chemicals via dietary ingestion. Short-tailed albatross rarely are observed in Washington, forage in open ocean waters, and for over 70 years have been known to breed only on two islands off the southern coast of Japan (although there is recent documentation of successful breeding on Midway Island in Hawaii). The U.S. Fish and Wildlife Service states that the northern extent of the breeding range of western snowy plover is Damon Point (http://www.fws.gov/arcata/es/birds/wsp/plover.html), the southeastern tip of the Ocean Shores peninsula in southwestern Washington, a location not near any of the hatcheries covered under this NPDES permit.

The federal Endangered Species Act listed fully aquatic and aquatic-dependent species present within the action area and evaluated within this Biological Evaluation are as follows. Only the species are listed below, multiple Evolutionarily Significant Units (ESU's) for individual species are not listed, as they have no impact on the toxicity assessment within this Biological Evaluation.

- Bocaccio (*Sebastes paucispinis*)
- Bull trout (*Salvelinus confluentus*)
- Canary rockfish (*Sebastes pinniger*)
- Chinook salmon (*Oncorhynchus tshawytscha*)
- Chum salmon (*Oncorhynchus keta*)
- Coho salmon (*Oncorhynchus kisutch*)
- Eulachon (*Thaleichthys pacificus*)
- Sockeye salmon (*Oncorhynchus nerka*)
- Steelhead (*Oncorhynchus mykiss*)
- Yelloweye rockfish (*Sebastes ruberrimus*)

Integration of Available Information on Hatchery Chemicals Used in Washington

For fully aquatic species, the available toxicity data was primarily identified from a search in EPA's online ECOTOX database (http://www.epa.gov/ecotox/). Any additional studies identified during a literature review by EPA Region 10 staff or identified by the Services were also evaluated.

ECOTOX is a comprehensive web-based database, compiled and maintained by EPA's Office of Research and Development (ORD), that provides information on the effects of single chemical exposures to ecologically relevant species. The database supports research in ORD and the broader scientific community, providing data to create and evaluate predictive effects models developed through intra- and extramural research efforts (e.g., advanced species, dose, and chemical extrapolation modeling). It is used by the Agency's Regional and Program Offices, as well as other Federal, State, Tribal and local government agencies, and the regulated community as a primary source of literature on ecological effects to meet responsibilities under Agency-delegated programs and/or data submissions and analyses required by EPA.

A publication is generally eligible for inclusion within the ECOTOX database if it reports (1) observed biological responses related to an exposure to a single chemical, and the chemical's name and Chemical Abstract Services Registry number can be verified in reliable chemical reference manuals; (2) a taxonomically verifiable test species that is an aquatic or terrestrial plant or animal with the exception of yeast, bacteria and viruses; (3) results based on the exposure of live, intact organisms; (4) a concurrent

environmental chemical concentration/dose or application rate with the exception of concentrations reported in the sediment without concurrent pore water concentrations and air exposures; and (5) a duration of exposure. A detailed description of the requirements for inclusion in ECOTOX is below.

Some publications obtained from literature searches do not meet minimum data requirements for the ECOTOX database. As publications are received and reviewed, the ECOTOX eligibility criteria in the text box below are applied. When a publication is identified as applicable to ECOTOX, it is assigned an ECOTOX reference number and retained in the ECOTOX literature holdings. Reasons for excluding a study from ECOTOX are summarized in Text Box 1.

Text Box 1: Reasons for Excluding Studies from the EPA ECOTOX Database.

Keyword	**Usage**
ABSTRACT	study results published as an abstract
BACTERIA	bacteria and microbes - for microbes, enter bacteria as keyword, Microbe in Reference Manager field 6 (Notes)
BIOLOGICAL TOXICANT	general biological toxicants including venoms, fungal toxins, Bacillus thuringiensis, and other plant, animal or microbial extracts or toxins not purified (Purified single chemicals (with CAS numbers) of biological origin may be applicable. See the following websites for examples of applicable toxicants with biological origin: www.hort.purdue.edu/newcrop/proceedings1990/v1-511.html and www.epa.gov/pesticides/biopesticides/ingredients/index.htm).
CAS # UNAVAILABLE	chemical is not verifiable, no CAS # is available
DRUG	testing for drug effects and side-effects on humans (drugs used as environmental toxicants are applicable)
EFFLUENT	includes sewage and polluted runoff
FATE	chemical distribution, metabolism
HUMAN HEALTH	studies with human subjects or with surrogate animal subjects for human health risk assessment
INCIDENT	reports of animal deaths by poison, etc.; lacks usable concentration and/or duration
INCOMPLETE CITATION	citation is not complete; order status ARCHIVE

INCORRECT CITATION	citation is wrong; order status ARCHIVE
IN VITRO	in vitro studies, including exposure of cell cultures and excised tissues
METABOLISM	what happens to the chemical rather than to the organism
METHODS	no usable toxicity tests; describes methods for conducting tests, purification or determination of chemicals, etc. Some methods publications are ordered for the ECOTOX methods information file (METHFILE); documentation provided for toxicology test methods, experimental design, statistical methods, standard terminology, and recently developed test methods. Methfile publications are chosen to support development and interpretation of coding guidelines and to assist in reviewer training.
MICROTOX	Microtox tests; studies conducted with bacteria
MIXTURE	no single chemical tests reported
MODELING	modeling only, no new organism exposure data; modeling studies may report original toxicity tests performed as comparisons or as a basis for extrapolation, if so, publications are ordered
NO CONC	no usable dose or concentration reported after examination of the entire publication; includes lead shot studies lacking dose information and which report only the number of pellets. Concentrations reported in log units only are not coded.
NO DURATION	no duration reported (entire publication examined)
NO EFFECT	no organism effect reported, including water quality studies with no effect on organisms reported
NO QUANTIFIABLE TOXICITY RESULT	no specific data values to code, authors used general statements such as “the animals decreased in weight”, used only for terrestrial publications
NO SOURCE	source of publication undetermined; order status ARCHIVE

NO SPECIES	-no organism present or tested -exposure of a dead organism -reviewer unable to verify species
NO TOX DATA	- chemicals in water, sediment or soil without organism effect data - ecological interactions with no toxicity tests - food studies - chemicals found in foods, food safety studies - genetics studies - including recombinant DNA and mutant strains - physiology - effects of the level of chemicals biologically present in an organism, including hormones and vitamins - risk assessment publications (related to regulation and legislation)
NO TOXICANT	no chemical toxicant - includes ambient air component chemicals (ozone, CO_2, SO_2) and pollution - includes vapor studies where the toxicant is delivered through inhalation/respiration -other ambient conditions including changes in conditions (other than chemical addition), including radioactivity, ultraviolet light (UV), temperature, pH, salinity, dissolved oxygen (DO), or other water, air or soil parameters
NON-ENGLISH	publication in a language other than English - (these publications receive ECOREF numbers UNLESS a second keyword is assigned); AUTH orders only (not ILL), if not received in 6 months, citations should be ARCHIVE
NUTRIENT	in situ chemicals tested as nutrients
OIL	only report toxic effects associated with exposure to oil and/or petroleum products
PUBL AS	publication was published in another journal or book, ECOREF number of other publication listed in Reference Manager citation Ex. PUBL AS ECOREF #####
QSAR	Quantitative Structure Activity Relationships; not primary source of data; bibliography skimmed to identify empirical studies

REVIEW	all toxicity tests reported elsewhere; REVIEW bibliography may be skimmed to identify relevant citations
SEDIMENT CONC	chemical concentration reported in sediment only (see applicable conditions)
SURVEY	measured chemical present, but lacking quantification of exposure; lacks usable concentration and/or duration
VIRUS	virus used as test organism
YEAST	yeast used as test organism

EPA attempts to obtain and review copies of all literature identified during our literature review. This is to ensure that individual studies contain results appropriate for use and of a sufficiently high quality for use in evaluating risks to threatened and endangered species. The final determinations regarding whether or not a study is of sufficiently high quality for use in a threatened and endangered species risk assessment are described in the EPA (2006) *Draft Framework for Conducting Biological Evaluations of Aquatic Life Criteria: Methods Manual*.

Below in Text Box 2, slightly modified from EPA (2006) is a list of rejection codes specific for use in separating out acceptable from unacceptable data for use in this Biological Evaluation. The rejection codes apply to both data downloaded from the ECOTOX public website and data found from other sources. The most common criteria used to select/reject data identified from ECOTOX and other sources will most likely be test Duration (Dur) (Note: test durations may deviate slightly from the Guidelines, but must be of sufficient duration to produce a reliable acute or chronic effect), Inappropriate Exposure or Inapp Exp (chronic tests that were not flow-through, especially for those chemicals that are highly biodegradable, hydrolyzable, oxidizable, reducible, or volatile, as is the case for many chemicals in this Biological Evaluation), or Detail (Det), which can refer to either insufficient information on analytical methodologies, contaminant concentrations or chemical form/species present in exposure water.

As a rule of thumb, EPA considers acute and chronic toxicity data for fish from 48 to 120 h and 21+ days, respectively. For cladocerans and most other invertebrate species, EPA considers acute and chronic toxicity data from 24 to 96 h and 7 to 14+ days, respectively. For algae, toxicity tests longer than 72 hours normally permit evaluation of effects on multiple algal generations, and are considered chronic toxicity tests, despite their duration being similar to that of acute tests with fish and invertebrates (ASTM 2012). These definitions of the terms acute and chronic exposure duration are also used to define the acute and chronic no effect concentrations of chemicals in the analysis and risk characterization phases of each chemical's risk assessment. Studies outside of these test durations are generally considered unacceptable for use in threatened and endangered species risk assessments, primarily because of the lack of a well-defined method to convert acute or chronic adverse effect concentrations to acute and chronic NOEC's for studies with exposure durations outside of these ranges.

Text Box 2: Rejection Codes Resulting in Exclusion of Studies from those Used to Evaluate Chemical Risks to Threatened and Endangered Species.

ACELLULAR (Ace)	Studies of acellular organisms (protozoa) and yeast.
BIOMARKER (Biom)	Studies reporting results for a biomarker having no reported association with a biologically significant adverse effect (survival, growth, or reproduction of an individual or population) and an exposure dose (or concentration)
CONTROL (Con)	Laboratory or field studies where no control is used or where survival of control organisms is unacceptable.
DETAIL (Det)	Insufficient detail regarding test methodology or statistical analysis.
FOREIGN LANGUAGE (Forgn)	Studies reported in non-English publication and where results in tables and figures indicate the data would not likely affect findings.
FORMULATION (Form)	Studies where the chemical is a primary ingredient in a commercial formulation, e.g., biocide, fertilizer, etc.
INAPPROPRIATE EXPOSURE (Inapp Exp)	Chronic studies that were not flow-through, especially for those chemicals that are highly biodegradable, hydrolyzable, oxidizable, reducible, or volatile.
LETHAL TIME (LT)	Laboratory studies reporting only lethal time to mortality, except under special conditions (no other applicable information is available for species pivotal in making a finding).
NO NOEC (XNoec)	A plant test in which all tested concentrations produce adverse effects, but the lowest tested concentration is far above the criteria concentrations (e.g., > 1 order of magnitude). Note: vascular plant median response measures are not used routinely in establishing acute criteria, but no-effect levels for all plants are considered in chronic criteria.
NOMINAL (Nom)	Chronic and bioaccumulation studies where test concentrations were not measured.
NON-AQUEOUS MEDIUM (Notaq)	Exposure to chemical via non-aqueous or artificial-aqueous medium, e.g., agar or soil, hydroponic exposure.
ROUTE OF EXPOSURE (EXP)	Un-natural exposure routes for aquatic chemicals, e.g., injection, spray, inhalation.
SECONDARY (Sec)	Review articles or other reports or papers containing no original data or only data reported more completely in a primary source.

STEADY-STATE (Steady)	Laboratory bioconcentration studies where the exposure duration could not be discerned or was too short for steady-state to be reached in the organism.
TOXICANT (Tox)	Inappropriate form of toxicant used or none identified in a laboratory or field study. Note: Inappropriate form includes mixtures.
UNRELATED (Unrel)	Studies that are unrelated to the contaminants and receptor groups of interest.
UNSUITABLE BCF (BCF)	Tissue chemical concentrations reported are not useful for BCF calculation, e.g., exposure concentrations used in the study were too high, or chemical concentrations were not measured in the whole animal or edible tissue. (Note: Studies where chemical concentrations were made only in organ tissue may be considered if enough data exists for the chemical to correlate the concentrations in the organ tissues to whole body concentrations.)
UNUSUAL DILUTION WATER (Dilut)	Laboratory or field studies where the dilution water contained unusual amounts or ratios of inorganic ions or was without addition of appropriate salts (i.e., distilled or de-ionized water). Dilution water containing > 5 mg/L dissolved organic carbon (DOC) is unacceptable unless it has been demonstrated that DOC has no effect on toxicity. Water quality characteristics that have been shown to be related to toxicity (e.g., hardness, pH, etc.) should be accounted for.
VARIABLE EXPOSURE (Var Exp)	Excessive variability in contaminant concentrations during the exposure period.

Stephan et al. (1985) is the guidance document describing how EPA develops water quality criteria for aquatic species, including guidelines for studies useable in deriving water quality criteria. Within this Biological Evaluation, chlorine is the only chemical with EPA aquatic life criteria, although chloride, released when sodium chloride is dissolved in water, also has EPA water quality criteria for freshwater (sodium chloride itself does not have an EPA water quality criterion). The guidance within Stephan et al. (1985) identifies acceptable studies through the following process:

A. Collect all available data on the material concerning (a) toxicity to, and bioaccumulation by, aquatic animals and plants, (b) FDA action levels, and (c) chronic feeding studies and long-term field studies with wildlife species that regularly consume aquatic organisms.

B. All data that are used should be available in typed, dated, and signed hard copy (publication, manuscript, letter, memorandum, etc.) with enough supporting information to indicate that acceptable test procedures were used and that the results are probably reliable. In some cases it may be appropriate to obtain additional written information from the investigator, if possible. Information that is confidential or privileged or otherwise not available for distribution should not be used.

C. Questionable data, whether published or unpublished, should not be used. For example, data should usually be rejected if they are from tests that did not contain a control treatment, tests in which too many organisms in the control treatment died or showed signs of stress or disease, and tests in which distilled or deionized water was used as the dilution water without addition of appropriate salts.

D. Data on technical grade materials may be used if appropriate, but data on formulated mixtures and emulsifiable concentrates of the material of concern should not be used.

E. For some highly volatile, hydrolyzable, or degradable materials it is probably appropriate to use only results of flow-through tests in which the concentrations of test material in the test solutions were measured often enough using acceptable analytical methods.

F. Data should be rejected if they were obtained using:

1. Brine shrimp, because they usually only occur naturally in water with salinity greater than 35 g/kg.
2. Species that do not have reproducing wild populations in North America.
3. Organisms that were previously exposed to substantial concentrations of the test material or other contaminants.

G. Questionable data, data on formulated mixtures and emulsifiable concentrates, and data obtained with non-resident species in North America or previously exposed organisms may be used to provide auxiliary information but should not be used in the derivation of criteria.

As will be discussed in the measures of effect section for each chemical, the small number of studies available for most of the chemicals evaluated in this Biological Evaluation meant that in order to have a sufficient number of studies available for risk characterization, EPA could not strictly follow the data quality requirements EPA normally employs in biological evaluations. Many studies used in this Biological Evaluation failed one or more of the data quality and information requirements EPA has set for using studies in derivation of national aquatic life criteria, or in other Biological Evaluation's of water quality criteria effects on threatened and endangered species. EPA was therefore in a position where we could either follow our data quality requirements and be unable to quantitatively evaluate risks of hatchery chemicals to threatened and endangered species, or use our best professional judgment to select studies for use in this Biological Evaluation, which adds a level of uncertainty to the subsequent quantitative risk assessment. EPA decided that, in order to perform risk characterization of hatchery chemicals, EPA could not strictly follow our data quality requirements normally used in Biological Evaluations or derivation of national aquatic life criteria. The less than optimal data quality of many of the individual studies used in this Biological Evaluation is one of the primary sources of uncertainty in the conclusions of this Biological Evaluation.

Mechanism(s) of Toxic Action of Hatchery Chemicals

The toxic mechanism(s) of action (i.e. the specific biochemical reaction(s) causing toxicity) of the hatchery disinfectant chemicals to aquatic life are related to their ability to oxidize organic matter. Different hatchery chemicals oxidize different types of molecules (Rutala and Weber 2008). Cellular enzymes containing sulfhydryl groups found in the amino acids cysteine and methionine are oxidized almost immediately by residual chlorine in both plants and animals. Due to the strength of the chemical bond formed between chlorine and proteins, enzyme activity is irreversibly terminated. This irreversible nature of chlorine reacting with enzymes likely explains its disinfecting properties, and also explains the observed irreversible toxicity of chlorine to fish once equilibrium has been lost (Alabaster and Lloyd 1982).

Chemically similar to chlorine, chemicals such as povidone-iodine that release iodine into solution also rapidly bind with and inactivate sulfhydryl containing proteins. Formalin disinfects by binding to sulfhydryl containing proteins via alkylation, and also alkylates amino groups of proteins and ring nitrogen atoms of purine bases. Hydrogen peroxide and potassium permanganate both disinfect by releasing oxygen and hydroxyl free radicals that attack cell membrane lipids. Although the mechanism of action of Chloramine-T historically has been debated in the scientific literature (slow release of hypochlorite ion vs. toxicity of Chloramine-T anion at pH > 7), the mode of action (i.e. a functional or anatomical change at the cellular, organelle or tissue level of biological organization) is the oxidation of cell membranes. The most recent studies on Chloramine-T have concluded that its effects are due to the release of elemental chlorine to water. As this appears to be the mode of toxic action, chlorine and Chloramine-T toxicologically should have the same mechanism of toxic action, although their environmental chemistries will differ until chlorine has been released from the organic portion of the Chloramine-T molecule.

What chlorine, iodine, formalin, hydrogen peroxide, potassium permanganate and Chloramine-T all appear to have in common is a toxic mode of action that operates on cell membranes, cell walls or viral envelopes (Rutala and Weber 2008, McDonnell and Russell 1999). As such, these chemicals when used as external disinfecting solutions are among the relatively few chemicals that do not require an internally bioaccumulated dose to elicit toxicity to aquatic life. The external toxic mode of action of these hatchery chemicals, combined with their short persistence in the environment led EPA to conclude that ***dietary ingestion and food web transfer of these chemicals is unlikely to occur.*** Thus, EPA does not evaluate risks from a multiple route of exposure pathway (e.g. water column exposure plus dietary ingestion), nor do we evaluate trophic transfer risks of these chemicals through the dietary ingestion of these chemicals by marine mammals.

Assessment Endpoint

EPA (1998) describes assessment endpoints in terms of an ecological entity (e.g. a species, feeding guild or aquatic community) and one or more attributes or characteristics of the ecological entity it is desired to protect. The Oregon Toxics Biological Evaluation (Shephard et al. 2008) based its assessment on ecologically relevant toxicological endpoints that could be related to either organism fitness (an organism's ability to perpetuate itself as measured by its reproductive success [Pianka 1983]), or adverse effects at population or higher levels of biological organization. Within the Oregon Toxics Biological Evaluation (Shephard et al. 2008), toxicological endpoints that met this ecological relevance guideline were organism survival, reproduction and growth. This is consistent with the approach used to derive aquatic life criteria under the Clean Water Act, which are also based on the survival, reproduction and growth of aquatic species.

Under the ecological risk assessment approach used herein, the only assessment endpoint for the evaluation of hatchery chemicals is: survival, reproduction and growth of federally threatened and endangered fish species in Washington that are exposed to chemicals discharged from fish hatchery operations

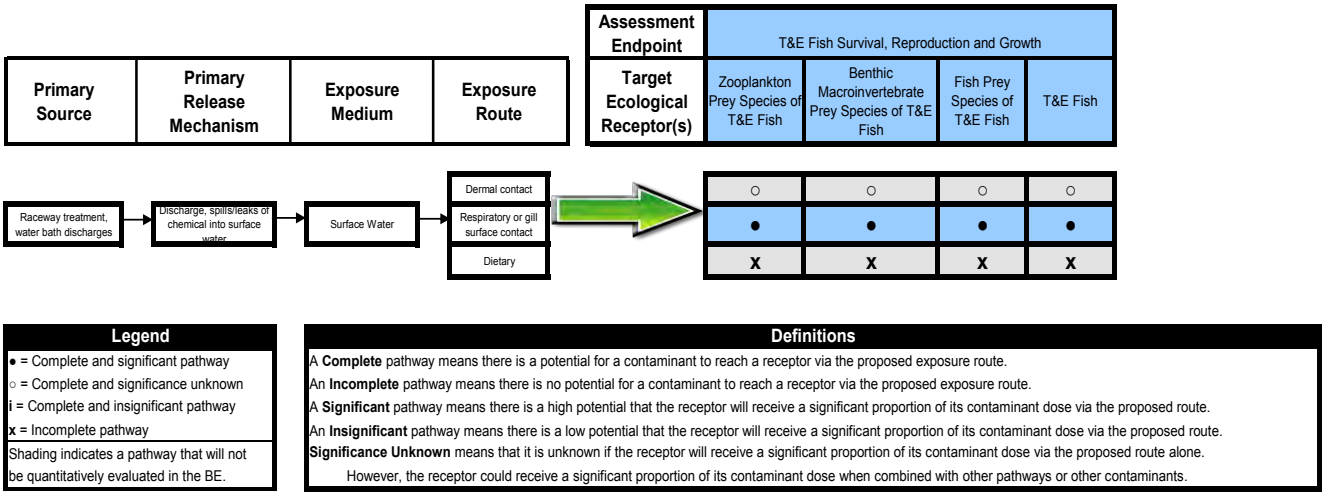
Conceptual Model

A conceptual model is a written description and visual representation of known or predicted relationships between ecological entities and the stressors to which they may be exposed. Conceptual models describe key relationships between contaminants and the Biological Evaluation assessment endpoint, the explicit

expression of environmental values to be protected. By describing links and relationships between contaminant sources and the exposure pathways by which threatened and endangered species and their prey are exposed to contaminants, the conceptual model provides a framework for predicting the effects of the stressors (chemical contaminants) evaluated in this Biological Evaluation.

Figure 4 provides a summary of how threatened and endangered species and their prey can potentially be exposed to chemicals released by hatcheries. Transport mechanisms and exposure pathways of chemicals are considered as part of the measures of exposure evaluation in the analysis plan. The toxicity assessment portion of the measures of effect focus on stressor effects on survival, growth and reproduction of threatened and endangered species.

Figure 4. Conceptual Model for Fish Hatchery Toxicity Assessment



The conceptual model for the disinfectant chemicals used at hatcheries is simple compared to conceptual models for other chemicals, for several reasons. The reactivity of disinfectants with other substances found in aquatic systems, combined with the volatility of several chemicals such as chlorine and formalin limits both the concentration and residence time of these chemicals in aquatic systems. Unlike most other chemicals discharged to aquatic systems, sediments do not serve as a sink for reactive disinfectants. Sediment is therefore not a medium by which aquatic species are exposed to disinfectants released by hatcheries. The combination of these factors serves to minimize the potential exposure of aquatic species to hatchery chemicals discharged to surface waters. The mode of toxic action of disinfectants described earlier in this section (i.e. external toxicants affecting cell walls, cell membranes and viral envelopes) further limits the exposure of aquatic species to hatchery chemicals, as it precludes exposure via the dietary ingestion exposure route. Ingestion via drinking water is an insignificant contaminant exposure pathway to freshwater fish, which are physiologically constrained from ingesting substantial quantities of water because of their need to maintain a higher internal solute content than found in their external freshwater environment.

The conceptual model for hatchery chemicals (Figure 4) illustrates that the toxicity assessment and ecological risk characterization should focus on surface water concentrations of hatchery chemicals that affect the respiratory surfaces of aquatic species. Dietary ingestion of and dermal contact with hatchery

chemicals are an insignificant exposure routes for threatened and endangered fish species, aquatic-dependent reptiles, birds, mammals, and the prey of threatened and endangered species. The epidermis, scales and mucus that are the external surfaces of fish are designed to prevent uptake of chemicals from the surrounding water.

Risk Hypotheses

Risk hypotheses are assumptions regarding what responses assessment endpoints will show when they are exposed to stressors, and how the exposure of ecological entities to stressors will occur. As a specific example of a risk hypothesis, within the risk characterization, a risk hypothesis under evaluation is:

1.) Long-term survival, reproduction and/or growth of threatened and endangered fish species in Washington will be adversely affected if they or their prey are exposed to hatchery chemical concentrations in surface water above that of a chronic no effect concentration (chronic NOEC).

Note that risk hypotheses are not the same as and do not take the form of a null hypothesis used in statistical hypothesis testing.

Analysis Plan

Literature Review and Empirical Toxicity Data Compilation

The analysis plan evaluates risk hypotheses to determine how they will be assessed using available and new information. The analysis plan includes a description of the toxicity assessment design, data needs, measures, and methods for conducting the analysis phase of the risk assessment.

This toxicity assessment of hatchery chemicals is based completely on existing information. The methodology used to obtain toxicity data from the online ECOTOX database has already been described in the problem formulation section of this methodology. It was employed for all chemicals assessed in this Biological Evaluation except for chlorine.

Chlorine toxicity data for aquatic species is that presented in the EPA (1985) water quality criteria document for chlorine, augmented by 2012 and 2013 EPA Region 10 ECOTOX literature searches for additional chlorine toxicity information published in the literature subsequent to the publication of the EPA (1985) chlorine criteria document. The chlorine literature review and evaluation was performed as part of a biological evaluation of proposed water quality standards of the Coeur d'Alene Tribe (EPA 2013). Unlike most other chemicals in this Biological Evaluation, the amount of chlorine toxicity data of suitable quality for use in deriving an EPA water quality criterion was sufficient that we did not have to use best professional judgment to select studies that failed to meet EPA data quality requirements for use in deriving national water quality criteria.

The assessment endpoint for this Biological Evaluation does not provide detail regarding how adverse effects of hatchery chemicals to threatened and endangered species are defined or identified. The remainder of this analysis plan details how the measures of exposure to hatchery chemicals by threatened and endangered species, and measures of effect of hatchery chemicals on threatened and endangered species are defined.

Measures of Exposure

With the notable exception of chlorine, for which there are both effluent limits and monitoring requirements in the permit, none of the Washington hatcheries have monitoring data for any of the chemicals evaluated in this Biological Evaluation. Thus, there are no empirical hatchery effluent concentrations of chemicals that can be used as a starting point for calculating the chemical concentrations in receiving waters to which threatened and endangered species are exposed (besides chlorine).

Furthermore, relatively few hatcheries have gaging stations at or near the hatcheries on the receiving waters to which they discharge. This means that only a few hatcheries have stream discharge information that could be used in conjunction with effluent monitoring data, if it even existed, to calculate the actual chemical concentrations to which threatened and endangered species in the environment are exposed (i.e. the diluted concentration of hatchery chemical discharges when threatened and endangered species are exposed to them). In the absence of empirical data with which to calculate exposure concentrations, we identified an (unrealistically conservative) estimation method by which we could calculate chemical concentrations in hatchery discharges at the point where the discharge enters a receiving water body. This point is termed the 'end of pipe' concentration in this Biological Evaluation, and does not account for any dilution in the receiving water.

In ecological risk assessment, the chemical concentration to which ecological receptors are exposed is generally termed the exposure point concentration (EPC). In many risk assessments, the exposure point concentration is empirically measured at the location(s) where the receptors of concern are found. Unfortunately, as noted above, little if any empirical data exists for the chemicals evaluated in this Biological Evaluation at the locations where the threatened and endangered species are present. Instead, EPA has had to estimate the chemical concentrations to which threatened and endangered species are exposed in the environment. Since the exposure concentrations are not measured, but instead are estimated using the procedures described in this section, this Biological Evaluation uses the term expected environmental concentration (EEC) to describe the chemical concentrations to which threatened and endangered species are exposed in receiving waters.

In this Biological Evaluation, we use the approach used by the U.S. Food and Drug Administration (FDA) in their environmental assessments of aquaculture drugs either currently in use or proposed for use at fish hatcheries. The specific methodology used in this Biological Evaluation is described in Schmidt et al. (2007), which is the FDA environmental assessment of Chloramine-T. FDA calculated what they called an environmental introduction concentration (EIC), equivalent to the expected environmental concentration (EEC) terminology used in risk assessment from the following information: the maximum proposed product label treatment concentration of a chemical; the maximum daily treated volume of water by the chemical; the total hatchery water discharge over 24 hours; and the effluent pond or waste treatment system volume.

EPA compiled chemical use data via site visits to 18 facilities, personal communications with hatchery managers from every facility covered by the permit, and by reviewing Annual Reports and Discharge Monitoring Reports (DMRs) for all facilities. Based on applicable label instructions and chemical treatment/use data provided by the hatcheries, EPA calculated expected environmental concentrations to which fish could be exposed. In some cases, EPA relied on hatchery calculations of maximum

concentration of chemical in the effluent, which facilities are required to provide in their Annual Reports as part of the existing NPDES general permit. EPA's use of the Schmidt et al. (2007) approach allowed us to estimate the chemical concentration at the point where hatcheries discharge into surface water (the 'end of pipe' chemical concentration), which we have used as a conservative (i.e. an overestimate) of the actual concentration of chemicals to which threatened and endangered species would be exposed in their natural environment. Washington hatcheries did provide EPA with a range of daily discharge volumes, thus, we were able to estimate EECs under low, average and the maximum water discharge for each hatchery. This information ultimately allowed us to calculate a range of EEC values.

This EEC calculation also does not take into account degradation (i.e. reduction of the EEC over time due to biological, chemical and/or physical processes in the environment) of hatchery chemicals described in the environmental fate portion of each chemical's Measures of Exposure section. This assumption adds another layer of conservatism to our EEC estimates.

The EEC is calculated as follows, based on the procedures described in Schmidt et al. (2007).

$$EEC = \frac{C \times V}{F + E}$$

Where: EEC = Expected environmental concentration (mg/L or µg/L)

C = Treatment concentration of chemical in the hatchery (mg/L or µg/L)

V = Volume of chemical used (gallons/day)

F = Volume of water discharged from hatchery to receiving water (gallons/day)

E = Effluent pond volume (gallons)

For the purposes of calculating the EECs, EPA has assumed that the effluent pond volume is zero, a third conservative assumption we have made when calculating EECs in the absence of empirical exposure data.

For chlorine and freshwater chloride, the only two chemicals in this Biological Evaluation with existing water quality criteria and standards, the chronic criteria concentration set as the effluent discharge limit in the Washington hatcheries NPDES general permit is set as the expected environmental concentration (EEC). As the permit sets the chlorine discharge limit at the chronic criterion, this approach assumes that the chlorine concentration to which threatened and endangered species are exposed is that at the 'end of pipe' and no dilution of hatchery effluent by receiving water occurs.

Measures of Effect

The primary measure of effect identified in this Biological Evaluation is a chronic no effect concentration (chronic NOEC) on the survival, reproduction and growth of both threatened and endangered species and their prey. The NOEC is defined as the highest concentration of a material in a toxicity test that has no statistically significant adverse effect on the exposed population of test organisms compared with the controls. Chronic refers to the temporal duration of an exposure, and can signify time periods from weeks to years, depending on the reproductive life cycle of the species. For the purposes of this Biological Evaluation, EPA considers a chronic NOEC to be a chemical concentration that poses no unacceptable risk to the survival, growth and reproduction of a threatened and endangered species or its prey under the

exposure conditions to which a species is exposed to the chemical in its natural environment. Survival, reproduction and growth are organism level toxicological endpoints, as opposed to population level endpoints. Risk is the statistical probability or frequency of adverse effect.

Note: Acute duration exposure concentrations and effects of acute exposures were not considered because acute duration exposure concentrations are not designed or intended to be protective of the long term survival, reproduction, or growth of aquatic species. Thus, they do not meet the need of the Biological Evaluation, which is to identify chronic long term concentrations protective of aquatic species, including threatened and endangered species.

Within this Biological Evaluation, there are several ways by which a chronic NOEC can be calculated. The ideal, preferred method would be the availability of one or more empirically measured chronic NOEC concentrations for the threatened and endangered species under evaluation. Unfortunately, with the exception of some empirical information for rainbow trout (steelhead), little or no empirical toxicity data are available for any of the threatened and endangered aquatic species exposed to hatchery chemicals used in Washington.

Given the paucity of empirical toxicity data for hatchery chemicals to threatened and endangered species, EPA could either choose to not quantitatively evaluate risks from exposure to hatchery chemicals, or use existing toxicity data for other species to estimate the response of threatened and endangered species to hatchery chemicals. Several methodologies have been identified by EPA to utilize empirical toxicity data for other species to evaluate risks to threatened and endangered species. Some of these approaches are preferable to others, thus, EPA developed the hierarchy of methods to calculate chronic NOECs shown in Figure 5.

Procedures in Figure 5 are ranked from the most to the least preferable for use in predicting the toxicity of hatchery chemical to threatened and endangered species and their prey. In order to obtain chronic NOECs, in some cases empirical toxicity data had to undergo data transformations. The data transformations used in this Biological Evaluation to obtain chronic NOECs, their derivation and sources, and rationale for their use are given immediately after Figure 5.

Figure 5. Hierarchy for Deriving Chronic NOEC Values from Empirical Toxicity Data for ESA Listed Species and their Prey.

First: Empirical chronic toxicity data are available for the ESA listed species.

- Where there is only one available test result, use the NOEC from that test.
- Where there is more than one test, use best professional judgment in selecting an appropriate NOEC, based on data quality. Best professional judgment could include calculation of a geometric mean of the various NOECs to derive the chronic NOEC.

Second: Empirical acute toxicity data are available for the ESA listed species.

- Where there is only one acute test, select the lower 95% confidence interval of the LC_{50} if one is available, otherwise select the LC_{50} itself.

- Where there is more than one acute test, use as much of the data as possible to calculate a species mean acute value (SMAV), or use best professional judgment in selecting an appropriate LC_{50}. SMAV's are calculated as geometric, not arithmetic means of the available LC_{50} values.
- Divide the acute NOEC by the chemical specific acute-chronic ratio (ACR) to obtain the chronic NOEC.
- If a chemical specific ACR is unavailable, divide the acute NOEC by 8.3, the default median national ACR from Raimondo et al. (2007).

Third: Interspecies Correlation Estimation (ICE) models are available for the ESA listed species.

- Use the ICE determination for acute toxicity to estimate an LC_{50} for the ESA listed species based on the lower 95% confidence interval of the ICE predicted LC_{50} for the listed species of interest, calculated from the empirical LC_{50} for a surrogate species.
- Divide the acute NOEC by the chemical specific acute-chronic ratio (ACR) to obtain the chronic NOEC.
- If a chemical specific ACR is unavailable, divide the acute NOEC by 8.3, the default median national ACR from Raimondo et al. (2007).

Fourth: ICE models are available for the genus of the ESA listed species.

- Use the ICE determination for acute toxicity to estimate an LC_{50} for the genus of the ESA listed species based on the lower 95% confidence interval of the ICE predicted LC_{50} for the listed genus of interest, calculated from the empirical LC_{50} for a surrogate species.
- Divide the acute NOEC by the chemical specific acute-chronic ratio (ACR) to obtain the chronic NOEC.
- If a chemical specific ACR is unavailable, divide the acute NOEC by 8.3, the default median national ACR from Raimondo et al. (2007).

Fifth: ICE models are available for the family of the ESA listed species.

- Use the ICE determination for acute toxicity to estimate an LC_{50} for the family of the ESA listed species based on the lower 95% confidence interval of the ICE predicted LC_{50} for the listed family of interest, calculated from the empirical LC_{50} for a surrogate species.
- Divide the acute NOEC by the chemical specific acute-chronic ratio (ACR) to obtain the chronic NOEC.
- If a chemical specific ACR is unavailable, divide the acute NOEC by 8.3, the default median national ACR from Raimondo et al. (2007).

Within the hierarchy for calculating chronic NOEC values, the acute-chronic ratio (ACR) is defined (Hoff et al. 2010) as its historical expression of an acute LC_{50} concentration divided by a chronic no effect concentration (chronic NOEC) or a chronic maximum acceptable toxicant concentration (MATC). An MATC is defined as the geometric mean of a chronic NOEC and a chronic lowest observed effect concentration (chronic LOEC). Within the last few years, EPA's Office of Water has also allowed the use of a chronic EC_{20} (concentration affecting 20% of individuals in a chronic toxicity test as derived from regression analysis) as the denominator of an ACR derivation (Hoff et al. 2010).

$$Acute - chronic\ ratio\ (ACR) = \frac{Acute\ LC_{50}}{Chronic\ NOEC}\ or\ \frac{Acute\ LC_{50}}{Chronic\ MATC}\ or\ \frac{Acute\ LC_{50}}{Chronic\ EC_{20}}$$

Depending on the available toxicity data from any given literature study, any one of the above three definitions of an acute-chronic ratio are used in this Biological Evaluation.

Measures of Effect: Data Transformations of Empirical Toxicity Data

This section describes how empirical toxicity data will be processed for use in the analysis and risk characterization phases of this toxicity assessment. If empirically measured acute and/or chronic toxicity data are available for the ESA listed species under evaluation, those chronic concentrations are directly compared with the chronic criterion. If only acute data are available, it is compared to the chronic criterion after undergoing the transformations described below.

Note: All of the data transformations and pre-processing used in this Biological Evaluation are standard toxicological and statistical procedures used with toxicity data.

Using an LC_{50} as a toxic effect threshold during the toxicity assessment of the acute criterion clearly would not be protective of threatened and endangered species. By definition, the LC_{50} represents the concentration lethal to 50% of test organisms under the conditions of the toxicity test.

Also by definition, the acute-chronic ratio is an acute LC_{50} divided by a chronic NOEC, MATC or EC_{20}. This ACR calculation assumes that the acute toxicity tests are of the standard duration (96 hours for fish, 48 hours for invertebrates). So for most chemicals in this Biological Evaluation, a fish 96 hour LC_{50} (or if available the lower 95% confidence limit of the LC_{50}) is divided by an ACR to calculate the chronic NOEC used to determine whether a chemical concentration adversely affects a threatened and endangered species.

For potassium permanganate, however, there are essentially no toxicity tests with fish of the standard 96 hour duration. Most permanganate mortality tests available in the literature were of between 1 – 24 hours exposure duration to $KMnO_4$. In some instances, the $KMnO_4$ exposure was for one hour (the exposure duration of a bath to treat parasites or disease organisms on fish at a hatchery), followed by transfer of the fish to water without $KMnO_4$ for the duration of the study, several of which extended out to 96 hours. Furthermore, a number of the potassium permanganate toxicity tests reported only LC_0 (acute no effect concentrations) and LC_{100} (complete mortality) concentrations, without any information on concentrations associated with partial mortality. In order to estimate chronic no effect concentrations for potassium permanganate, a modification had to be made to the above hierarchy in order to transform short-term LC_{100} values to chronic NOEC values.

To convert potassium permanganate short-term (i.e. shorter than 96 hour exposures for fish or 48 hour exposures to invertebrates) LC_{100} values to a chronic NOEC, we have employed a two-part data transformation. The two parts of the data transformation are:

1. Conversion of a short duration LC_{100} to a short duration LC_{50}
2. Conversion of the short duration LC_{50} to a 96 hour LC_{50}

The first part of the data transformation, converting a LC_{100} to a LC_{50}, is based on the procedure used in the Oregon toxics Biological Evaluation (Shephard et al. 2008) to convert LC_{50} data to LC_0 to LC_{10}

concentrations (termed LC_{LOW} values in both Shephard et al. (2008) and this Biological Evaluation. The conversion is based on an assumption that dose-response curves are symmetrical around the LC_{50} concentration. The converted short duration LC_{50} value is then transformed to a 96 hour LC_{50} using a time-concentration effect (TCE) model, also termed a time to effect (TTE) model in the literature.

A generalized dose-response curve is shown in Figure 6. Note that the sigmoid curve is symmetrical around the LC_{50} concentration. This symmetry allows us to convert a short duration LC_{100} to a short duration LC_{50}. This is because the ratio between the LC_{100} and the LC_{50} is the same as the ratio between the LC_{50} and the LC_{LOW}. The basis for this ratio, set at a value of 2.27, is presented in the next several paragraphs.

To convert an short duration LC_{50} value to an 96 hour LC_{50} for toxic effects to fish threshold, the lower 95% confidence interval (if available) of the LC_{50} from a single study is divided by 2.27. If multiple LC_{50} values are available for a species, the geometric mean LC_{50} is calculated, then divided by 2.27. These procedures were used by USEPA for the Oregon toxics criteria consultation (Shephard et al. 2008). The following rationale was provided in Shephard et al (2008, page 5-21).

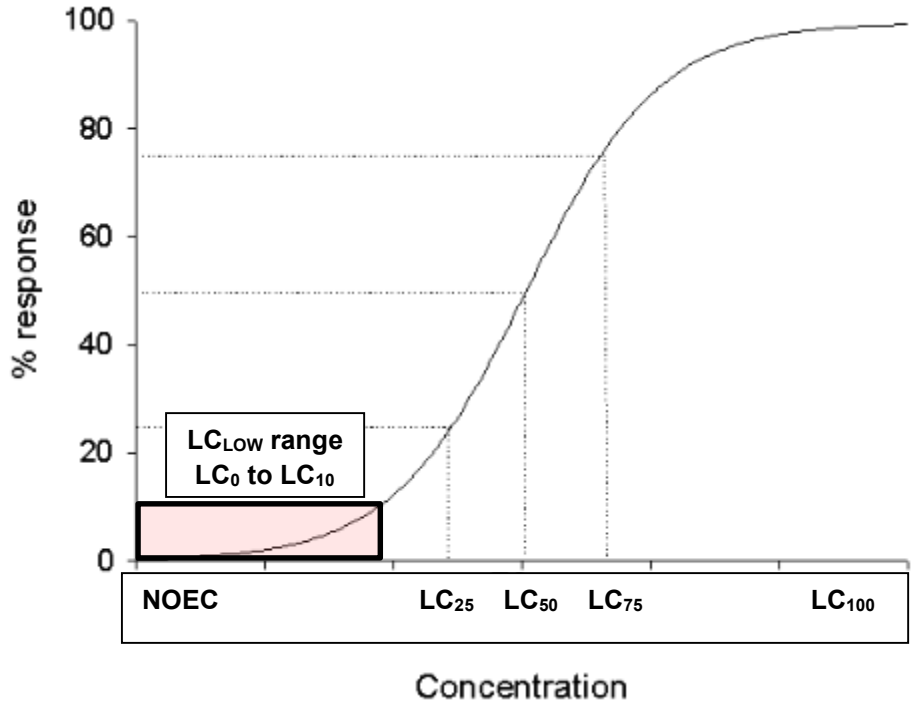


Figure 6. A generalized dose-response curve showing several effect concentrations.

The value of 2.27 is intended to convert the LC_{50} concentration to an "LC_{LOW}" value that should result in little or no toxicity to the test species. The LC_{LOW} is the concentration posing between 0 - 10% mortality among test species, equivalent to the range of control mortality allowable in standard EPA and ASTM acute toxicity testing methods for fish species.

The basis for the 2.27 adjustment factor used to convert LC_{50} values to LC_{LOW} values is an analysis of data from 219 acute toxicity tests showing that the mean concentration lethal to 0-10% of the test population

was 0.44 times the LC_{50} or its inverse, the LC_{50} divided by 2.27. The data and analysis on which the 2.27 value is based is described in the Federal Register on May 18, 1978 (43 FR 21506-21518). Briefly, the analysis consisted of calculating the geometric mean of the ratios of the highest concentration (HC) affecting or killing 0-10% of organisms divided by the LC_{50} or EC_{50} for the same organisms in the same acute test (i.e. the geometric mean of 219 HC/LC_{50} ratios from toxicity tests with a variety of chemicals). A rounded down to 2.0 value of 2.27 is used in the derivation of EPA's water quality criteria (Stephan et al. 1985) to adjust LC_{50} data before it is used to calculate the final acute value during criteria derivation.

Independent validation of the 2.27 adjustment factor was obtained from a study by Dwyer et al. (2005). Their work with five chemicals and 17 species, including some ESA listed species, shows the average multiplier to calculate a no- or low-effect concentration from an LC_{50} varies among pollutants and species from 0.50 to 0.66, with a geometric mean factor for all species of 0.56. Use of the Dwyer et al. (2005) geometric mean LC_{50} multiplier is mathematically equivalent to dividing the LC_{50} by 1.8 (the inverse of 0.56 to two significant figures). All computations of the mean LC_{50}/LC_{10} presented in Dwyer et al. (2005) result in low- or no-acute effect concentrations higher than are calculated through the use of EPA's 2.27 adjustment factor. In other words, use of the Dwyer et al. (2005) multiplier of 0.56 results in a less conservative estimate of the acute no effect concentration (Dwyer et al. 2005 terminology) than does use of the EPA 2.27 adjustment factor used to calculate LC_{LOW} concentrations (EPA terminology) used in this Biological Evaluation. This observation suggests that the EPA developed adjustment factor of 2.27 is a protective method that can be used to convert LC_{50} concentrations to LC_{LOW} concentrations.

For potassium permanganate analyses, the short duration LC_{50}, calculated as the LC_{100} divided by 2.27, still needs to be converted to a 96 hour LC_{50}. This is accomplished by applying a time-concentration effect (or time to effect) conversion that transforms the short duration LC_{50} to a 96 hour LC_{50}. Hoff et al. (2010) recommended a safety factor of 0.2 be applied to acute toxicity data to calculate a survival NOEC under normal acute toxicity test durations. Time-concentration effect calculations require one or more partial mortality response in order to perform the calculations, information that is not generally present in the potassium permanganate toxicity literature. In the absence of any other specific information, we multiply the recommended 0.2 safety factor by the short duration LC_{50} to obtain a 96 hour survival NOEC. We then use this survival NOEC in interspecies correlation estimation models as a 96 hour LC_{50} value for species without empirical toxicity data, then divide either the empirical $KMnO_4$ 96 hour LC_{50} or the estimated survival NOEC by the acute-chronic ratio to obtain the chronic NOEC values for potassium permanganate. We believe this procedure should allow calculation of a conservative chronic NOEC for $KMnO_4$.

If empirical chronic toxicity data is already reported in the literature for the listed species, the NOEC from the study is directly compared to the chronic criterion (for chlorine) or to the expected environmental concentration (for all other chemicals). If only empirical acute toxicity data are available for the listed species, the above procedures to convert LC_{50} to chronic NOEC values are used. Once the acute LC_{50} is obtained (or for potassium permanganate, the acute 96 hour NOEC), it is divided by a chemical specific acute-chronic ratio (ACR) to yield the chronic NOEC concentration for the listed species. If a chemical specific or threatened and endangered species specific ACR is unavailable for a given chemical – threatened and endangered species pair, a default ACR = 8.3 from Raimondo et al. (2007) is used to convert the acute NOEC to a chronic NOEC.

If empirical toxicity data are unavailable for an ESA listed species of interest, the next section of this measures of effect portion of the ecological risk assessment analysis phase describes how toxicity data for surrogate species is used to characterize risks to the ESA listed species of concern.

Note: Implications of exceeding 96 hours of exposure are chemical and species specific. They relate to the steepness of the concentration-response curve for each chemical-species pair. For acutely lethal chemical concentrations, extending the exposure past 96 hours eventually results in the determination of an endpoint called the incipient lethal level (ILL). The ILL is defined as the concentration lethal to 50% of the test population within a sufficiently long time period that mortality has ceased. For chemicals such as chlorine, with very steep concentration-response curves, an ILL may be reached within 24 – 48 hours. For other chemicals with shallow concentration-response relationships, such as dioxin, it may take weeks to reach the ILL.

Measures of Effect: Interspecies Correlation Estimation (ICE) Methodology

It is impractical for toxicologists to perform laboratory toxicity studies on all aquatic species present in North America with all chemicals to which they are exposed in the environment. This is particularly true for ESA listed species, whose rarity or limited distribution in the environment generally precludes their use as test organisms in aquatic toxicology, except for limited research purposes. Interspecies correlation estimation (ICE) models are statistical regressions that permit estimations of LC_{50}s to be made for a species or higher taxa (genus, family) having no measured acute toxicity information from a species for which five or more LC_{50}s have been measured. The detailed description of how ICE models were developed and their use to estimate LC_{50}s for taxa for which no toxicity information is available is given in Raimondo et al. (2013).

ICE models between two taxa are linear regressions of the form shown in Equation 1.

Equation 1: $\log_{10} X_2 = a + (b \times [\log_{10} X_1])$

Where: X_1 is a measured LC_{50} for an aquatic species (e.g. coho salmon, *Daphnia magna*)
X_2 is the predicted LC_{50} for the taxa (species, genus or family) without toxicity data

The current version of ICE, called WebICE, is freely available from EPA on the Internet at: http://www.epa.gov/ceampubl/fchain/webice/

The endangered species module of WebICE contains species, genus and family level regressions for all threatened and endangered salmonid species found in Washington. Empirical toxicity data has been identified for at least rainbow trout (steelhead) for all hatchery chemicals undergoing detailed analysis in this Biological Evaluation, although not all of the empirical rainbow trout data meets current EPA data quality requirements for use in national water quality criteria development. Therefore, ICE models can be developed for all chemicals and all salmonid species evaluated in this Biological Evaluation, permitting quantitative evaluation of risks from hatchery chemicals. Both the LC_{50} and the lower 95% confidence interval of the modeled species LC_{50} is calculated by the ICE model. The acute LC_{50} for each threatened and endangered species is then transformed into chronic NOEC values using either a chemical specific or a national default acute-chronic ratio, as described earlier in the measures of effect section. Each threatened and endangered species chronic NOEC, whether from empirical data or calculated from ICE, is used in the risk characterization of hatchery chemicals to threatened and endangered species.

Risk Characterization: Description of How Risks are to be Characterized

The basic approach for evaluating the potential adverse effects of hatchery chemicals released to the environment to ESA listed species is the standard ecological risk assessment hazard quotient (HQ) approach (EPA 1998). This approach has been used in other ESA consultations, such as the Oregon Toxics Biological Evaluation (Shephard, et al. 2008). A chronic NOEC value, either from empirical data or estimated by one of the methods presented in the analysis plan section of this Biological Evaluation, is the chemical concentration that represents the highest concentration having no measurable effect on a threatened and endangered species. The chronic NOEC is specific to each chemical – threatened and endangered species pair. The chronic NOEC for each listed species was then compared to the expected environmental concentration (EEC) of that chemical, defined as the estimated or anticipated maximum concentration of a hatchery chemical in surface water after its discharge or release from a hatchery. The comparison is in the form of a hazard quotient (HQ) as shown in Equation 2:

Equation 2:

$$HQ = \frac{EEC}{\text{Chronic NOEC}}$$

Where:
HQ = hazard quotient (unitless)
EEC = Expected environmental concentration, the calculated concentration of a chemical in a receiving body of water after its release from a hatchery, in units of µg/L
Chronic NOEC = Either the measured or calculated chronic (long-term) no effect concentration for a threatened or endangered species, in units of µg/L

Interpretation of a hazard quotient is as follows:

If the HQ < 1, a determination of "may effect, not likely to adversely affect (NLAA)" is made (i.e. the EEC is expected to be lower than the chemical concentration expected to elicit toxicity in an ESA listed species)

If the HQ ≥ 1, a determination of "may affect, likely to adversely affect (LAA)" is made.

As discussed in the introduction to this methodology, chemicals with an HQ ≥ 1.0 for one or more chemical – threatened and endangered species pairs is considered to be a chemical of potential ecological concern (COPEC) in this evaluation. An HQ ≥ 1.0 can occur in one of the following situations:

1. The EEC is within the known range of adverse effect concentrations to threatened and endangered species and/or their prey. This is the worst case situation for exposure to hatchery chemicals.
2. The EEC is above the chronic NOEC but below a known or estimated adverse effect concentration for a threatened and endangered species and/or its prey. This is the situation where interpretation of a HQ is most problematic or uncertain.
3. The EEC is above the chronic NOEC or a known adverse effect concentration for one or more threatened and endangered species and/or its prey, but below the chronic NOEC for other species. The chemical would be a COPEC for some, but not all threatened and endangered species.

Receiving water flows and discharges are unavailable for the aquatic systems on which most Washington hatcheries are located, at least within the immediate location of the hatcheries. This lack of information precludes calculation of a receiving water EEC for most Washington hatcheries. There is no quantitative method to estimate dilution of hatchery effluent concentrations at the 'end of pipe' by receiving waters without information on flow in receiving water. In these cases, EPA has assumed that the chemical concentration estimated at the 'end of pipe' of a hatchery is the concentration to which threatened and endangered species are exposed in the environment.

This approach of using the 'end of pipe' chemical concentration as an EEC results in a very conservative estimate of the EEC in receiving waters. However, if the hazard quotient calculated from this EEC is less than 1, then the concentration of chemical to which threatened and endangered fish are exposed to in the receiving water environment will also be less than 1. Indeed, the receiving water HQ will always be smaller than the HQ derived using the 'end of pipe' concentration as the EEC due to dilution of hatchery discharges by the receiving water. The conclusion EPA will draw from an 'end of pipe' EEC lower than a chronic NOEC for a chemical is not likely to adversely affect, even without an estimate of a receiving water EEC.

For chlorine, one of the only two chemicals in this Biological Evaluation with national water quality criteria and Washington water quality standards (freshwater chloride ion is the other), a slightly different approach is used to describe risks. In this case, the EEC is defined as the chronic criterion (either freshwater or marine) for chlorine, a value that is written into the NPDES permit. The EEC for chlorine is not the calculated concentration discharged from a hatchery at the end of pipe, because there are effluent limits and monitoring requirements for chlorine in the permit. In other words, the chlorine concentration permitted to be discharged from a hatchery must be lower than the chronic chlorine criterion for a hatchery to be in compliance with the NPDES permit. If the concentration of chlorine (the chronic NOEC) required to elicit toxicity in a threatened and endangered species is lower than the chlorine criterion, the criterion is not protective of that species.

Description of How Risks are to be Characterized – Toxicity to Prey of ESA Listed Species

The range of chronic NOEC values for the following broad groups of prey species are summarized in tabular form in the text: algae, aquatic macrophytes, aquatic invertebrates, amphibians, and fish. The aquatic invertebrate section is further subdivided to present the range of chronic NOEC values for zooplankton, aquatic insects, non-zooplankton crustaceans, molluscs, and other invertebrate species such as oligochaetes. Chronic NOEC values for prey species are calculated in the same manner as are chronic NOEC values for ESA listed species or their surrogate species.

Not all of these taxonomic groups may be prey for all of the ESA listed species evaluated in this Biological Evaluation. However, given the anticipated lack of toxicological information for most of the chemicals evaluated, EPA believes it is most informative to include all available toxicological information about non-ESA listed species, whether or not they are prey of listed species.

In the analysis on the effects of hatchery releases on prey species, the chronic NOEC range of values for prey species are compared to the EEC for each chemical. If the lowest chronic NOEC for a prey species exceeds the EEC of a chemical, no further analyses are made.

No quantitative determinations are made regarding whether or not reductions in prey species richness are likely to adversely affect ESA listed species. Instead, the analysis of a meaningful reduction in the diet of ESA listed species includes a qualitative discussion of which prey species have acute or chronic toxicity values below the criteria concentration, a discussion of whether those prey species are primary prey species of ESA listed species, and whether or not the loss of those prey species is likely to adversely affect the listed species.

Uncertainties Associated with the Chemical Toxicity Assessment of Threatened and Endangered Species

By design, risk assessments are conservative in the face of uncertainty. In this context, "conservative" means efforts were made to minimize the chances of underestimating exposure, effects, or risk. The uncertainty analysis portion of each chemical's toxicity assessment is intended to illustrate the degree of confidence in the conclusions of the assessment.

Uncertainty in a risk assessment has four components:

1. **Variation** (e.g. a fish is exposed to a range of chemical concentrations in water, not to a constant concentration of a chemical);
2. **Model uncertainty** (e.g. use of a single species or several target ecological receptors to represent the sensitivity of a threatened and endangered species to a chemical introduces uncertainty because of the considerable amount of interspecies variability in sensitivity to a chemical);
3. **Decision rule uncertainty** (e.g. use of a dichotomous decision framework to determine chemical effects (i.e. NLAA vs. LAA) instead of calculating the probability of an adverse effect at the expected environmental concentrations); and
4. **True unknowns** (e.g. the toxic effects of chlorine in water on bull trout survival, growth, and reproduction have never been studied, and are unknown).

Consistent with the methods of the problem formulation, receptor-contaminant pairs subject to potentially unacceptable risk from exposure to chemicals in surface waters were identified using conservative methods and assumptions. Examples of conservatism include assumptions that chemical contaminant concentrations are 100% bioavailable, and assumptions that the most reliable evaluation of chemical toxicity to threatened and endangered species in the absence of empirical threatened and endangered species toxicity data generally comes from basing the assessment only on the most closely taxonomically related species to a particular threatened and endangered species that had available and high quality empirical toxicity data.

The largest single uncertainty in any toxicity assessment is the absence of any measured toxicity data for a species of interest. This is a true unknown, and required the use of toxicity data for surrogate species to estimate chemical effects on threatened and endangered species evaluated within this Biological Evaluation. In some cases, no empirical data are available permitting toxicity estimates using ICE models. This situation occurs when no or insufficient empirical toxicity data are available from the family of a threatened and endangered fish species to permit a family level ICE model to be run.

Much of the risk characterization is based on the output of ICE models. ICE models are generated from a database of empirical LC_{50} values for a large number of chemicals. To generate an ICE model, all species LC_{50}s are paired with each other by common chemical. Three or more common chemicals per pair are

required to develop an ICE model. The more LC_{50} pairs that are available to develop an ICE model, in general the less uncertain are model predictions and the more statistical power model predictions have (statistical power is the probability that a hypothesis test will correctly reject a null hypothesis that is false).

Uncertainty in the ICE models is described by the percent cross-validation success statistic. According to Raimondo et al. (2013), the percent cross-validation success rate for each model is the proportion of data points that are predicted within 5-fold of the actual LC_{50} value. There is a strong relationship between taxonomic distance and cross-validation success rate, with uncertainty increasing with larger taxonomic distance between the surrogate and predicted taxa. This is the primary reason that one of the more preferred methods for estimating toxicity to threatened and endangered species is the species level ICE model. For fish and aquatic invertebrates, ICE models overall predict within 5-fold and 10-fold of the actual LC_{50} value with 91 and 96% certainty for surrogate and predicted taxa within the same family, and for 86 and 96% within the same order. Although perhaps arbitrary, EPA prefers not to use ICE models in this Biological Evaluation with less than a 90% cross-validation success statistic, unless all ICE models have a less than 90% cross validation success statistic value. This is also the primary reason why the ICE model does not evaluate taxa further apart than the family level.

EPA's aquatic life criteria are designed to protect 95% of aquatic genera from adverse effects, not 100% of aquatic species. Given this design, it is possible that one or more important prey species of a threatened and endangered species within the action area not tested may be subject to toxic effects at chemical concentrations lower than the chronic NOEC. Loss of such species could reduce the prey base available to threatened and endangered species.

Use of acute-chronic ratios to convert 96-hr LC_{50} data to chronic maximum acceptable toxicant concentrations (MATC's) introduces uncertainties into the evaluation of the chronic criteria. A study by Raimondo et al. (2007) determined a geometric mean acute-chronic ratio of 8.3 from a data set of 456 same-species pairs of acute and maximum acceptable toxicant concentrations for metals, narcotics, pesticides, and other organic chemicals. ACR's smaller than 8.3, such as the chlorine ACR of 3.345, are often indicative of a chemical with a relatively steep dose response curve, meaning the difference between adverse and no adverse effect concentrations for a given species may be small.

5.2 CHLORINE

Freshwater acute (CMC) criterion = 19 µg/L
Freshwater chronic (CCC) criterion = 11 µg/L

Marine water acute (CMC) criterion = 13 µg/L
Marine water chronic (CCC) criterion = 7.5 µg/L

CAS ID: Elemental Chlorine 7782-50-5; CAS ID numbers for chemical forms of chlorine combined with other elements presented in Measures of Effect section

Chemical formula: Cl_2; chemical formula for chemical forms of chlorine combined with other elements presented in Measures of Effect section

Synonyms / Trade names: Chlorine dioxide, sodium hypochlorite, bleach, Clorox, HTH chlorine, calcium hypochlorite

Hatchery use: Four hatcheries covered by the permit (Makah, Quinault, Quilcene, and Spring Creek National Fish Hatcheries) report using chlorine to disinfect effluent from isolation incubation buildings that house fish from another watershed (to prevent disease spread from basin to basin). This use of chlorine could potentially result in its release to receiving waters where threatened and endangered species are present, although facilities are required to meet the chlorine limits in the permit. Chlorine solutions are often neutralized with sodium thiosulfate before discharge into the environment.

Little White Salmon National Fish Hatchery reports spraying chlorine on dewatered raceways at the end of the season for disinfection purposes, then allows the chlorine solution to dry for at least 24 hours. Dried chlorine solutions on raceway walls (indeed on any surface exposed to outdoor ambient light) rapidly degrade so that the concentration of biologically active chlorine is reduced to zero. The chemistry of these reactions is described in detail in the environmental chemistry and fate of chlorine section later in this chapter. It is not anticipated that any chlorine from its use on dewatered raceways would reach receiving waters where threatened and endangered species are present. None of the hatcheries currently using chlorine discharge directly to estuarine or marine systems. Thus, under current use conditions, threatened and endangered species in marine waters are not exposed to chlorine releases from hatcheries.

The NPDES permit for Washington hatcheries contains effluent limitations set at the Washington chronic chlorine standards of 11 µg/L (discharges into freshwater) or 7.5 µg/L (discharges into estuarine or marine waters). It is these chlorine permit limits that are evaluated for risks to threatened and endangered species in this Biological Evaluation. Chlorine discharges at concentrations exceeding the chronic criteria will violate the NPDES permit limit for Washington hatcheries, and will not be allowed. Therefore, evaluation of the acute chlorine criteria is not germane to hatchery discharges in this Biological Evaluation BE.

Introduction

Unlike all other hatchery chemicals used in Washington and evaluated in this Biological Evaluation, chlorine has both acute and chronic water quality standards within both the fresh and marine waters of Washington State. The Washington chlorine standards (Ecology 2012) are identical to the EPA (1985)

water quality criteria for chlorine. The chlorine standards do not apply to chloride ion concentrations in freshwater, for which both EPA and Washington State have separate chloride criteria and standards. The freshwater chloride standards are discussed in the Problem Formulation section of this Biological Evaluation.

The only previous biological evaluation of the chlorine water quality criteria of which EPA is aware was performed for proposed water quality standards of the Coeur d'Alene Tribe in Idaho (EPA 2013). The Coeur d'Alene Tribe chlorine standards, numerically the same as the Washington State chlorine standards, apply only to freshwaters where bull trout was the only threatened or endangered species present. The Biological Evaluation for the Coeur d'Alene Tribe water quality standards concluded that the proposed Tribal chlorine standard was not likely to adversely affect bull trout, a conclusion with which the USFWS concurred. No threatened or endangered species for which NMFS has trust responsibilities are present within waters subject to the Coeur d'Alene Tribe water quality standards, thus NMFS did not prepare an opinion on the Coeur d'Alene Tribe chlorine standards.

The current NPDES general permit for Washington hatcheries, as well as the new permit, both have effluent limitations for chlorine set at the chronic criterion chlorine concentration of 11 µg/L and 7.5 µg/L for fresh and marine waters, respectively.

Problem Formulation

Objective of the Biological Evaluation of the Chlorine Aquatic Life Criteria

The objective of this section of the Biological Evaluation is to determine whether an EPA approval of the proposed NPDES permit limit for chlorine, which is equivalent to the chronic chlorine national water quality criterion and Washington chronic chlorine standard, is protective of threatened and endangered species.

Mechanism of Toxic Action of Chlorine

The toxic mechanism(s) of action of residual chlorine to aquatic life are not fully understood, but are likely related to the ability of chlorine to oxidize organic matter. Intracellular enzymes containing sulfhydryl groups are oxidized almost immediately by residual chlorine in both plants and animals. Due to the strength of the chemical bond formed between chlorine and proteins, enzyme activity is irreversibly terminated. This irreversible nature of chlorine reacting with enzymes likely explains the observed irreversible toxicity of chlorine to fish once equilibrium has been lost (Alabaster and Lloyd 1982).

In fish, gills are believed to be the primary site of toxic action of chlorine. This is based on multiple observations of damage to gill epithelium following exposure to chlorine. Cairns et al. (1975) concluded that the mode of toxic action of chlorine to fish is gill tissue damage combined with accumulation of mucus on the gills. The combination of physical damage to gill tissue and coating of gill tissue by mucus inhibits oxygen uptake, resulting in suffocation of the fish.

If the mechanism of toxic action proposed by Cairns et al. (1975) is correct, chlorine is one of the relatively few chemicals that does not require an internally bioaccumulated dose to elicit toxicity to aquatic life. The mechanism of toxic action of chlorine limits the exposure of both fully aquatic and aquatic-dependent species to chlorine, as it precludes exposure via the dietary ingestion exposure route. Ingestion via drinking water is an insignificant contaminant exposure pathway to freshwater fish, which are

physiologically constrained from ingesting water because of their need to maintain a higher internal solute content than found in their external freshwater environment.

Conceptual Model of Chlorine Toxicity to Threatened and Endangered Species and their Prey

The reactivity of chlorine with other substances found in aquatic systems, combined with the volatility of chlorine gas limits both the concentration and residence time of chlorine in aquatic systems. Unlike most other chemicals discharged to aquatic systems, sediments do not serve as a sink for chlorine. Sediment is therefore not a medium by which aquatic species are exposed to chlorine. The combination of these factors also serves to limit the complete and significant exposure pathway of aquatic species to chlorine discharged to surface waters to direct contact, primarily with respiratory surfaces of aquatic species.

Consistent with the mode of toxic action for chlorine, dietary ingestion of chlorine is considered an insignificant exposure route for both threatened and endangered fully aquatic and aquatic-dependent species, as well as their prey in this Biological Evaluation. Therefore, the dietary ingestion exposure route will not be quantitatively evaluated for any species in this Biological Evaluation.

Measures of Exposure

As described in the methodology section, the expected environmental concentration (EEC) of chlorine is set at either 11 µg/L (freshwater) or 7.5 µg/L (estuarine or marine water). These values are the respective chronic criteria for chlorine in fresh and marine waters. They are also the proposed Washington hatcheries NPDES permit effluent discharge limits at 'end of pipe'.

Environmental Chemistry and Fate of Chlorine

Chlorine is a chemical element, atomic number 17, atomic weight 35.453. Except for minute amounts released to the atmosphere from volcanic eruptions, elemental chlorine is not found in a free state in nature due to its reactive nature. Elemental chlorine is a yellowish green gas under all conditions normally found in the environment except for extreme cold temperatures (boiling point = -34°C or -29°F). Elemental chlorine is most commonly produced by the chloralkali process, which is the electrolysis of sodium chloride dissolved in water. Electrolysis of brine produces diatomic or elemental chlorine (Cl_2), hydrogen gas and sodium hydroxide.

Use of chlorine in hatcheries for disease control purposes mimics its use in public health. Use of chlorine since the early 1900's as a disinfectant in both drinking water and sewage before it is discharged to surface water, with the concomitant reduction or elimination of many waterborne infectious diseases has been identified as one of the top ten advances in public health of the 20th century (CDC 1999).

The water chemistry of chlorine in freshwater is among the most complex of any contaminant evaluated in this Biological Evaluation. In addition to having a complex chemistry, there are multiple names in the literature for the same or similar combinations of chlorine chemical forms, necessitating this discussion of chlorine chemistry and terminology used in this Biological Evaluation.

The EPA aquatic life criteria for chlorine describes the toxicity of total residual chlorine (TRC), which is the combined concentration of different chemical forms of chlorine able to react with other substances, or which can interconvert among each other. Within the literature, TRC is generally synonymous with reactive chlorine (RC), combined residual chlorine (CRC), and total available chlorine (TAC).

Total residual chlorine includes free available chlorine (FAC; hypochlorous acid [HOCl] and the hypochlorite ion [OCl^-]; also referred to as free residual chlorine [FRC]) and combined available chlorine (CAC; organic and inorganic chloramines [NH_2Cl or monochloramine, $NHCl_2$ or dichloramine, and NCl_3 or nitrogen trichloride]). Chloramines are also often termed N-chloramides.

In ambient freshwater, the dominant reactive chlorine species are hypochlorous acid and its associated hypochlorite anion in waters with low ammonia or nitrogen concentrations. The hypochlorite anion is one of several compounds or anions that collectively are called chlorine oxides, the best known of which may be the perchlorate anion ($HClO_4^-$). Hypochlorous acid and its associated hypochlorite anion, along with chlorine dioxide (ClO_2) are by far the chlorine oxides most commonly utilized in water disinfection. Chlorine dioxide is also commonly used in the industrial bleaching of wood pulp.

Like elemental chlorine, chlorine dioxide is also a gas at temperatures found in the environment. Rather than hydrolyzing in water as chlorine does, chlorine dioxide forms a true solution in water under typical surface water conditions. Chlorine dioxide is volatile and is easily lost from water. Chlorine dioxide is a powerful oxidant but unlike chlorine, does not readily combine with ammonia to form chloramines. Chlorine dioxide also does not form trihalomethanes such as chloroform. Due to its reactive nature, chlorine dioxide is produced on-site at locations where it is used as a disinfectant.

Monochloramine can be a dominant chemical form if sufficient nitrogen, particularly in the form of ammonia/ammonium ion is present in surface water. Di- and trichloramines are only formed in water at pH < 6 and when the Cl_2:NH_3 is at least 5:1 (Hankin 2001). Free chlorine gas (Cl_2) becomes the dominant chemical form only in low organic content waters with a pH < 2. Chlorine can also react with naturally occurring organic matter in water to form a number of disinfection byproducts, including trihalomethane compounds such as chloroform.

The initial chemical reaction when Cl_2 is added to surface water is one of hydrolysis (EPA 1976):

$$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^-$$

Hypochlorous acid (HOCl) is a weak acid, and undergoes a pH dependent dissociation:

$$HOCl \leftrightarrow H^+ + OCl^-$$

The release of hydrogen ions from hydrolysis of Cl_2 and the dissociation of hypochlorous acid are the reasons chlorination of surface water tends to reduce the pH of the water. The ratio of HOCl to OCl^- is pH dependent, with 96% HOCl present at pH 6, 75% HOCl at pH 7, 22% HOCl at pH 8, and only 3% HOCl at pH 9. The proportion of HOCl present in water is significant, as HOCl is the chemical form most effective as a disinfectant (Shannon et al. 2008).

Analytical determination of the various chemical species within TRC is generally not performed, and is generally not feasible at the low µg/L concentrations of toxicological relevance in surface waters. This is the reason the EPA aquatic life criteria are expressed in terms of TRC, not as criteria for the individual chemical forms comprising TRC.

Without continuous addition of chlorine to water, TRC concentrations in water can be quickly reduced through several chemical, physical and biological processes. In addition to the chemical reactions in the

water column described above, these processes include volatilization, photodegradation, adsorption on solids, and reactions with aquatic life.

Degradation rates of chlorine species in natural waters are generally rapid, ranging between seconds and hours. The half life of chlorine gas (Cl_2) in surface water has been reported as 0.005 second (EPA 1994). Cooper et al. (2007) have performed a number of photodegradation half life studies with HOCl / OCl^- mixtures under various pH values and water depths, and at several dissolved organic matter concentrations. The light intensity used was based on that at solar noon in both summer and winter at the latitude of Miami, Florida (24° N). In distilled water, the photodegradation half life of a HOCl / OCl^- mixture ranged between 41 minutes at pH 5.0 to 17 minutes at pH 7.0 to six minutes at pH 12.0. Half lives of a HOCl / OCl^- mixture were shortest in waters exposed to higher light intensity (i.e. summer light intensities), in waters with the lowest dissolved organic matter concentrations, and in waters of the shallowest depths. Shortest half lives of just over nine minutes occurred under conditions of summer light intensity in surface water at 0 meters depth and with dissolved organic matter concentrations of either 0.53 or 17.6 mg C/L. The only half lives longer than 10 hours observed by Cooper et al. (2007) occurred under conditions of water with a depth ≥ 1 meter with a dissolved organic matter concentration of 17.6 mg C/L under either summer or winter light intensity. In water containing 0.53 mg C/L dissolved organic matter and with depth ≤ 5 meters, all HOCl / OCl^- mixture half lives were 5.85 hours or shorter under all light intensities tested.

Historically at fish hatcheries, sodium thiosulfate has been used to neutralize and remove residual chlorine from water in which fish are eventually to be held. The reaction of sodium thiosulfate with chlorine produces sodium chloride as the end product of the residual chlorine, with the thiosulfate being converted to sodium tetrathionate, as follows:

$$Cl_2 + 2Na_2S_2O_3x5H_2O \rightarrow Na_2S_4O_6 + 2NaCl + 10\ H_2O$$

The stoichiometric ratio of sodium thiosulfate to residual chlorine to completely neutralize the chlorine without the addition of excess sodium thiosulfate is 6.99:1 (i.e. 6.99 mg/L sodium thiosulfate pentahydrate neutralizes 1 mg/L chlorine).

Sodium thiosulfate also neutralizes hypochlorous acid and monochloramines according to the following reactions:

$$2Na_2S_2O_3 + HOCl \rightarrow Na_2S_4O_6 + NaCl + NaOH$$

$$NH_2Cl + 2Na_2S_2O_3x5H_2O \rightarrow Na_2S_4O_6 + 2NaOH + NH_3 + HCl + 3H_2O$$

Several reactions occur in chlorine solutions which are sprayed on hatchery surfaces such as raceways, then allowed to dry, that reduce chlorine to non-toxic chemical forms. The two most common reactions both involve transformation into sodium chloride, with either sodium chlorate ($NaClO_3$) or elemental oxygen as byproducts. These reactions are illustrated using sodium hypochlorite as the chlorine solution sprayed onto surfaces, as follows:

$$3NaOCl \rightarrow 2NaCl + NaClO_3 \text{ (chlorate formation)}$$

$$2NaOCl \rightarrow 2NaCl + O_2 \text{ (elemental oxygen formation)}$$

Warmer temperatures, higher hypochlorite concentrations and higher ionic strength (i.e. the concentration of salts in water) all serve to increase the reaction rate of the breakdown of hypochlorite solutions to sodium chloride and either sodium chlorate or elemental oxygen. The conversion of hypochlorite to chlorate is the more common of the two reactions. The formation of sodium chloride and sodium chlorate accounts for the white powder often observed on surfaces after hypochlorite solutions have dried. Sunlight also speeds up the decomposition of hypochlorite solutions through the process of photolysis.

$$2OCl^- + \text{ultraviolet light} \rightarrow 2Cl^- + O_2 \quad \text{(photolysis)}$$

$$2NaOCl + \text{ultraviolet light} \rightarrow 2NaCl + O_2 \quad \text{(photolysis)}$$

In water with a pH of 8.0, the half-life of sodium hypochlorite undergoing the above photolysis reactions is 12 minutes (Oltchim 2011). The photolysis half-life of chlorine in water varies with pH, chlorine/hypochlorite concentration, light intensity and water temperature, with pH having the largest effect. Forsyth (2012) evaluated the half-life of chlorine during photolysis under a range of pH (6, 7 and 8), water temperatures (10° and 25°C), light intensities up to full natural sunlight intensity during May at latitude 47°N, and chlorine chemical forms, and observed a range of half-lives between 9 – 96 minutes. The half-life of chlorine under photolysis gets longer as the pH becomes more acidic. Metal cations in water, including iron, nickel, cobalt and copper also catalyze the breakdown of hypochlorite anion (OCl^-) to chloride anion (Cl^-) and oxygen. Allowing chlorine solutions to completely dry on surfaces, particularly outdoor surfaces for a 24 hour period before they are rinsed or refilled with water should reduce the concentration of biologically active chlorine forms to non-toxic levels.

In marine and estuarine waters, the chemistry of chlorine is, if anything, even more complex than it is in freshwater. Full strength (35‰) seawater contains roughly 70 mg/L bromide ion, mostly in the form of sodium bromide. This is substantially higher than the EPA chronic chlorine criterion for saltwater of 7.5 µg/L. But because elemental chlorine (Cl_2) has a higher standard reduction potential (i.e. is a stronger oxidant) than does elemental bromine (Br_2), chlorine can displace bromine from sodium bromide via the following reaction:

$$2\ NaBr + Cl_2 \rightarrow 2NaCl + Br_2$$

Other chemical forms of chlorine, including hypochlorous acid can also rapidly react with bromide in seawater to form a series of brominated compounds (Singleton 1989). If ammonia is present, the brominated compounds can form a series of bromamines analogous to the chloramines formed in freshwater. Dibromamine is the most commonly formed bromamine in sea water of pH = 8. Several of the more important chlorine and bromine reactions in sea water are shown below.

$$HOCl + Br^- \leftrightarrow HOBr + Cl^- \quad \text{(hypochlorous acid converts to hypobromous acid)}$$
$$OCl^- + Br^- \leftrightarrow OBr^- + Cl^- \quad \text{(hypochlorite converts to hypobromite)}$$
$$HOBr + NH_3 \leftrightarrow NH_2Br + H_2O \quad \text{(monobromamine formation)}$$
$$HOBr + NH_2Br \leftrightarrow NHBr_2 + H_2O \quad \text{(dibromamine formation)}$$

These reactions are important to understand the disinfecting ability of chlorine in sea water. The rapid formation of brominated compounds in sea water after the addition of chlorine means in practice much of the disinfecting capacity of chlorine in sea water is actually due to bromine compounds, not chlorine

compounds. As acknowledgement of the role of bromine in disinfection in marine and estuarine systems, the term 'chlorine produced oxidants' is often used to describe the sum of the concentrations of all oxidative chemical forms of chlorine and bromine in saltwater. The standard analytical methods (most commonly amperometric titration) used to measure total residual chlorine in freshwater also detect the various chemical forms of bromine in saltwater. However, due to the presence of both chlorinated and brominated compounds in saltwater with disinfecting properties, results of the analysis for chlorine in saltwater are often expressed in units of µg/L chlorine produced oxidants, not µg/L total residual chlorine as is the case in freshwater.

The short persistence of chlorine in water relative to the duration of standard toxicity tests with fish and invertebrates has direct bearing on the experimental design of toxicity studies useable to evaluate chlorine toxicity to threatened and endangered fish species. In order to maintain a consistent concentration of chlorine in laboratory toxicity tests, flow through studies where chlorine concentrations are constantly replenished are needed. EPA's water quality criteria are designed to apply in situations of continuous exposure to a contaminant. They are not designed to be applied in situations of intermittent contaminant exposure. Much of the available aquatic toxicity data for chlorine describes information generated during either very short term studies (three hours or shorter), from exposure to chlorinated sewage effluent (an unacceptable dilution water) or from intermittent exposures. These short term and intermittent studies are not suited for EPA water quality criteria development or evaluation of effects on threatened or endangered species, as they are not representative of effects from continuous exposure to chlorine. The chlorine effects determination within this Biological Evaluation are therefore based only on continuous flow through exposures of acceptable duration (96 hours for acute mortality studies with fish).

Measures of Ecosystem and Receptor Characteristics

Section 4 describes the range, critical habitat, life history, population trends and status of the threatened and endangered species evaluated in this toxicity assessment.

Measures of Effect

To characterize ecological effects, it must first be verified that the stressor elicits adverse effects on ecological entities of interest. Once verified, the adverse effects elicited by the stressor are described, and then evaluated in terms of how the magnitude of adverse effect changes as the concentration of the stressor changes. Finally, it is confirmed that the observed effects are consistent with the environmental values to be protected as described in the assessment endpoints, as well as confirming that the exposure conditions under which the observed adverse effects occur are consistent with the conceptual model.

This chlorine toxicity assessment, the primary focus of this Measures of Effect section, is based completely on existing information. The toxicity data for aquatic species is that presented in the EPA (1985) water quality criteria document for chlorine has been augmented by 2012 and 2013 EPA literature searches for additional toxicity information published in the literature subsequent to the publication of the EPA (1985) chlorine criteria document. The toxicity assessment infers or extrapolates chlorine effects on threatened and endangered fish species and their prey from this existing data.

All measures of effect in this toxicity assessment are laboratory toxicity tests where empirically measured chlorine concentrations in water were associated with adverse effects on survival, reproduction or growth of aquatic species. Mixture studies where chlorine was part of a mixture of contaminants to which a test

species was exposed are not included in the measures of effect data, as it is generally not possible to attribute the proportion of the response due to chlorine.

Specifically, mixture studies where aquatic species were exposed to sewage or wastewater disinfected with chlorine were excluded as a primary line of evidence in this Biological Evaluation. This exclusion is because there is no quantitative method for separating the adverse effects of other contaminants in sewage from the adverse effects of chlorine. Unfortunately, a review of the studies used to derive the 1985 EPA aquatic life criteria for chlorine found that a number of the toxicity studies used to derive the criteria were performed on treated wastewater. The publication of the 1985 EPA chlorine criteria document predates the Stephan et al. (1985) guidance document which contains the procedures, including the data acceptability requirements of toxicity literature, currently used to derive EPA's water quality criteria. If the data acceptability requirements of Stephan et al. (1985) had been employed in the 1985 EPA chlorine criteria document, a number of the studies used to derive the chlorine criteria would have been excluded from criteria derivation.

The EPA 1985 chlorine criteria document is the basis for the chlorine effluent permit limit in the Washington hatcheries general NPDES permit. As such, we have chosen to evaluate the studies in the EPA 1985 chlorine criteria document using a line of evidence not employed for any other chemical in this Biological Evaluation. The ranked genus mean acute values in Table 3 of the EPA 1985 chlorine criteria document include species mean acute values for two freshwater threatened and endangered species under evaluation in this Biological Evaluation: coho salmon and steelhead (rainbow trout).

For marine waters, the 1985 EPA criteria document includes a species mean acute value for coho salmon of acceptable data quality (Thatcher 1978). The primary line of evidence in this evaluation of chlorine is the use of high quality acute toxicity data with the Interspecies Correlation Estimation (ICE) model and with acute-chronic ratio (ACR) for chlorine to calculate chronic no effect concentrations. With the exception of the saltwater coho salmon study of Thatcher (1978), all other evaluations in this section have been performed with ICE models where acute LC_{50} data with non- threatened and endangered salmonid species in Washington has been used as the input into the ICE model.

We have also for the purposes of the evaluation of chlorine in this Biological Evaluation assumed that all of the acute toxicity data in the EPA 1985 chlorine criteria for prey species of the threatened and endangered species met current data quality requirements. This assumption allowed us to convert species mean acute values from the criteria document into chronic no effect concentrations using an acute-chronic ratio. Although not based on as high a quality literature information as desired for this Biological Evaluation, the approach used to evaluate information from the 1985 EPA chlorine criteria document provides a secondary line of evidence in the toxicity assessment and risk characterization of chlorine. This secondary line of evidence provides an additional level of support for our conclusions regarding the protectiveness of the chlorine effluent limit in the Washington hatcheries NPDES general permit.

The three sources of measures of effect are 1.) The acute and chronic toxicity data for aquatic species in the EPA (1985) Ambient Water Quality Criteria for Chlorine, specifically Tables 1 and 2 (empirical acute and chronic toxicity, respectively) and 3 (empirical rank ordered genus and species mean acute toxicity data) from the chlorine criteria document; 2.) The additional toxicity data identified by EPA during its 2012 literature review on chlorine toxicity, and; 3.) A supplemental EPA 2013 literature review that searched specifically for toxicity information on chloramines and other chlorine chemical forms not searched for during the EPA 2012 literature review. The 2012 and 2013 literature reviews were originally performed

for the Coeur d'Alene Tribe Biological Evaluation for their water quality standards. The 2013 EPA literature review in ECOTOX searched for all freshwater animal toxicity data for the following chlorine chemical forms listed in Table Chlorine-1, an expanded list from the search performed in 2012.

Table Chlorine-1. Chemicals for which Aquatic Toxicity Data Searches were Performed in ECOTOX.

Chemical	Chemical Abstracts Service ID
Chlorine (same CAS ID as TRC)	7782-50-5
Chlorine dioxide	10049-00-4
Monochloramine	10599-90-3
Dichloramine	3400-09-7
Trichloramine (nitrogen trichloride)	10025-85-1
Hypochlorous acid	7790-92-3
Hypochlorite anion	14380-61-1
Sodium hypochlorite	7681-52-9

Chlorine dioxide is reported as chlorine oxide in the ECOTOX output. Monochloramine is reported as chloramine in the ECOTOX output. Sodium hypochlorite is reported as hypochlorous acid, sodium salt (1:1) in the ECOTOX output.

No additional chronic toxicity data meeting current EPA data quality criteria requirements were found in addition to those already identified in Table 2 of the EPA (1985) chlorine water quality criteria document. Division of an LC_{50} by an acute-chronic ratio provides an estimate of a chronic no effect concentration in the absence of empirical chronic toxicity data for aquatic species. This is based on the standard ACR definition EPA historically has used, as described in Raimondo et al. (2007). "The ACR is calculated as the ratio of the median lethal concentration (LC_{50}) and a chronic no-observed-effect concentration (NOEC) or the maximum acceptable toxicant concentration (MATC). The MATC is the geometric mean of the NOEC and the lowest-observed-effect concentration (LOEC) determined from growth, reproduction, or survival endpoints."

Lines of Evidence

Information derived from different sources or by different techniques that can be used to describe and interpret risk estimates are called lines of evidence in ecological risk assessments. Sometimes more than one line of evidence is needed to reasonably demonstrate that stressors are likely to cause adverse effects on the assessment endpoint. This situation arises when either the amount of information available for a line of evidence is limited, or if substantial uncertainties exist regarding the information to be used in risk characterization. If multiple lines of evidence are evaluated and some lines of evidence conflict with others, professional judgment is needed to determine which data should be considered more reliable or relevant to the questions.

Once there is agreement on which lines of evidence are required to answer questions concerning the assessment endpoint, the measures of effect by which the risk hypotheses will be examined can be selected.

Empirical Toxicity Data Line of Evidence

Unfortunately, there are no empirical acute or chronic chlorine toxicity data for any of the freshwater threatened and endangered species under evaluation that meet current EPA data quality requirements for use in derivation of EPA water quality criteria. The only freshwater salmonid studies with chlorine that meet current EPA data quality requirements are a series of LC_{50} tests with brook trout (*Salvelinus fontinalis*) by Thatcher et al. (1976), and several LC_{50} tests with brook trout and cutthroat trout (*Oncorhynchus clarki*) performed by Larson et al. (1978). These two studies are the sources of the acute LC_{50} data used with the Interspecies Correlation Estimation (ICE) line of evidence described in the next section.

For marine systems, a chlorine acute LC_{50} study of acceptable data quality was performed by Thatcher (1978) on coho salmon. The LC_{50} from this study was used directly with the chlorine ACR to derive a chronic NOEC for coho salmon in marine systems. The coho salmon LC_{50} from Thatcher (1978) was used with the ICE model to estimate chlorine toxicity to the remaining salmonid species in marine waters. No chronic toxicity studies of acceptable data quality were identified for any threatened and endangered species under evaluation in the marine waters of Washington.

As described in the methodology, once an acceptable acute LC_{50} is identified for a threatened and endangered species with an existing water quality criterion, it is divided by the acute-chronic ratio (ACR) for that chemical to convert an acute LC_{50} into a chronic NOEC concentration.

Interspecies Correlation Estimation (ICE) Methodology Line of Evidence

It is impractical for toxicologists to perform laboratory toxicity studies on all aquatic species present in North America with all chemicals to which they are exposed in the environment. This is particularly true for ESA listed species, whose rarity or limited distribution in the environment generally precludes their use as test organisms in aquatic toxicology, except for limited research purposes. ICE models are statistical regressions that permit estimations of LC_{50}s to be made for a species or higher taxa (genus, family) having no measured acute toxicity information from a species for which five or more LC_{50}s have been measured. The detailed description of how ICE models were developed and their use to estimate LC_{50}s for taxa for which no toxicity information is available is given in Raimondo et al. (2013).

ICE models between two taxa are linear regressions of the form shown below:

$$\log_{10} X_2 = a + (b \times [\log_{10} X_1])$$

Where: X_1 is a measured LC_{50} value for an aquatic species (e.g. coho salmon, Daphnia magna)
X_2 is the predicted LC_{50} value for the taxa (species, genus or family) without toxicity data

The current version of ICE, called WebICE, is freely available from EPA on the Internet at: http://www.epa.gov/ceampubl/fchain/webice/

Based on the current data quality requirements for literature to be used in the derivation of EPA aquatic life criteria, a study of chlorine toxicity to brook trout by Thatcher et al. (1976), and a study of chlorine toxicity to brook trout and cutthroat trout by Larson et al. (1978) are the only freshwater studies with a salmonid that meets present day data quality requirements. The endangered species module of WebICE contains regressions between either brook trout or cutthroat trout and all other threatened and endangered species of the family Salmonidae under evaluation in this Biological Evaluation. The Thatcher et al. (1976) brook trout study was therefore used with WebICE to generate the regressions used to

estimate LC_{50} values for bull trout, while the Larson et al. (1978) cutthroat trout results were used with WebICE to generate the regressions used to estimate LC_{50} values for all of the threatened and endangered Oncorhynchus species in freshwater under evaluation in this Biological Evaluation. The lower 95% confidence interval of the surrogate species empirically measured LC_{50} is calculated by the ICE model. If the study that is the source of the LC_{50} for the surrogate species does not report a lower 95% confidence interval, the LC_{50} itself is used as the input into ICE.

The ICE calculated lower 95% confidence interval of the LC_{50} for the threatened and endangered species of interest (derived from the empirical LC_{50} of a surrogate species) is then divided by the chlorine acute-chronic ratio (ACR) of 3.345, as presented in the EPA (1985) chlorine criteria document. The quotient resulting from dividing the LC_{50} by the ACR is the chlorine chronic no effect concentration (chronic NOEC) for the threatened and endangered species of interest. The risk characterization portion of this assessment compares the chronic NOEC for each threatened and endangered species to the chronic chlorine criterion to determine whether the NPDES permit limit for chlorine is protective of threatened and endangered species.

A second study by Thatcher (1978) contains 96 hour LC_{50} results in saltwater meeting current EPA data quality requirements for one of the threatened and endangered salmonid species under evaluation in this Biological Evaluation: coho salmon. The procedures described in the previous paragraph for relating a brook trout freshwater 96 hour LC_{50} to a chronic NOEC were used with the saltwater coho salmon 96 hour LC_{50} to generate chronic NOECs for the remaining threatened and endangered salmonid species in saltwater.

Species Mean Acute Values (SMAVs) from the 1985 EPA Ambient Water Quality for Chlorine – 1984 Line of Evidence

The EPA (1985) chlorine criteria document (the title of the chlorine criteria document indicates it was completed in 1984, but was not released until January 1985) calculated acute criteria for freshwater species from a species sensitivity distribution containing LC_{50} data from 28 different genera. Two of the freshwater threatened and endangered species under evaluation in this Biological Evaluation were included in the criteria derivation: coho salmon and rainbow trout (steelhead). At the time the criteria were published, rainbow trout were considered to be in a separate genus (Salmo) from coho salmon (Oncorhynchus), although today taxonomists consider both species to be in the genus Oncorhynchus. Cutthroat trout were also considered to be a Salmo species in the EPA chlorine criteria document, but are also currently considered to be members of the genus Oncorhynchus.

Among the 28 freshwater genera, coho were the 6th most sensitive to chlorine, rainbow trout and cutthroat trout (both considered as Salmo in the 1985 criteria document) the fourth most sensitive. The species mean acute values are available from the EPA (1985) chlorine criteria document. They are listed as 74.79 µg/L for coho, and 61.92 µg/L for rainbow trout. Within EPA water quality criteria documents, toxicity data when possible are carried to four significant digits, while the criteria values themselves are only reported to two significant digits.

A number of the studies used in the EPA (1985) chlorine criteria document to derive species mean acute values or genus mean acute values during the derivation of the acute chlorine criterion for freshwater do not meet current EPA data quality requirements. The studies not meeting current data quality requirements and the reason they do not meet the requirements are shown in Table Chlorine-2.

Table Chlorine-2. Data quality rationale for excluding chlorine acute toxicity studies from the EPA (1985) chlorine criteria document as a primary line of evidence in this Biological Evaluation

Reference	Species	Data Quality Requirement Not Met
Lamperti 1976	Coho salmon	Control response not reported
Arthur et al. 1975	Coho salmon Brook trout	Dilution water quality not acceptable (fish exposed to chlorinated sewage effluent)
Ward et al. 1976	Coho salmon Rainbow trout Lake trout	Dilution water quality not acceptable (fish exposed to chlorinated sewage effluent)
Ward and DeGraeve 1978	Coho salmon Rainbow trout Lake trout	Dilution water quality not acceptable (fish exposed to chlorinated sewage effluent)
Rosenberger 1972	Coho salmon	Inappropriate test endpoint and duration (LT_{50} instead of LC_{50})
Merkens 1958	Rainbow trout	Exposure duration too short (2 hr.)
Wolf et al. 1975	Rainbow trout	Fish exposed to combination of thermal shock and chlorine
Buckley et al. 1976	Coho salmon (salt water)	Dilution water quality not acceptable (fish exposed to chlorinated sewage effluent)

As an additional, but secondary line of evidence in this Biological Evaluation, EPA has assumed that the fish studies in meet EPA data quality requirements (see Appendix A and Appendix B), and the chronic NOEC values derived from these studies for rainbow trout and coho salmon are compared to the freshwater chronic chlorine criteria in risk characterization. The freshwater chronic chlorine criterion (11 µg/L) is equivalent to the effluent discharge permit limit in the Washington hatcheries NPDES permit.

Risk Characterization

Risk characterization is the final phase of ecological risk assessment. It combines and integrates the products of the problem formulation and analysis phases to estimate and describe any identified adverse ecological effects related to the assessment endpoints. The relationships between stressors, effects, and ecological entities are used to reach conclusions regarding the occurrence of exposure and the adversity of existing or anticipated effects.

After estimating the risk, risk estimates are described in the context of the significance of any adverse effects and lines of evidence supporting their likelihood. Finally, the uncertainties of the risk assessment are described, followed by the conclusions and determinations of the risk characterization.

The approaches used in this risk characterization to assess chlorine toxicity to threatened and endangered species and their prey are summarized in Table Chlorine-3.

Table Chlorine-3. Summary of Assessment Endpoints, Measures of Effect and Lines of Evidence Used in Toxicity Assessment of Chlorine.

Assessment Endpoint	Measures of Effect	Lines of Evidence
Survival, reproduction and growth of threatened and endangered species	For chronic effects: calculated chronic NOEC (no empirical chlorine NOEC data exists for any threatened and endangered species under evaluation)	Empirical high quality LC_{50} data for threatened and endangered species divided by acute-chronic ratio (ACR) to derive chronic NOEC
		Interspecies Correlation Estimation (ICE) model at species, genus or family level to estimate acute LC_{50} for threatened and endangered species without empirical toxicity data, which is then divided by the ACR to derive chronic NOEC
		For threatened and endangered species with empirical acute LC_{50} data that does not meet current EPA acceptable data quality criteria for water quality criteria derivation, assume such data does meet data quality criteria, then divide the acute LC_{50} by the ACR to derive chronic NOEC
	For effects on prey species: LC_{50}, EC_{50}, EC_{20}, NOEC, LOEC, calculated MATC, calculated acute and chronic EC_A	Comparison of acute and chronic EC_A for prey species to acute and chronic water quality criteria
	For multiple routes of exposure:	Not evaluated, bioaccumulated dose of chlorine not required to elicit toxicity, dietary ingestion is an incomplete or insignificant exposure pathway for aquatic species

Chronic Freshwater Chlorine Criterion

Empirical Data Line of Evidence

No empirical data are available that describe either the acute or chronic exposure responses of any of the threatened and endangered species under evaluation to chlorine in freshwater. Therefore, no risk

characterizations have been made for any threatened and endangered species in freshwater based on direct measurements of chlorine toxicity to the threatened and endangered species.

Interspecies Correlation Estimation (ICE) Line of Evidence

Because of the complete absence of high quality empirical freshwater acute or chronic toxicity data for any threatened and endangered species under evaluation, high quality toxicity data from surrogate species has been used with the ICE model (Raimondo et al. 2013) to estimate chlorine toxicity to threatened and endangered species in freshwater. As discussed in the toxicity assessment, no high quality chronic toxicity data exists for any species which could be used as a surrogate species for a threatened and endangered species under evaluation.

The EPA (1985) chlorine criteria document and the 2012 and 2013 ECOTOX searches completed by EPA all identified the studies of Thatcher et al. (1976), which reported the effects of temperature changes on chlorine toxicity to juvenile brook trout (*Salvelinus fontinalis*), and that of Larson et al. (1978) with cutthroat trout (*Oncorhynchus clarkii*) as containing high quality 96 hour LC_{50} acute toxicity that can be used with ICE to estimate chlorine toxicity to all of the threatened and endangered salmonid species under evaluation.

Brook trout are the same genus as bull trout (*Salvelinus confluentus*), and thus are expected to have similar sensitivity to contaminants as do bull trout. Brook trout, a species in the genus *Salvelinus* known to hybridize with bull trout, is used as a surrogate species for bull trout in the ICE model as the starting point to derive a chronic NOEC for bull trout. Cutthroat trout are the same genus (*Oncorhynchus*) as the remaining five threatened and endangered species under evaluation (Chinook, chum, coho and sockeye salmon, steelhead). Cutthroat trout is used as the surrogate species in ICE for all freshwater threatened and endangered species of the genus *Oncorhynchus*. The rationale for these choices is shown in Appendix C (ICE predictions for chlorine) and the following discussion.

Within the Thatcher et al. (1976) study, six 96-hr LC_{50} studies performed at either 10°C or 15°C provide suitably high quality data that can be used to evaluate TRC toxicity to brook trout. LC_{50} values for the four tests run at 10°C and the two tests run at 15°C ranged between 131 – 179 µg/L. Temperature had no statistically distinguishable effect on the six LC_{50} values, so they were pooled to calculate a geometric mean 96-hr LC_{50} of 152 µg/L, with a 95% lower confidence limit of the mean LC_{50} of 136 µg/L.

Similarly, Larson et al. (1978) generated five 96 hour LC_{50} values for cutthroat trout exposed to chlorine. The geometric mean of these five LC_{50}s was 85 µg/L, with a 95% LCL of the mean LC_{50} of 75 µg/L. The 95% LCL of the mean LC_{50} estimates for brook trout and cutthroat trout were used as input into ICE in order to estimate LC_{50} values for the threatened and endangered salmonid species in freshwater.

As described in the Methodology section, EPA (2006) uses the term risk ratio to quantify potentially unacceptable risks to threatened and endangered species from exceedance of national acute or chronic water quality criteria. The risk ratio for evaluating the protectiveness of chronic water quality criteria in practical terms is defined as the chronic criterion value divided by the chronic NOEC for a threatened and endangered species as shown below, where the threatened and endangered species chronic NOEC is either taken from the empirical literature, or is estimated or modeled.

$$R = \frac{C_A \text{ or chronic criterion}}{E_{CA} \text{ or chronic NOEC}}$$

Where: R = Risk ratio

C_A = Assessment exposure concentration (EPA 2006) or chronic water quality criterion

E_{CA} = Assessment effects concentration (EPA 2006) or chronic NOEC

In this Biological Evaluation, an E_{CA} is defined as a chronic no effect concentration (chronic NOEC), which is the standard approach for EPA risk assessment determinations.

The risk ratio approach is the inverse of the hazard quotient (HQ) calculation normally performed in ecological risk assessments (Wenmei et al. 2012), as shown below. In a hazard quotient calculation, a water quality criterion is the assessment exposure concentration (C_A) or toxicity reference value (the denominator) of a hazard quotient, not the numerator as it is in the EPA (2006) risk ratio calculation for water quality criteria.

$$HQ = \frac{EEC}{TRV \text{ or } C_A \text{ (set to equal the chronic water quality criterion)}}$$

Where: HQ = Hazard quotient

EEC = Expected environmental concentration (chemical concentration likely to occur in the environmental media to which organisms are exposed)

TRV = Toxicity reference value (a numerical expression of a chemical's concentration-response relationship with organisms)

C_A = Assessment exposure concentration (EPA 2006). Same definition as used for risk ratio

In ecological risk assessment terms, the EPA (2006) risk ratio for evaluating the protectiveness of water quality criteria to threatened and endangered species is a safety factor, not a hazard quotient. The hazard quotient approach is used to describe risks from all other chemicals in this Biological Evaluation. The reason EPA (2006) presents results as risk ratios for chemicals with water quality criteria such as chlorine, and hazard quotients for all chemicals without water quality criteria is so that the interpretation of protectiveness is the same for both chemicals with and without water quality criteria. In both cases, a risk ratio and an HQ < 1 indicates a chemical whose environmental concentration poses acceptable levels of risk. (EPA risk assessment policy and guidance states that if adequate information exists permitting a risk characterization to conclude ecological risks are acceptable, defined in this Biological Evaluation as estimated environmental concentrations being lower than chronic no observed effect concentrations, the ecological risk assessment process ends with appropriate documentation to support the conclusion.) Conversely, a risk ratio and an HQ ≥ 1 is interpreted as a chemical concentration which poses unacceptable levels of ecological risk.

Note: The difference in terminology between risk ratio and hazard quotient is due to the use of risk ratio specifically to evaluate protectiveness of water quality criteria. In ecological risk assessments of chemicals with water quality criteria, the chronic criterion value is the denominator of the hazard quotient calculation, and the EEC is the concentration to which species are exposed in the environment. We want the exposure concentration (the EEC) to be lower than the water quality criterion, when it is, the hazard quotient is less than unity. In this example, the EEC is lower than the concentration that is protective of aquatic species (the water quality criterion), and the risk characterization conclusion is acceptable levels of risk (due to the statistical nature of concentration-response relations, the only concentration at which no risk is present is a zero concentration of the chemical in water). When evaluating the protectiveness

of a water quality criterion itself with respect to an endangered species, the water quality criterion becomes the numerator of the risk ratio (not the denominator as it is in a hazard quotient calculation). The denominator of a risk ratio calculation is the chronic no effect concentration for the specific endangered species under consideration. If the water quality criterion is protective of the endangered species, it will be lower than the chronic no effect concentration for the endangered species, and the risk ratio will be less than unity. *Thus, the risk ratio and hazard quotients are inverses of each other due to whether the water quality criterion is used as the numerator (risk ratio) or denominator (hazard quotient) of the risk characterization.* But both the risk ratio and hazard quotient are interpreted in the same way: a numeric value less than unity is protective from adverse effects, a numeric value greater than or equal to unity is interpreted as potentially posing unacceptable risks.

Entering the lower 95% confidence limit (95% LCL), 136 µg/L of the geometric mean 152 µg/L 96-hr LC_{50} for brook trout (Thatcher et al. 1976) into WebICE yielded a predicted bull trout LC_{50} of 114 µg/L, with a 95% lower confidence interval of 45 µg/L (Table 14). Within ICE, the estimated bull trout LC_{50} derived from the empirical brook trout LC_{50} resulted from a family Salmonidae level regression, the only taxonomic comparison ICE was able to perform between bull trout and brook trout. When divided by the chlorine ACR of 3.345, the bull trout 95% lower confidence interval of 45 µg/L yielded an assessment effects concentration (EC_A), equivalent to a chronic NOEC, of 13 µg/L. For completeness, Table 14 also shows the results of the bull trout – cutthroat trout ICE regression estimates of the bull trout chlorine LC_{50}. The ICE regressions selected for use to derive the acute LC_{50}s for all threatened and endangered species are highlighted in green in Appendix C.

Using the terminology of the Oregon Toxics Biological Evaluation (Shephard et al. 2008) and the EPA (2006) national guidance for performing Endangered Species Act – Clean Water Act consultations on national EPA water quality criteria, the assessment exposure concentration (C_A, which equals the chronic criterion of 11 µg/L) divided by the assessment effects concentration (EC_A) of 13 µg/L results in a risk ratio of 0.85. A risk ratio less than one indicates that adverse effects are not expected if bull trout are exposed to the chronic chlorine criterion of 11 µg/L. The interspecies correlation estimation line of evidence indicated that the national chronic chlorine criterion is protective of bull trout.

Of the five Salmonidae species whose acute LC_{50}s were estimated using ICE model regressions between the threatened and endangered species and the empirical cutthroat trout chlorine LC_{50} (Table 14) from Larson et al. (1978), none of the model predicted EC_As (chronic NOECs) were lower than the freshwater chronic chlorine criterion of 11 µg/L. Results for the risk ratio calculations for protectiveness of the chronic chlorine criteria to all freshwater threatened and endangered species that could be quantitatively evaluated are presented in Table Chlorine-4.

Table Chlorine-4. Risk estimates for the chronic chlorine criterion to threatened and endangered fish species in freshwater

Species	ICE estimated LC_{50} (µg/L)	95% LCL of ICE estimated LC_{50} (µg/L)	ACR	Risk ratio	Conclusion
Bull trout	114	44.53	3.345	0.83	Not likely to adversely affect
Chinook salmon	73	56.04	3.345	0.66	Not likely to adversely affect
Chum salmon	73	56.04	3.345	0.66	Not likely to adversely affect
Coho salmon	73	56.04	3.345	0.66	Not likely to adversely affect

Steelhead	74	55.60	3.345	0.66	Not likely to adversely affect
Sockeye salmon	73	56.04	3.345	0.66	Not likely to adversely affect
ICE = Interspecies Correlation Estimation LCL = Lower Confidence Limit ACR = Acute-chronic ratio					

The conclusion of the Interspecies Correlation Estimation line of evidence for freshwater threatened and endangered salmonids is that the freshwater chronic chlorine criterion, which is the Washington hatcheries NPDES permit limit for chlorine discharges to fresh water, is not likely to adversely affect any of the threatened and endangered salmonid species in freshwater.

Note: The chlorine criteria has already gone through consultation for bull trout in Idaho, and the USFWS concurred with a not likely to adversely affect determination (NLAA). Since the chlorine criteria is protective of bull trout in Idaho, so it should be protective of bull trout in Washington, as well.

Species Mean Acute Value Line of Evidence

As discussed in the toxicity assessment, a number of 96 hour LC_{50} values exist from studies that do not meet current EPA data quality requirements for use in deriving water quality criteria. Results of these studies are presented in the EPA (1985) *Ambient Water Quality Criteria for Chlorine – 1984* document, Tables 1 and 3, and are available for two freshwater threatened and endangered species: rainbow trout (steelhead) and coho salmon. Although the rainbow trout and coho salmon studies listed in Table 1 of EPA (1985) were used to derive the chlorine acute water quality criterion, Text Boxes 1 and 2 in the Methodology section of this Biological Evaluation lists the reasons the studies do not meet present day EPA data quality requirements for inclusion in data sets used to derive EPA national water quality criteria. As shown in Text Boxes 1 and 2, many of the studies not meeting present day data quality requirements were rejected because the dilution water used was chlorinated sewage effluent, a dilution water not considered to be of sufficiently high quality for use in laboratory toxicity tests.

Despite these data quality shortcomings, for the purposes of this Biological Evaluation we have assumed that the rainbow trout and coho salmon acute toxicity studies listed in EPA (1985) do meet present day data quality requirements. This assumption allows us to use empirical acute toxicity data of less than optimal data quality as a secondary line of evidence to derive chronic NOEC estimates using the procedures previously described (i.e. 95% LCL of the empirical LC_{50} divided by the ACR to estimate the chronic NOEC).

The species mean acute 96 hour LC_{50} values and the 95% lower confidence limit of the LC_{50} for coho salmon and rainbow trout were recalculated from the information in Table 1 of EPA (1985) after exclusion of the coho salmon results from Rosenberger (1971) and the rainbow trout results of Merkens (1958). In both cases, these results were excluded because the exposure durations were not 96 hours. The recalculated geometric mean 96 hour LC_{50}s for coho salmon and rainbow trout, and the 95% confidence limits of the geometric mean LC_{50}s were:

Coho salmon – 96 hour LC_{50} = 72 µg/L (65.40 – 79.23 µg/L)
Rainbow trout – 96 hour LC_{50} = 56 µg/L (42.50 – 74.49 µg/L)

Division of the 95% LCL concentrations by the ACR of 3.345 yielded chronic NOEC estimates of 19.55 µg/L and 12.71 µg/L for coho salmon and rainbow trout, respectively. The risk ratios and the conclusions

regarding the protectiveness of the freshwater chronic chlorine criterion to coho salmon and steelhead from this secondary line of evidence are as follows:

Coho salmon – Risk ratio = 0.56, not likely to adversely affect
Steelhead – Risk ratio = 0.87, not likely to adversely affect

Chlorine Effects on Prey Species

This section evaluates the potential for adverse effects on threatened and endangered species due to direct toxicity to their prey, followed by the loss of food items from the aquatic system. Results are presented in Table Chlorine-5 for the prey of threatened and endangered fish species, and are expressed as a range of acute EC_A and chronic EC_A toxicity values for various categories of prey species.

Table Chlorine-5. Toxicity of Chlorine to Food Items of Threatened and Endangered Species

Assessment Exposure Concentrations (C_A): Freshwater Acute = 19 µg/L, Freshwater Chronic = 11 µg/L		
Organism Type	**Acute EC_A Range (µg/L)**	**Chronic EC_A Range (µg/L)**
Fish	20 – 313	13 - 212
Amphibians	No data	No data
All aquatic invertebrates	5.1 - 1418	3.5 - 957
Aquatic insects	5.1 - 1410	3.5 - 957
Crustaceans	5.9 – 1418	4.0 – 201
Zooplankton	12 – 34	8.3 – 23
Molluscs	31 – 105	21 – 71

No freshwater fish species had acute or chronic EC_A values lower than the respective acute or chronic water quality criteria. This finding supports a conclusion that the chlorine criteria should not have any adverse effect on prey of adult salmonids in freshwater (primarily applicable to bull trout in this Biological Evaluation), which normally feed on fish. The range of chlorine concentrations causing toxicity to invertebrates appears comparable in both fresh and salt water. It also appears evident that at least some crustaceans and insects have a higher tolerance to chlorine than do fish, particularly during short term acute exposures.

As described in the Measures of Ecosystem and Receptor Characteristics section, juvenile and subadult salmonids feed on a variety of invertebrate species before switching over to the primarily fish diet of adult salmonids. The favored prey appears to be mayflies and dipteran larvae. Table 15 indicates that both the lowest calculated acute and chronic EC_A values are lower than the respective acute and chronic chlorine criteria for aquatic insects, crustaceans and zooplankton. Among aquatic insects, data for two of the six available insect species, both of which are mayflies, yielded both acute and chronic EC_A values lower than the respective acute and chronic criteria. A third mayfly species had acute and chronic EC_A values higher than the acute and chronic criteria, as did a caddisfly and two beetle species. Of the remaining 13 invertebrate species with available data (three zooplankton species, three molluscs and seven non-zooplankton crustaceans), only one zooplankton species (*Daphnia magna*, the single most sensitive

species to chlorine) and one crustacean (*Gammarus minus*) had calculated acute and chronic EC_A concentrations lower than the respective acute and chronic water quality criteria.

Most aquatic species, including the threatened and endangered fish species evaluated in this Biological Evaluation, tend to be opportunistic feeders. Numerous alternative prey species with chronic NOEC or EC_A values above the chlorine criteria exist for threatened and endangered fish. This would minimize the potential for adverse effects on threatened and endangered fish species from chlorine toxicity to their prey. Therefore, EPA believes that the chronic chlorine criteria will not result in a meaningful reduction in the available prey for threatened and endangered fish species.

Risk Characterization of Chlorine in Marine and Estuarine Waters

At the present time, none of the hatcheries in Washington that report the use of chlorine in their operations discharge into estuarine or marine waters. Thus, marine threatened and endangered species should not be exposed to chlorine releases from hatcheries. Without exposure to chlorine discharges from hatcheries, there will be no effect of chlorine on the eulachon and three rockfish species currently listed as threatened or endangered in Washington. This conclusion would need to be revisited if one or more hatcheries discharging to marine waters would begin to use chlorine in their operations.

Chlorine Multiple Routes of Exposure Assessment

As discussed above, chlorine is one of the relatively few chemicals that does not require an internally bioaccumulated dose to elicit toxicity to aquatic life. EPA's ECOTOX database contains no information on the bioaccumulation of chlorine, chlorine oxide or chloramines, indirectly supporting the premise that chlorine is an external toxin whose toxicity is elicited externally on the gill surfaces of fish, not an internal toxin. This implies that exposure to waterborne chlorine is the only exposure route of importance to the threatened and endangered species under evaluation in this Biological Evaluation. Dietary toxicity from chlorine residues in prey species, or from bioaccumulation of chlorine in the tissues of threatened and endangered species are not routes of exposure for chlorine.

Uncertainties Associated with the Chlorine Toxicity Assessment

By design, risk assessments are conservative in the face of uncertainty. In this context, conservative means efforts were made to minimize the chances of underestimating exposure, effects, or risk. The uncertainty analysis portion of this chlorine toxicity assessment is intended to illustrate the degree of confidence in the conclusions of the assessment.

Uncertainty in a risk assessment has four components:

1. **Variation** (e.g. a fish is exposed to a range of chemical concentrations in water, not to a constant concentration of a chemical);
2. **Model uncertainty** (e.g. use of a single species or several target ecological receptors to represent the sensitivity of a threatened and endangered species to chlorine introduces uncertainty because of the considerable amount of interspecies variability in sensitivity to a chemical);
3. **Decision rule uncertainty** (e.g. use of a dichotomous decision framework to determine chlorine effects (i.e. NLAA vs. LAA) instead of calculating the probability of an adverse effect at the criteria concentrations); and

4. **True unknowns** (e.g. the toxic effects of chlorine in water on bull trout survival, growth, and reproduction have never been studied, and are unknown).

Consistent with the methods of the problem formulation, receptor-contaminant pairs subject to potentially unacceptable risk from exposure to chlorine in surface waters were identified using conservative methods and assumptions. Examples of conservatism include assumptions that chlorine contaminant concentrations are 100% bioavailable, and assumptions that the most reliable evaluation of chlorine toxicity to a threatened and endangered species in the absence of empirical toxicity data for that threatened and endangered species comes from basing the assessment on the most closely taxonomically related species to the threatened and endangered species of concern that had available and high quality empirical toxicity data.

Not all uncertainties create a conservative bias. Some may lead to underestimation of risk, for example the unavailability of exposure data within the action area. Without empirical data, it may be possible that a spill or other release of chlorine may result in concentrations in localized portions of action area waters near known chlorine dischargers that exceed either the chronic or acute chlorine criteria. As an exposure assumption in this Biological Evaluation is that threatened and endangered species are not exposed to concentrations of criteria chemicals higher than the criteria values, a chlorine release could result in this assumption not being met.

The largest single uncertainty in the chlorine toxicity assessment is the absence of high quality measured toxicity data for any of the threatened and endangered species evaluated in this Biological Evaluation, with the exception of coho salmon in saltwater. This is a true unknown, and required the use of toxicity data for surrogate species to estimate chlorine effects on threatened and endangered species within this Biological Evaluation. Furthermore, the complete absence of empirical toxicity data for any of the threatened and endangered rockfish species, eulachon and Oregon spotted frog, as well as for any taxonomically related surrogate species to rockfish, eulachon and Oregon spotted frog made it impossible to employ the ICE model to estimate toxicity for these species from empirical toxicity data of a surrogate species.

Much of the risk characterization is based on the output of ICE models. ICE models are generated from a database of empirical LC_{50} values for a large number of chemicals. To generate an ICE model, all species LC_{50}s are paired with each other by common chemical. Three or more common chemicals per pair are required to develop an ICE model. The more LC_{50} pairs that are available to develop an ICE model, the less uncertain are model predictions and the more statistical power model predictions have (statistical power is the probability that a hypothesis test will correctly reject a null hypothesis that is false). Use of the ICE model to estimate chronic NOEC data from empirical toxicity data of a surrogate species is an example of model uncertainty in risk assessment.

Uncertainty in the ICE models is described by the percent cross-validation success statistic. According to Raimondo et al. (2013), the percent cross-validation success rate for each model is the proportion of data points that are predicted within 5-fold of the actual LC_{50} value. There is a strong relationship between taxonomic distance and cross-validation success rate, with uncertainty increasing with larger taxonomic distance. For fish and aquatic invertebrates, ICE models overall predict within 5-fold and 10-fold of the actual LC_{50} value with 91 and 96% certainty for surrogate and predicted taxa within the same family, and for 86 and 96% within the same order. All ICE models used in the chlorine toxicity assessment had cross-validation success rates greater than 86%, which would be the minimum acceptable cross validation percentage for any ICE model run between two species of the order Salmoniformes. Current fish

taxonomy (Eschmeyer 1998) recognizes the family Salmonidae as the only family with currently living species within the order Salmoniformes.

EPA's aquatic life criteria are designed to protect 95% of aquatic genera from adverse effects, not 100% of aquatic species. Given this design, it is possible that one or more important prey species of threatened and endangered species within the action area not tested may be subject to toxic effects at chlorine concentrations lower than the chronic criteria. Loss of such species could reduce the prey base available to juvenile and subadult age classes of threatened and endangered species. Four of 19 invertebrate species (21%) for which empirical chlorine toxicity data are available were affected by TRC concentrations lower than the chronic criteria.

Use of acute-chronic ratios to convert 96-hr LC_{50} data to chronic NOECs or maximum acceptable toxicant concentrations (MATC's) introduces uncertainties into the evaluation of the chronic criteria, as the empirical data from which the geometric mean 3.345 ACR in EPA (1985) was derived differ for the three species ACR's used to calculate the geometric mean ACR. An ACR of 3.345 is low compared to the ACR of most other chemicals. A study by Raimondo et al. (2007) determined a geometric mean acute-chronic ratio of 8.3 from a data set of 456 same-species pairs of acute and maximum acceptable toxicant concentrations for metals, narcotics, pesticides, and other organic chemicals. The chlorine ACR of 3.345 may be indicative of a chemical with a relatively steep dose response curve, meaning the difference between adverse and no adverse effect concentrations for a given species may be small. Steep dose-response curves for chlorine have been empirically identified for fish species (Tsai et al. 1990).

Although not as much an issue with chlorine compared to other chemicals used at Washington fish hatcheries and evaluated in this Biological Evaluation, the limited quantity of stream flow data in the vicinity of most Washington hatcheries, combined with the complete absence of any water quality monitoring for hatchery chemicals in receiving waters where threatened and endangered fish are located, renders any estimate of environmental concentrations to which threatened and endangered species are exposed a true unknown in this Biological Evaluation. Permittees are required to adhere to the effluent limitations and monitoring requirements for chlorine in the permit, and for the purposes of this analysis we used the chlorine criteria.

Chlorine Effects Determinations and Summary

The conclusions of this Biological Evaluation for the threatened and endangered species where chlorine risks could be quantified are as follows:

Freshwater:
Bull trout – not likely to adversely affect
Chinook salmon – not likely to adversely affect
Chum salmon – not likely to adversely affect
Coho salmon – not likely to adversely affect
Steelhead – not likely to adversely affect
Sockeye salmon – not likely to adversely affect

Note that the chlorine criteria has already gone through consultation for bull trout in Idaho, and the USFWS concurred with a not likely to adversely affect determination (NLAA). Since the chlorine criteria is protective of bull trout in Idaho, so it should be protective of bull trout in Washington, as well.

The following species are not exposed to chlorine releases from Washington hatcheries. Therefore, a no effect determination from chlorine released by hatcheries is warranted for the following species:

Eulachon: No effect

Bocaccio: No effect

Canary rockfish: No effect

Yelloweye rockfish: No effect

Toxicological information is not available which permits risks to be quantified to the remaining fully aquatic threatened and endangered species in Washington.

Evaluation of chlorine toxicity to other fish species, some of which are potential prey species of the threatened and endangered salmonids that could be quantitatively evaluated in this BE, indicated that both the acute and chronic criteria are protective of all fish species for which empirical toxicity data are available. Evaluation of chlorine toxicity to invertebrate species indicates that although adverse effects have been observed on several invertebrate species at chlorine concentrations lower than the acute and chronic criteria, numerous alternative prey species exist for salmonids with acute and chronic EC_A values above the chlorine criteria.

Bioaccumulation risks and dietary ingestion risks from chlorine are unlikely, based on the no to low likelihood of chlorine bioaccumulation in either threatened or endangered species or their prey. This is true for both fully aquatic and aquatic-dependent species.

5.3 CHLORAMINE-T

CAS ID: 127-65-1

Chemical formula: $C_7H_7ClNNaO_2S.3H_2O$

Synonyms / Trade names: Sodium p-toluenesulfonamide; chloramine-T trihydrate; HAMALID®AQUA

Active Ingredient: Chloramine-T trihydrate; 98% w/w (minimum)

Primary metabolite: p-Toluenesulfonamide (*p-TSA*)

Chemical composition: 100% Benzene sulfonamide, N-chloro-4-methyl, sodium salt

Hatchery Use: For the control of mortality in freshwater reared salmonids due to bacterial gill disease associates with *Flavobacterium spp*. For the control of mortality in walleye (*Sander vitreus*) and freshwater reared warm water finfish due to external columnaris disease associated with *Flavobacterium columnare*.

EPA Water Quality Criteria: None

FDA Water Quality Benchmark: 0.13 mg/L (Acute)

Measures of Exposure: According to the product label issued by Western Chemical, Chloramine-T (or Hamalid®Aqua) is administered to salmonids, walleye and other freshwater finfish at different concentrations, but for the same frequency and duration. Salmonids are treated at a concentration ranging from 12 to 20 mg/L; walleye are treated at (10 to 20 mg/L); and other freshwater finfish are treated at 20 mg/L. Chloramine-T is applied in a continuous flow-through or static bath 60 minute treatment on consecutive or alternate days for three treatments.

The potential for exposure of an ESA-listed species to Chloramine-T depends on a number of factors:

1) Persistence in the receiving water, including its polarity, causing it to adsorb to solids or remain in the dissolved form.
2) Transformation products including metabolites and degradates and their persistence.
3) Lipophilicity of the compound and its potential to bioaccumulate.

The decision to analyze the effects of a parent compound and/or transformation products depends on the likelihood of their presence in the aquatic environment at sufficient concentrations and duration to be a risk to ESA-listed species and/or their prey.

Environmental Fate of Chloramine-T: Chloramine-T is an organic N-chloramine disinfectant and sanitizer used by the aquaculture industry. Chloramine-T can be present in three states; it can remain unchanged, release its chlorine as aqueous free chlorine, or release its chlorine directly to create chloramines or other chlorinated organic-N or non-N compounds (Schmidt et al. 2007). Below, we discuss the environmental fate of Chloramine-T, and its common degradates: free chlorine, chloramines and *p-TSA*.

Free or Total Residual Chlorine – The environmental fate of the chlorine released by Chloramine-T is identical to that of elemental chlorine itself, which is described in detail in the chlorine chapter of this document, and is not repeated here. The chlorine chapter also contains a discussion of chlorine reaction products such as chloramines, which also will not be repeated in this section. The environmental fate discussion in this section is thus limited to a discussion of chloramines and p-toluenesulfonamide (p-TSA), the primary transformation product of the parent Chloramine-T molecule in water after it has released its chlorine.

It should be noted that dried chlorine solutions on raceway walls (indeed on any surface exposed to outdoor ambient light), including any that may result from the use of Chloramine-T, rapidly degrade so that the concentration of biologically active chlorine is reduced to zero before a raceway is re-watered.

Chloramines

The kinetics of chloramine hydrolysis are slow, rate-limiting and usually produces chloramines which are less toxic than free chlorine (Isaac and Morris 1983 as cited in Schmidt et al. 2007), but more toxic than Chloramine-T.

Monochloramine can be a dominant chemical form if sufficient nitrogen, particularly in the form of ammonia/ammonium ion is present in surface water. In the presence of ammonia, Chloramine-T can exchange into inorganic chloramines (mostly monochloramine), but this occurs over weeks. According to the proper equilibrium ratios (Yoon and Jensen 1993, as cited by Schmidt et al. 2007) the amount of total ammonia-N concentration in the receiving water influences the amount of monochloramine produced. Yoon and Jensen (1993) modeled the production of monochloramine at an ammonia concentration typical of wastewater treatment plants (100:1 total ammonia-N to organic-N) and demonstrated that higher ratios were need to detect monochloramine. It is highly unlikely that the total ammonia concentration in aquaculture facilities would be greater than that measured at wastewater treatment plants, due to the toxicity of ammonia to fish. Therefore, we will not consider the creation of monochloramine further in this Biological Evaluation.

Because Schmidt et al. (2007) conclude that the generation of other organic N-chloramine or chloramine products can be evaluated by considering the toxicity of Chloramine-T, and the primary degradation product is para-toluenesulfonamide (p-TSA), we will focus our analysis on these compounds.

p-TSA

P-TSA is the dechlorinated remainder and a primary breakdown product of the Chloramine-T molecule as it loses chlorine atoms. Appendix G of Schmidt et al. (2007) contains a detailed assessment of *p-TSA,* including degradation, persistence and toxicity, as it relates to aquatic organisms. Schmidt et al. (2007) summarize studies describing the low degradation of *p-TSA* through hydrolysis, photolysis and biodegradation, and volatization from the water surface. Although *p-TSA* is relatively stable, its predicted bioconcentration factor is 2.5, meaning that it will not bioaccumulate in aquatic organisms (NIEHS 2002).

Residue studies are routinely conducted to determine the uptake of chemicals used in aquaculture in fish raised for human consumption; these studies are reported by NIEHS (2002). When immersed in a 20 mg/L concentration of radio-labeled Chloramine-T, the compound was undetected in fish muscle, bile, and residual carcass tissue. The Chloramine-T had been rapidly reduced to *p*-TSA, which was detected at 4.0 µg/kg in muscle tissue. Other studies are cited in NIEHS (2002), which reports injection and pond exposures. The conclusions of these studies are that Chloramine-T was poorly absorbed from water and that *p*-TSA does not appear to bioaccumulate in fish.

Estimated Environmental Concentrations (EECs)

We are focusing this analysis on Chloramine-T and *p-TSA* because of the likelihood that ESA-listed species and their prey may be exposed to these compounds from aquaculture discharges. We use the estimated environmental concentration (EEC) terminology throughout this Biological Evaluation because of the near complete absence of empirical measurements for the chemicals evaluated in this Biological Evaluation in hatchery effluents or discharges to receiving waters.

As per its label instructions, Chloramine-T is administered at concentrations ranging for 12 to 20 mg/L in a continuous flow water or as a static bath for one hour on consecutive or alternative days for three treatments. The potential for exposure to non-target species is through the discharge of Chloramine-T from treated ponds or raceways. The rate of discharge is dependent upon the size and number of ponds or raceways treated. The treatment is administered for up to one hour, after which the treated water is replaced by clean water. According to Annual Reports, INAD agreements, and personal communications, four facilities covered by this permit have used Chloramine-T during the past 5 years. The permitted facilities that have used Chloramine-T are: Colville Tribal Hatchery, Keta Creek Hatchery Complex, Ford State Fish Hatchery, and Spokane Tribal Hatchery. These facilities do not use Chloramine-T prophylactically, but to minimally and to treat outbreaks. Colville Tribal Hatchery used the chemical for 3 days in 2011. Spokane Tribal Hatchery used Chloramine-T for approximately 9 days in 2010, but has not used it since 2013. Ford State Fish Hatchery used it for approximately 9 days per year during 3 of the last 5 years, for an annual total of 13.2 kg of the chemical, but neutralized it with sodium thiosulfate before it reached the receiving water. Keta Creek Hatchery Complex uses Chloramine-T for approximately 30 days per year.

Since Keta Creek Hatchery Complex uses Chloramine-T more often than any other facility covered by this permit, it represents a reasonable worst case scenario. In 2011, Keta Creek Hatchery Complex reported using Chloramine-T for 35 days, for a yearly total of 9.066 kg. The facility calculated the maximum concentration in its end of pipe effluent to be 1.018 ppb. This end of pipe concentration serves as a conservative estimated environmental concentration (EEC) because it does not take into account the considerable dilution provided by the receiving water.

Another source of water quality data for Chloramine-T is not specifically related to Washington, but is nonetheless useful due to the comprehensive nature of the data set. The second source of receiving water concentration data collected through a survey conducted at the U.S. Geological Survey's Upper Midwest Environmental Sciences Center (UMESC). The UMESC survey contains data from 100 public and

private hatcheries representing freshwater fish culture activities in 25 states to calculate what they term the estimated introductory concentration (EIC) for Chloramine-T. The methodology used by the UMESC survey to calculate estimated introductory concentrations (EICs) is identical to the procedure EPA uses in this Biological Evaluation to calculate estimated environmental concentrations (EECs). Thus, the terms EIC and EEC are identical. To be consistent with the remainder of this document, EPA uses the estimated environmental concentration (EEC) terminology throughout this Biological Evaluation. The hatcheries reported treating an average of 10 therapies/year with an average of 3.7 treatment days per therapy (Schmidt et al. (2007). The EEC's generated from data collected during the UMESC Survey are presented in Table Chloramine-T 1. Results from the 1 and 5-day scenarios are similar for all summary statistics, while the 21-d values are substantially lower. According to Schmidt et al. (2007), the one-day EEC estimates for application assumed that a single one-hour treatment would have been administered over one-day while the five day or 21 day EEC's assumed four 1-hour treatments on consecutive days over a five- or 21-day period. Again, none of the predicted end of pipe EEC's exceed 1.0 mg/L (ppm).

Table Chloramine-T 1. Summary Statistics for the Estimated Environmental Concentrations (herein EECs) calculated based on the USGS Upper Midwest Environmental Science Center Survey Data for 60 Hatcheries (from Schmidt et al. (2007).

Parameter (mg/L)	1-d EEC		5-d EEC		21d-EEC	
	Typical	Worst-Case	Typical	Worst-Case	Typical	Worst-Case
Mean (mg/L)	0.37	0.42	0.35	0.40	0.09	0.10
Median (50th percentile)	0.40	0.40	0.35	0.50	0.09	0.12
75th Percentile	0.70	0.70	0.60	0.60	0.15	0.15
95th Percentile	0.80	0.80	0.70	0.70	0.16	0.16
Number of EECs< 0.1 mg/L	8/60	8/60	7/60	6/60	31/60	25/60
Number of EECs< 0.2 mg/L	21/60	14/60	19/60	12/60	60/60	60/60
Number of EECs< 0.2 mg/L	34/60	41/60	34/60	42/60	0/60	0/60
Number of EECs< 0.5 mg/L	17/60	21/60	20/60	19/60	0/60	0/60

The difficulty in estimating EECs is a ubiquitous problem, and anyone attempting to determine the safety of drugs used in aquaculture to non-target species in effluent receiving waters struggles to predict exposure concentrations. USGS scientists (Gaikowski et al. 2004) at the UMESC designed a study to validate two simple dilution models to estimate Chloramine-T EECs, and then compared these estimates to measured concentrations at two locations in the waste stream as it moved through the facility. They applied four treatments of 20 mg/L Chloramine-T or 100 µg/L rhodamine WT (fluorescent dye) for 60 minutes and then collected samples every 15 minutes for approximately 180 minutes[13] from the treated raceway and at the two sampling points mentioned above. They got good agreement (bounded by 90% confidence intervals) at the two sampling points between the predicted (2.8 and 1.3 mg/L) and measured

[13] After at which time the CL-T was below the limit of quantitation.

EECs (2.7 and 1.3 mg/L). The study conclusions confirmed the utility of the dilution model to predict EECs at the UMESC.

Acute Toxicity of Chloramine-T – Salmonids

The FDA calculated a water quality benchmark for use in NPDES permitting actions where Chloramine-T may be discharged. They used the procedure established by EPA in Stephan et al. (1985)[14]. The derivation of Ambient Water Quality Criteria developed by EPA is a data-driven process that follows strict data acceptability criteria presented in detail in the Sections identified below in Stephan et al. (1985). To derive a criterion for freshwater aquatic organisms and their uses, the following should be available:

1. Results of acceptable acute tests with at least one species of freshwater animal in at least eight different families such that all of the following are included:

   a. the family Salmonidae in the class Osteichthyes

   b. a second family in the class Osteichthyes, preferably a commercially or recreationally important warm water species (e.g., bluegill, channel catfish, etc.)

   c. a third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)

   d. a planktonic crustacean (e.g., cladoceran, copepod, etc.)

   e. a benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)

   f. an insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)

   g. a family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)

   h. a family in any order of insect or any phylum not already represented.

2. Acute-chronic ratios with species of aquatic animals in at least three different families provided that one of the three species:
   a. at least one is a fish
   b. at least one is an invertebrate
   c. at least one is an acutely sensitive freshwater species (the other two may be saltwater species).
3. Results of at least one acceptable test with a freshwater alga or vascular plant. If plants are among the aquatic organisms that are most sensitive to the material, results of a test with a plant in another phylum (division) should also be available.
4. At least one acceptable bioconcentration factor determined with an appropriate freshwater species, if a maximum permissible tissue concentration is available.

[14] http://water.epa.gov/scitech/swguidance/standards/criteria/aqlife/upload/85guidelines.pdf

Schmidt et al. (2007) found that the existing data were not sufficient to use the Tier I approach from Stephan et al. (1985), so they used the Tier II methodology described in the Great Lakes System guidance, which relied on data for fewer species (21 CFR 132, Appendix A; EPA 1995). The Tier II aquatic life methodology is used to derive Tier II values, which can be calculated with fewer toxicity data than Tier I. Tier II values can, in certain instances, be based on toxicity data from a single taxonomic family, provided the data are acceptable. The Tier II methodology generally produces more stringent values than the Tier I methodology, to reflect greater uncertainty in the absence of additional toxicity data. As more data become available, the derived Tier II values tend to become less conservative.

Schmidt et al. (2007) were able to use the Tier II methodology because Chloramine-T toxicity data were available for *Daphnia magna*, along with three other data points that met the data requirements stipulated in Stephan et al. (1985). They also used toxicity data at pH 6.5 so that the resulting criterion were protective for receiving waters with lower pH values. Schmidt et al. (2007) calculated the species acute value (SAV) by dividing the lowest genus mean acute value (GMAV) (1.8 mg/L) by the factor for 4 data requirements satisfied (7.0) to yield a species acute value (SAV) of 0.26 mg/L. Using this methodology, the Secondary Maximum Concentration (SMC) for Chloramine-T is one half of the SAV of 0.26 mg/L or 0.13 mg/L. This value, the SMC, is the acute benchmark value for Chloramine-T. The approach used is presented in Section 8.7 of the EA (Schmidt et al. 2007).

In the derivation of the water quality benchmark, Schmidt et al. (2007) used data from a variety of species as stipulated in Stephan et al. (1985) and EPA (1995). Some of these data are germane to the analysis of species in Washington. In the case of salmonids, they relied primarily on a study conducted by USFWS (Bills et al. 1988). In addition to salmonids, Bills et al. (1988) tested striped bass (*Morone saxatilis*), juvenile channel catfish (*Ictalurus punctatus*) and fathead minnow (*Pimephales promelas*) fry.

EPA found the necessary peer reviewed toxicity data lacking for Chloramine-T and *p-TSA*. Other than the documents cited in Schmidt et al. (2007), the primary sources of toxicity data are available through the United States Department of the Interior, U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program (Bowker et al. 2011) and the U.S. Geological Survey, Upper Midwest Environmental Sciences Center (Gaikowski et al 2008; 2009). These data were generated to obtain FDA-approved and EPA-compliant new animal drugs for use in federal, state, tribal and private aquaculture programs. Field studies were conducted at various federal and state fish hatcheries throughout the U.S. to generate these data. The studies also included target animal safety studies examining cumulative mortality of test species [rainbow trout (*Oncorhynchus mykiss*), lake trout (*Salvelinus fontinalis*)] at various exposure concentrations and durations (USFWS 2008).

Both Bowker et al. (2011) and Bills et al. (1988) examined the toxicity of Chloramine-T to rainbow trout at multiples (one to five times) of the therapeutic concentration (12mg/L and 20 mg/L). Bowker et al. (2011) exposed fry, fingerling and juvenile rainbow trout for three one-hour intervals. Bills et al. (1988) included more exposure durations including, one hour, three hours, six hours, 12-hours, 24-hour and 96-hours. Bowker et al. (2011) conducted eight separate target animal safety tests to estimate the margin of safety for administering Chloramine-T to rainbow trout according to a specified treatment regime using federal Good Laboratory Practices. These target animal safety tests are routinely conducted on the most sensitive

life-stage of the fish up to 10 times the treatment dose and three times the treatment duration. Both lethal and sublethal endpoints are measured and provided to the FDA for consideration in the drug approval process. Bowker et al. (2011) pooled and analyzed the data using a model to help estimate the Chloramine-T margin of safety by calculating the probability of survival.

Bowker et al. (2011) exposed fry, fingerling and juvenile rainbow trout to Chloramine-T concentrations of 0 mg/L, 20 mg/l, and 30 mg/L up to 100 mg/L/ for three alternating or consecutive days; fish were then observed for 14 days post-exposure. There was 100 percent survival in fry, fingerlings and juveniles exposed to the therapeutic concentration of 20 mg/L. Survival for the within-experiment results was 97 to 100 percent for fry and fingerlings up to 100 mg/L and 60 mg/L, respectively and; 100 percent for juveniles up to 40 mg/L (Table Chloramine-T 2). The relationship between Chloramine-T concentration and probability of survival was not significant for fingerlings. The authors attribute the maximum exposure concentration of 60 mg/L to this result. Finally, the probability of juvenile survival was always adversely affected at 80 and 100 mg/L, and sometimes at 60 mg/L for juvenile rainbow trout. Based on these results, Bowker et al. (2011) determined that the margin of safety for fry, fingerling and juvenile rainbow trout was 100 mg/L, 60 mg/L and 50 to 60 mg/L, respectively.

Table Chloramine-T 2. Cumulative percent survival of various fish species exposed to Chloramine-T in target animal safety experiments (Bowker et al. 2011 and Gaikowski et al. 2008).

			Mean Nominal Concentration (SD) dose (mg/L) administered								
Common Name	Lifestage	Exposure duration (hours)	0	20	30	40	50	60	70	80	100
Rainbow Trout	Fry	3 alt	99	100	ND	ND	ND	100*	ND	ND	97*
	Fingerling	3 alt	99	100	100	100	100	100	ND	ND	
	Juvenile	3 alt	96	100	ND	ND	98	91	87	62*	0*
		3 con	100	100	ND	100	ND	100	ND	66*	10*
Largemouth[1] Bass	Fry	1	100	100	ND	ND	ND	100	ND	ND	93.3 (11.5)
					ND	ND	ND	ND	ND	ND	
Channel[1] Catfish	Fry	1	100	100	ND	ND	ND	100	ND	ND	100
	Fingerling	1	100	100	ND	ND	ND	100	ND	ND	96.7 (5.7)
Northern Pike[1]	Fry	1	96.7 (5.7)	100	ND	ND	ND	96.7 (5.7)	ND	ND	96.7 (5.7)
Walleye[1]	Fry	1	100	100	ND	ND	ND	100	ND	ND	100
	Fingerling	1	100	100	ND	ND	ND	100	ND	ND	53.5
Lake Sturgeon[1]	Fry	1	100	100	ND	ND	ND	100	ND	ND	100

ND: No data

[1]: Survival 96 hours after exposure to Chloramine-T for 1 hour.

Bills et al. (1988) examined environmental factors as they relate to toxicity of Chloramine-T to rainbow trout, channel catfish and fathead minnows. They considered the effects of pH, water hardness, and

temperature in addition to the persistence of toxicity over a four-week period. Although they did not use statistical analysis to determine if the effect of water characteristics resulted in a significant difference in toxicity, toxicity appeared to be inversely related to pH (Figure Chloramine-T3). Bills et al. (1988) demonstrated that at a pH of 7.5 and 8.1 and a temperatures of 12°C the LC_{50} was 43 mg/L to greater than 60 mg/L after one hour exposure to Chloramine-T. While at pH 6.5, the LC50 ranged from 48.4 to 1.63 mg/L over the 96- hour exposure period (Figure 6).

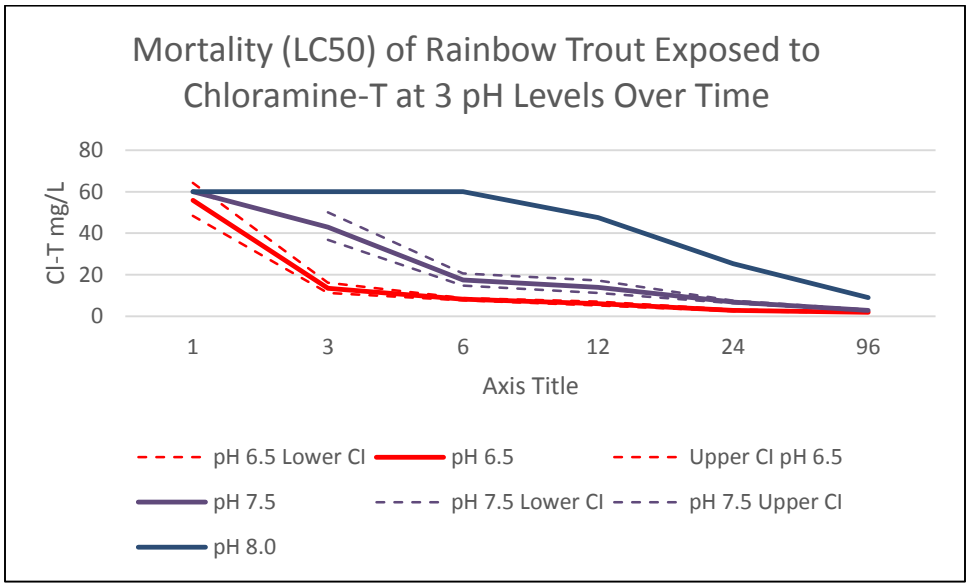


Figure 6. Toxicity (LC_{50} mg/L and 95% CI) of Chloramine –T to rainbow trout exposure to Chloramine-T at 1, 3 and 5 times the treatment concentration (from Bills et al 1988).

Powell and Harris (2004) studied the acute (within 12 hours) toxicity of freshwater- and seawater-acclimated Atlantic salmon (*Salmo salar*) smolts under aerated (100% air saturation with O2) and oxygen super-saturation conditions. They exposed Atlantic salmon smolts to concentrations of 5, 10, 25 and 50 mg/L for 12 hours. Chloramine-T was more acutely toxic to salmon in seawater than to those in freshwater, and oxygen super-saturation enhanced the toxicity in both sea- and freshwater. In aerated freshwater, the median lethal times (LT_{50}s) for Chloramine-T concentrations of 50 and 25 mg/L were 166.8 and 474.3 min, respectively; in aerated seawater they were 119.1 and 297.3 min, respectively. However, in freshwater at 200% air saturation with oxygen, the 50 and 25 mg/L LT_{50}s were 133.6 and 190.9 min, respectively. Because mortality did not occur at the 5 mg/L and 10 mg/L concentrations, it was not possible to calculate the LT_{50} for these exposures (Powell and Harris 2004).

Targeted animal safety studies were conducted by the USFWS' National Investigational New Animal Drug Office to generate data necessary for the FDA to approve the use of Chloramine-T to control bacterial gill disease in salmonids at hatcheries. The salmonids tested included three life stages of rainbow trout and fingerling lake trout. Rainbow trout fry, fingerling, and juveniles were acclimated to water temperatures of 8 °C and 14 °C to determine if water temperature was a contributing factor to mortality.

Chloramine-T was administered in static bath treatments at concentrations of 0 mg/L to 100 mg/L and 0 mg/L to 300 mg/L for rainbow trout and lake trout, respectively. Doses were administered for one hour per day every other day, or every day for three days, to rainbow trout and lake trout, respectively.

Cumulative mortality was recorded every 30 minutes during treatment, and 24-hours and 14 days post treatment. Statistical analysis was performed on the rainbow trout data using GENMOD in SAS. Cumulative mortality was observed every 30 minutes during treatments.

According to USFWS, there were no "clinically relevant differences" in mortality between the rainbow trout acclimated to 8 ^{o}C or 14 ^{o}C. Statistically significant mortality was detected after 2.5 hours of treatment. The safe level of Chloramine-T was determined to be 20 mg/L, administered as a 60 minute bath for three consecutive or alternate days, to rainbow trout and lake trout.

The final chronic NOEC values for all listed-salmonids used to calculate risk are presented in Table Chloramine-T 3. The 96-hr LC_{50} for rainbow trout used to calculate the NOEC's for ESA-listed salmonids was the lowest (1.63 mg/L) reported by Bills et al. (1988). The NOEC predicted using the EPA Web-ICE model and the National ACR (0.16 mg/L) is very similar to the water quality benchmark developed by FDA 0.13 mg/L (Schmidt et al. 2007). The Web-ICE model was not used for the steelhead; instead we used the national ACR of 8.3 (Raimondo et al. 2007) to adjust the 1.63 mg/L to a chronic NOEC of 0.2. The NOEC values will be compared to the expected EECs to develop hazard quotients in the risk characterization section to follow.

Table Chloramine-T 3. Chronic no observed effect concentrations (NOEC) for Chloramine-T based on 96-hr LC50 data calculated using the EPA Web-ICE Model (see Appendix D).

Species	Chronic NOEC (mg/L)	Source of Chronic NOEC
Bull Trout	0.16	Family
Chinook salmon	0.16	Family
Chum salmon	0.16	Family
Sockeye salmon	0.16	Family
Steelhead	0.2	Empirical

Summary – Chloramine-T is administered at 20 mg/L for a one hour duration as a flow through or static bath treatment every day or every other day for 3 days to control for bacterial gill disease in fish. Targeted animal safety trials are designed to demonstrate the safety of a therapeutic drug over the standard treatment period at various multiples of the dose. Standard acute toxicity tests are conducted for a 96-hour duration in order to determine the LC_{50} of a chemical. In the process of approving a drug for use in aquaculture, FDA will conduct a risk assessment utilizing conservative assumptions and data to protect non-target organisms including ESA-listed species.

Targeted animal safety studies were conducted by USFWS (2008), which demonstrated the safety of Chloramine-T at 20 mg/L for rainbow trout (3 life stages) and lake trout. Bowker et al. (2011) determined that the margin of safety for fry, fingerling and juvenile rainbow trout was 100 mg/L, 60 mg/L and 50 to 60 mg/L, respectively. Bills et al. (1988) demonstrated that pH affected toxicity, and measured a 24-hour

LC_{50} for rainbow trout at a pH of 9.5 of 60 mg/L, while at a pH of 8.1 the LC_{50} was 20 mg/L to 25.5 mg/L. The 96-hour LC_{50}'s are in some cases an order of magnitude lower ranging from 1.6 to 16.5 mg/L.

Powell and Harris (2004) demonstrated that in aerated freshwater, the median lethal times (LT_{50}s) for Chloramine-T concentrations of 50 and 25 mg/L were 166.8 minutes (2.7 hours) and 474.3 minutes (7.9 hours), respectively. These results are consistent with USFWS' when they found that significant differences in mortality between treatment and control didn't manifest until at least 2.5 to 3.0 hours after treatment commenced. *At the standard treatment duration (1 hour) and treatment concentration (20 mg/L), Chloramine-T is not acutely toxic to salmonids- even at sensitive life stages. Nor was it toxic to the warm water species tested.*

Toxicity of Chloramine-T – Other Aquatic Organisms

Very little toxicity data have been generated for invertebrates and Chloramine-T. Some data exist for *Daphnia magna,* (see Table Chloramine-T 4). Schmidt et al. (2007) evaluated the invertebrate data for Chloramine-T. In addition to studies in the primary literature, they had access to proprietary data submitted presumably for registration of the compound. EPA reviewed the study with *Daphnia magna* conducted by Kühn et al. (1989); they reported both acute and chronic effects at durations of 24 hours and 21 days, respectively. The 24-hour acute EC_0 and EC_{50} test results were 2.7 mg/L and 4.8 mg/L, respectively. Reproduction and appearance of the first offspring were the most sensitive endpoints for the 21-day exposure, with a NOEC of 1.3 mg/L.

Schmidt et al. (2007) report results from another independent laboratory studying the acute toxicity of Chloramine-T to *Daphnia magna*. The 48 hour exposure resulted in an LC_{50} of 4.5 mg/L (Blok 1981; Appendix H in Schmidt et al. (2007)), which is similar to the 24-hr EC_{50} (immobilization) of 4.8 mg/L reported in Kühn et al. (1989).

Table Chloramine-T 4. Toxicity of Chloramine –T (mg/L) to Non-target Aquatic Species over a range of test durations. (Data from Schmidt et al. 2007).

Test Species	Endpoint	Exposure Duration (hours)			
		24	48	96	21-day
Scenedesmus subspicatus	Growth (EC10)		0.11		
S. subspicatus	Growth (EC50)		0.31		
Selenastrum capricornutum	Growth (NOEC)			0.2	
S. capricornutum	Growth (LOEC)			0.6	
Daphnia Magna	Immobilization (EC50)	4.8)			3.1
D. magna	Mortality (LC50)	4.5			
D. magna	Reproduction (NOEC)				1.1

Toxicity of Free or Residual chlorine

Please see the chlorine section of this document for a detailed analysis on the toxicity of chlorine to salmonids and other non-target species.

P-Toluenesulfonamide – Toxicity

Toxicity data generated for *p-TSA* are cited by Schmidt et al. (2007), but the primary studies were submitted as unpublished reports, presumably for the registration of the compound, and are therefore proprietary. We were unable to find published literature on the toxicity of *p-TSA* to aquatic organisms. We have not been able to review the studies for inclusion in this document we have instead summarized the data presented in Schmidt et al. (2007) in Table Chloramine-T 5.

Studies have been conducted with fish, invertebrates and algae. The compound tested was Santicizer®9 which is a mixture of *o*-TSA and *p*-TSA, others were reported as Axcentive Proprietary (Axcentive is the manufacturer). Of the species tested, aquatic vegetation are the most sensitive to *p-TSA,* with an EC_{50} for growth inhibition of 23 mg/L. The 24-hour LC_{50}s for fish range from 200 to 420 mg/L with a 24 to 96-hour LC_0 of 324 mg/L for killifish. The species presented in Table Chloramine-T 5 are intended to be representative of species within the aquatic food web.

Table Chloramine-T 5. Toxicity of *p-TSA* to Aquatic Organisms reported as Proprietary with Exceptions[2].

Group	Species	Exposure Duration (hours)	Endpoint	Concentration (mg/L)
Fish	Rainbow trout	24	LC_{50}	200
		48	LC_{50}	120
		96	LC_{50}	100, 120
	Bluegill	24	LC_{50}	370, 420[1]
		48	LC_{50}	370, 420[1]
		96	LC_{50}	260[1], 370
	Killifish[2]	24	LC_0, LC_{50}	324, 435
		48	LC_0, LC_{50}	324, 435
		72	LC_0,LC_{50}	324, 435
		96	LC_0, LC_{50}	324, 435
Invertebrates	*Daphnia magna*[2]	24	EC_0, EC_{50}, EC_{100}	32, 150, 320
		21 days	Immobilization and reproduction, NOEC /LOEC	47/150
Algae	*Selanastrum capricornutum*	72	EC_{50} growth inhibition	23
	Chlorella pyrenoidosa	96	EC_{50} growth inhibition	80

[1] Chemical is Santicizer®9 which is a mixture of o-TSA and p-TSA

[2]Data from the Office of Economic Cooperation and Development as cited in Schmidt et al. (2007)
Endpoint for Daphnia is immobilization
Information in this table from Schmidt et al. (2007)

We calculated the 96-hour LC_{50} for the ESA-listed salmonids using the lowest reported values (100 mg/L) generated with rainbow trout (Table Chloramine-T 7). Using this as an input to the Web-ICE model, we were able to calculate 96-hour LC_{50}s and chronic NOECs using the National ACR (Table Chloramine-T 6).

Table Chloramine-T 6. Chronic no observed effect concentrations (NOEC) for *p-TSA* based on 96-hr LC_{50} data calculated using the EPA Web-ICE Model.

Species	96-hr LC_{50} mg/L	Chronic NOEC mg/L	Source of Chronic NOEC
Bull Trout	52.9	6.4	Family
Chinook salmon	52.9	6.4	Family
Chum salmon	52.9	6.4	Family
Coho salmon	52.9	6.4	Family
Sockeye salmon	52.9	6.4	Family
Steelhead	100	12.0	Empirical Data

Table Chloramine-T 7. Chronic no observed effect concentrations (NOEC) for *p-TSA* based on 96-hr LC50 data calculated using the National Acute to Chronic Ratio and Empirical data.

Species	**96-hr LC50 (mg/L)**	**EC_{50} (mg/L)**	**Chronic NOEC (mg/L)**
Bluegill	260	NA	31.3
Killifish	435	NA	324
Daphnia magna[1]	NA	NA	32
Selanastrum capricornatum	NA	23[2]	NA
Chlorella pyrenoidosa	NA	80[3]	NA

[1] Endpoint is immobilization

[2]72-hour duration – growth inhibition

[3] 96-hour duration – growth inhibition

Sublethal Toxicity of Chloramine-T and *p-TSA* – Salmonids

Numerous sublethal endpoints have been measured in chronic toxicity tests that may or may not be interpreted as meaningful sublethal effects in ESA-listed fish exposed to Chloramine-T or *p-TSA* in receiving waters. Gross lesions in eye, skin, liver, gill and kidney have been measured as indicators of sublethal effects in fish (Bowker et al. 2011; Gaikowski et al 2009; USFWS 2008). Histopathological and hemodynamic changes, including acid base and ion flux in walleye, catfish and rainbow trout, have been reported for Chloramine-T and *p-TSA* (Powell and Perry 1998; Gaikowski et al. 2009). Pathological changes in organs and tissues are considered biomarkers of exposure in individuals exposed to contaminants and are less interpretable endpoints than changes in growth and reproduction (standard sublethal endpoints). Other, more direct, measures of fitness, including growth performance and condition indices have been measured in rainbow trout (Sanchez et al. 1996).

Because it is difficult to attribute variations in ion flux and hematological parameters to an assessment endpoints such as growth, fitness or reproduction, we did not discuss the Powell and Perry (1998) paper. Similarly, we did not include a discussion of the Gaikowski et al. (2009) paper, as the endpoints measured

included histologic changes to the spleen, degenerative changes (e.g. necrosis) and inflammatory and hemodynamic changes in blood chemistry. These endpoints are difficult to translate up to an effect that could be attributed to an ecologically meaningful response. We do discuss the development of gross lesions on eyes, skin and gills as these affects are likely to reduce fitness by compromising sight, disease resistance, communication and stamina.

Bowker at al. (2011) measured histopathological changes in fish at realistic exposure durations and multiples of the concentrations. There was no mortality in the 0 to 40 mg/L treatment groups, so they did not evaluate sublethal effects in these treatment groups. Exposure to Chloramine-T did not result in gross lesions or histopathological changes in eye or skin tissues, with the exception of one fish from the 80 mg/L treatment. Only mild histopathological changes were observed in gills of the reference population and all surviving fish from the three exposure periods (Bowker et al. 2011). Pale gills (considered a gross lesion) were apparent in moribund fish collected at exposure concentrations in excess of 40 mg/L.

USFWS (2008) also evaluated histopathology data in the targeted safety study of Chloramine-T. They exposed 540 fingerling rainbow trout to six concentrations of Chloramine-T from 0 mg/L to 100 mg/L in multiples of 20 mg/L for one-hour durations on 3 alternating days. The Service examined pathologies in the gill, eye, and skin, they also measured behavior. They reported that all fish in the control, 20 mg/L and 40 mg/L treatment groups were healthy throughout the study. Fish in the higher treatment groups 60 mg/L, 80 mg/L and 100 mg/L exhibited moderate to severe pathologies of the gill. USFWS (2008) concluded that the margin of safety for juvenile rainbow trout reared at water temperatures ~ 14 ^{o}C is at least 40 mg/L.

Sanchez et al. (1996) exposed rainbow trout to Chloramine-T at 10 mg/L twice weekly for 11 weeks. Growth indices (specific growth rate, fork length and condition index) were calculated over the entire 11 weeks; differences between the treated and control groups were significant. Growth rate was lower in the treatment groups during weeks 1-4, but not for the remaining weeks of the study. Feed consumption was not affected by Chloramine-T treatment; the slower growth rate was attributed to reduced feed conversion efficiency. Although control fish weighed more than treatment fish, there was no significant difference in the condition index between the groups. Finally, Sanchez et al. (1996) recorded eye and fin lesions. They found that there was no significant difference between the control and treatment fish in the prevalence of eye lesions with only a negligible number of fish developing corneal opacity. However, as noted with growth rate, the prevalence of fin lesion was not significantly different between the treatment and control groups by the end of the 11-week study.

Summary

According to the results of numerous studies, 40 mg/L appears to be considered the safe level. Repeated exposure of fish at Chloramine-T concentrations at or below 40 mg/L did not result in gross lesions or degenerative changes in eye or skin tissues. The USFWS (2008) concluded that the margin of safety for juvenile rainbow trout reared at water temperatures ~ 14 ^{o}C is at least 40 mg/L.

Risk Characterization

Risks to ESA-listed Fish Species from Chloramine-T and *p-TSA*

Risks to ESA-listed fish species for which toxic concentrations of Chloramine-T and p-TSA that can be identified from the literature were calculated using a standard ecological risk assessment hazard quotient approach. In the hazard quotient approach, the EEC is divided by the chronic NOEC for each ESA-listed species to calculate a hazard quotient (HQ). Hazard quotients less than 1.0 are indicative of acceptable levels of ecological risk. In the context of this Biological Evaluation, an acceptable ecological risk is represented as an EEC which, if not exceeded, results in no discernable effect on the survival, reproduction, and growth of an ESA-listed species. Hazard quotients greater than or equal to 1.0 are indicative of a potential for unacceptable ecological risks to ESA-listed species.

Note that acceptable chronic NOEC values do not vary between salmonid species. The reasons for the lack of variance is due to the limited species-specific data for Chloramine-T. Rainbow trout was the only salmonid species tested for toxicity to Chloramine-T. Consequently, it was the only surrogate species available with which to predict acute LC_{50}s for ESA-listed salmonids. Because the relationships between the genus and species within the Salmonidae family are similar, the predicted LC_{50} concentrations were also similar and generated the same chronic NOEC when divided by the National ACR (See Web-ICE output in Appendix D). Because the NOEC values do not differ between species, the HQs also represent all salmonid species.

Chloramine -T

Hazard quotients were calculated using Keta Creek Hatchery Complex's maximum effluent concentration of Chloramine-T (1.018 ppb) and toxicity data for the ESA-listed salmonid species are presented in Table Chloramine-T 10. With the exception of steelhead, surrogate species were used to calculate acute LC_{50}s, as species-specific data were lacking for bull trout, sockeye salmon, chum salmon, Chinook salmon and Coho salmon. These data are presented in Table Chloramine-T 3 and consist of the reasonable worst-case concentration to which ESA-listed species could be exposed (should receiving water dilution not be a factor). Empirical data are available for rainbow trout (steelhead). Therefore, we were able to calculate hazard quotients for steelhead using empirical data (Table Chloramine-T 8).

Table Chloramine-T 8. Hazard quotients (HQ) for ESA-listed salmonids exposed to the expected environmental concentration (EEC) of Chloramine-T discharged by aquaculture facilities

Species	EEC (µg/L)	Chronic NOEC (µg/L)	Hazard Quotient
Bull trout	1.018	160	0.0064
Chinook salmon	1.018	160	0.0064
Chum salmon	1.018	160	0.0064
Coho salmon	1.018	160	0.0064
Sockeye salmon	1.018	160	0.00644
Steelhead	1.018	200	0.0051

All hazard quotients in Table Chloramine-T 8 are substantially lower than 1.0, indicative of acceptable levels of ecological risk to the species under all hatchery discharge scenarios. Further, there are a number of reasons why this calculation is highly conservative:

1) The rainbow trout 96-hour LC_{50} used as the input (1.63 mg/L) is the lowest effect level available.
2) An exposure duration of 96-hours is highly unlikely given that Chloramine-T is administered for less than 1.0 hour and has been shown to be undetectable after 180 minutes (Gaikowski et al. 2004).
3) The EECs are the end of pipe concentrations, not accounting for dilution or degradation of Chloramine-T in the receiving water.
4) Most of the data (even sublethal) indicate a 40 to 60 mg/L margin of safety for short-term exposure to Chloramine-T.

The most sensitive prey species (or member of the aquatic community in which threatened and endangered fish species reside) for which Chloramine-T toxicity data is available is the green alga *Scenedesmus subspicatus,* which has an EC_{10} of 110 µg/L (with growth as the endpoint for a 48 hour exposure duration). Assuming *Scenedesmus subspicatus* is exposed to the maximum Chloramine-T EEC of 1.018 µg/L, the maximum *Scenedesmus subspicatus* hazard quotient is 0.0093. As the highest prey species' hazard quotient is significantly lower than 1.0, we have concluded that Chloramine-T releases from Washington hatcheries are unlikely to adversely affect prey species of threatened and endangered fish species. Based on this analysis, algae, invertebrates (represented by *Daphnia*), and fish do not appear to be at risk when exposed to Chloramine-T at the levels discharged by Washington hatcheries.

p-TSA

Since *p-TSA* is the primary breakdown product, we do not have empirical information about the actual amount discharged. Therefore, we conservatively assumed that all the Chloramine-T is in the form *p-TSA,* yielding an EEC of 1.018 µg/L. When compared to the chronic NOEC concentrations for p-TSA from Table Chloramine-T 8 (12,000 µg/L for steelhead, 6400 µg/L for all other ESA listed salmonids) the hazard quotients for Chloramine-T (Table Chloramine-T 9) are so low that p-TSA is unlikely to present unacceptable ecological risk to ESA listed salmonids.

Table Chloramine-T 9. Hazard quotients (HQ) for ESA-listed salmonids exposed to the expected environmental concentration (EEC) of p-Toluenesulfonamide (p-TSA) discharged by aquaculture facilities

Species	EEC (µg/L)	Chronic NOEC (µg/L)	Hazard Quotient
Bull trout	1.018	6400	0.00016
Chinook salmon	1.018	6400	0.00016
Chum salmon	1.018	6400	0.00016
Coho salmon	1.018	6400	0.00016
Sockeye salmon	1.018	6400	0.00016
Steelhead	1.018	12,000	0.000085

Conclusion
EPA concludes that the use of Chloramine-T at therapeutic concentrations and treatment durations and periods is not likely to adversely affect listed-salmonids based on the following:

Salmonids – There are no exceedance of the HQs for salmonids and algae potentially exposed to Chloramine-T EEC. HQs were calculated using conservative assumptions, including the use of the: 1) lowest 96-hour LC_{50} (1.63 mg/L) to derive the chronic NOEC, 2) 96-hour exposure period for the test duration, 3) end of pipe EECs without accounting for receiving water dilution, and no consideration of the 40 to 60 mg/L margin of safety reported by USFWS.

Other species – Non-salmonid fish and invertebrates do not appear to be at risk from exposure to Chloramine-T at end of pipe EECs. Algae are more sensitive to Chloramine-T, but there are no HQs greater than 1.0.

p-TSA

EPA conservatively assumed that the concentrations of *p-TSA* discharged were equivalent to the concentrations of Chloramine-T EECs. There were no exceedances of a HQ of 1.0 for any species, EEC or exposure duration.

Chloramine-T Effects Determinations and Summary

The conclusions of this Biological Evaluation for the threatened and endangered species where Chloramine-T risks could be quantified are as follows:

Bull trout – not likely to adversely affect
Chinook salmon – not likely to adversely affect
Chum salmon – not likely to adversely affect
Coho salmon – not likely to adversely affect
Steelhead – not likely to adversely affect
Sockeye salmon – not likely to adversely affect

Eulachon: No effect

Bocaccio: No effect

Canary rockfish: No effect

Yelloweye rockfish: No effect

See the chlorine chapter for more detailed information on effects of chlorine compounds to species.

5.4 FORMALIN

CAS ID: 50-00-0
Chemical formula: C_2H_20
Synonyms / Trade names: Formacide-B™, Formalin-F, Paracite-S®

Chemical composition
Formalin (100 %) is a generic term that describes a solution of 37% formaldehyde gas dissolved in water. Approximately 10% to 15% methanol is added to inhibit the formation of paraformaldehyde, a precipitate considered toxic to fish. Because the formaldehyde solution will polymerize, methyl alcohol is used as a stabilizer. The Food and Drug Association (FDA) has approved three formulations of formalin for use in aquaculture: Formalin F, Formacide –B™ and Paracite –S®. All contain 37% by weight formaldehyde gas in water. Formacide –B is 37% formaldehyde, 6 to 14% methanol and 49-57% water and inert ingredients. All of these formulations have the same CAS number and the toxicity data does not specify which trade names were tested only formalin with methanol, and formaldehyde.

Formalin is prepared in various formulations for use in aquaculture which vary only in the amount of methanol composition. The formulations listed above have been approved for use by the FDA on fish intended for human consumption (Francis-Floyd 1996). Apparently formalin does not persist in fish tissue at concentrations of concern, as there is no legal withdrawal time from when the chemical is administered and when the fish can be slaughtered for consumption.

Aquaculture Use
The Parasite-S formulation is administered in a bath treatment to control for external protozoa (*Chilodonella spp., Costia spp., Epistylis spp., Ichthyophthirius spp., Scyphidia spp. and Trichodina spp*.), and the monogenetic trematode parasites *(Cleidodiscus spp., Dactylogyrus spp., and Gyrodactylus spp*.) on all finfish. It is also used for the control of fungi of the family *Saprolegniaceae* on all finfish eggs (Western Chemical Label, no date).

Formalin is commonly used in hatch houses/incubation buildings in Washington facilities, and is also used to treat juvenile and adult fish – as needed.

As previously stated, formalin is a mixture of formaldehyde, methanol and water. It is administered for aquaculture purposes in parts per million (ppm) concentrations. These ppm concentrations are reported both on a volume to volume (µl/L) and weight to volume (mg/L) basis in practice and in toxicity testing, which depend on the formulation (formalin or formaldehyde) being tested. The use of these different units has led to some confusion, so we will explain their relationship (see also Appendix E):

Volume of water	**Equivalent mass of water**	**Fraction of one liter**
1 Liter (=1000 milliliters)	1 kilogram (= 1000 grams)	1 / 1
1 milliliter (mL) = 1 cubic centimeter (cm^3) = 0.001 liter	1 gram	0.001 / 1 = 1 part per thousand
1 microliter (µL) = 0.000001 liter	1 milligram (mg)	0.000001 / 1 = 1 part per million

1 µl/L volume to volume equals 1 ppm because 1 Liter (10000 ml) of water (density = 1.0) weighs 1000 grams. Therefore 1 ml equals 1 gram and 1 µl weighs 1 mg. Thus, on a weight to volume basis, 1 µl/L equals 1 mg/L or 1 ppm.

To express formalin toxicity in terms of the formaldehyde content, we take into account both the percentage of formaldehyde in the formalin (37%) mixture, and the density difference between water and formalin (1.089 g/cm3).

$$X\frac{mg}{L}Formalin = X\frac{\mu l}{L}\ formalin * \frac{1.089g}{ml} * 0.37$$

Measures of Exposure

Formalin is administered to salmon and trout as a bath treatment. The standard dosage recommended in the INAD #9013 Protocol to prevent or control fungus on fish and eggs is 15 - 2000 µl/L in a static-bath or flow-through treatment. Eggs are treated daily or every other day until hatch. Fish are treated every other day to weekly[15] for 30 to 60 minutes, and then transferred to clean water (Francis-Floyd 1996) . The formalin concentration is water temperature dependent and 50° F is the cutoff for the two treatment concentrations. Salmon and trout are treated up to 170 µl/L at water temperatures above 50° F and 250 µl/L at temperatures below 50° F. All other finfish are treated up to 250 µl/L regardless of temperature. Treatment is not recommended to exceed 1.0 hour (FDA 1995).

Because formalin removes oxygen from the water, its use in closed systems in discouraged. Formalin removes dissolved oxygen (DO) at a 5:1 ratio, for every 5 mg/L (ppm) formalin applied, 1 mg/L of DO is removed from the water. Formalin is also toxic to algae, reducing photosynthesis through increasing respiration and decomposition (Francis-Floyd 1996). The manufacturer's label directs the user to dilute the formalin treated water by 10 and 100 times when discharging from ponds, raceways and hatch houses, respectively (Western Chemical, Inc., no date).

Environmental Fate of Formalin

If released into water, formaldehyde in the formalin is not expected to adsorb to suspended solids and sediment based upon the estimated K_{oc}. Formaldehyde readily biodegrades under both aerobic and anaerobic conditions in the environment, assuming that the concentration is not toxic to microbes. In a die-away (biodegradation) test using water from a stagnant lake, degradation was complete in 30 and 40 hours under aerobic and anaerobic conditions, respectively. Volatilization from water surfaces is not expected to be an important fate process based upon the Henry's Law constant (3.4 x 10-7 atm-m^3/mole). Formaldehyde is not expected to bioaccumulate in aquatic organisms, as it is metabolized and transformed by them through various metabolic pathways[16].

[15] http://www.fws.gov/fisheries/aadap/summaryHistoryFormalin.htm accessed 8/12/2014
[16] http://www.inchem.org/documents/ehc/ehc/ehc89.htm#SectionNumber:1.3

An estimated bioconcentration factor of 3 suggests the potential for bioconcentration in aquatic organisms is also low. Microbial degradation is the main breakdown mechanism because of the lack of hydrolysable functional groups[17].

According to FDA (1995) formalin:

- contains 37 to 40% formaldehyde with 10 to 15% added methanol;
- has molecular weight of 30.03;
- is miscible with water, alcohol and acetone, and
- has a pH of 2.8 to 4.0.

Common chemical reactions consist of:

1) $H_2C = O + H_2O \rightarrow H_2C - (OH)_2$ $(Formalin\ or\ Formaldehyde\ hydrate)$

2) $2H_2C = O + O_2 \rightarrow 2HCOOH \rightarrow (Formic\ Acid)$

3) $HCOOH + O_2 \rightarrow CO_2 + H_2O$ $(Carbon\ Dioxide\ and\ Water)$

Equation #1 depicts the formation of formalin through the mixture of formaldehyde gas and water. Equation #2 presents the oxidation of formalin or formaldehyde to formic acid which is broken down by microorganisms to carbon dioxide and water as shown in equation #3. The ultimate mineralization of formaldehyde to carbon dioxide and water indicates that environmental impacts will be minimal. The primary concern is the potential for impacts to sensitive aquatic organisms upon exposure to elevated surface water or expected environmental concentrations (EIC).

Expected Environmental Concentrations

Formalin is administered at concentrations ranging from 170 to 250 µl/L to fish, and up to 2000 µl/L to eggs. The therapeutic concentration selected depends on the temperature and density of organisms in the treatment structure, as both along with the formalin influence the level of DO (Table F-1).

Table F-1. Dosage (ppm) of formalin for the control of external parasites on fish and fish eggs.

Aquatic Species	**Administer in tanks and Raceways for up to 1 hour (µl/L)**	**Administer in Earthen Ponds indefinitely (µl/L)**
Salmon and Trout Up to 50 ^{o}F Below 50 ^{o}F	 up to 170 up to 250	 15 to 25 15 to 25
All other finfish	up to 250	15 to 25

[17] http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=712&loc=ec_rcs#x351

Eggs of all finfish except Acipenseriformes	1000 to 2000 for 15 minutes	
Eggs of Acipenseriformes	up to 1500 for 15 minutes	

The potential for exposure of non-target species is through the discharge of formalin-treated raceways or hatchery house water. The rate of discharge depends upon the size of the treatment pond, raceway or proportion of the egg stacks treated.

Formalin is applied to raceways after the water supply is turned off, and the appropriate amount of formalin is added along with aeration to facilitate mixing. The treatment is administered for up to one hour, after which raceway water is replaced by clean, aerated water.

Formalin is applied to ponds in a dilute formulation using a pump, sprayer, boat bailer or other mechanism to assure mixing. In most cases a single treatment is efficacious; however if retreatment is necessary, it occurs after 5 to 10 days (FDA 1995). The use of formalin in ponds is likely to result in a lower receiving water concentrations, as formalin is applied at initial concentrations of 15-25 ppm, the half-life is approximately 30 hours, and flow-through would be minimal until the formalin is dispelled. If the parasite is *Ichthyophthirius sp*., formalin is administered for two-day intervals until control of the pathogen is achieved (FDA 1995).

As described in the Chloramine-T section of this Biological Evaluation, EPA used the model developed by the USGS at the UMESC to calculate the end-of-pipe EICs for formalin and formaldehyde. EPA ecological risk assessment guidance specifies the use of the 95th percentile upper confidence limit (UCL) of the mean to generate exposure point concentrations (EPA 2002). Therefore, in addition to reporting the summary statistics generated by the USGS survey data and model, we calculated the EICs from the 95% UCL for use as exposure point concentrations for each dosing regimen for formalin and formaldehyde. These EICs represent the range of dose concentrations over time and as anticipated degradation occurs (Tables F-2 – F-7).

In the interest of maintaining a conservative assessment; not overwhelming the reader with more data than necessary; and, because EICs for facilities without ponds are higher than the EICs for facilities with holding ponds, we reported and used EICs calculated for facilities without holding ponds (see Appendix F).

Table F-2. Environmental Introduction Concentrations based on formalin doses of 250 mg/L and 170 mg/L.

	Administration Dose							
	250 mg/L				**170 mg/L**			
Summary Statistic	**24-hr**		**96-hr**		**24-hr**		**96-hr**	
	Typical	Worst-Case	Typical	Worst Case	Typical	Worst-Case	Typical	Worst Case

mean	5.96	6.93	5.97	6.93	4.06	4.72	4.06	4.72
median	6.10	8.15	6.10	8.15	4.15	5.55	4.15	5.55
75%ile	10.4	10.4	10.4	10.4	7.10	7.10	7.10	7.10
95% ile	10.4	10.4	10.4	10.4	7.10	7.10	7.10	7.10
95% UCL	7.88	8.63	7.88	8.64	5.36	5.88	5.36	5.88

Table F-3. Environmental Introduction Concentrations based on degradation of formalin administered at a dose of 250 mg/L.

	Typical Average Concentrations			**Worst-Case Average Concentrations**		
	EICs over time 1/2 - life 30-hr increments					
	30-hr	60-hr	90-hr	30-hr	60-hr	90-hr
mean	2.86	1.43	0.72	3.37	1.69	0.84
median	3.05	1.53	0.76	3.95	1.98	0.99
75%ile	5.13	2.56	1.28	5.20	2.60	1.30
95%ile	5.20	2.60	1.30	5.20	2.60	1.30
95% UCL	3.84	1.92	0.96	4.25	2.12	1.06

Table F-4. Environmental Introduction Concentrations based on degradation of formalin administered at a dose of 170 mg/L.

	Typical Average Concentrations			**Worst-Case Average Concentrations**		
	EICs over time 1/2 - life 30-hr increments					
	30-hr	60-hr	90-hr	30-hr	60-hr	90-hr
mean	2.86	1.43	0.72	3.37	1.69	0.84
median	3.05	1.53	0.76	3.95	1.98	0.99
75%ile	5.13	2.56	1.28	5.20	2.60	1.30
95%ile	3.55	1.78	0.89	3.55	1.78	0.89
95% UCL	3.84	1.92	0.96	4.25	2.12	1.06

Table F-5. Environmental Introduction Concentrations based on formaldehyde doses of 100.7 mg/L and 68.5 mg/L.

	Administration Dose							
	100.7 mg/L				**68.5 mg/L**			
Exposure Period	24-hr		96-hr		24-hr		96-hr	
Summary Statistic	Typical	Worst-Case	Typical	Worst Case	Typical	Worst-Case	Typical	Worst Case
mean	2.40	2.80	2.40	2.80	1.54	1.70	1.63	1.90
median	2.50	3.30	2.50	3.30	1.55	1.75	1.70	2.25
75%ile	4.20	4.20	4.20	4.20	2.83	2.68	2.80	2.83
95%ile	4.20	4.20	4.20	4.20	2.90	2.90	2.90	2.90
95% UCL	3.18	3.48	3.18	3.48	2.06	2.18	2.16	2.38

Table F-6. Environmental Introduction Concentrations based on degradation of formaldehyde administered at a dose of 100.7 mg/L.

	Typical Average Concentrations			Worst-Case Average Concentrations		
	EICs over time 1/2 - life 30-hr increments					
	30-hr	60-hr	90-hr	30-hr	60-hr	90-hr
mean	1.20	0.60	0.30	1.40	0.70	0.35
median	1.25	0.63	0.31	1.65	0.83	0.41
75%ile	2.10	1.05	0.53	2.10	1.05	0.53
95%ile	2.10	1.05	0.525	2.10	1.05	0.52
95% UCL	1.59	0.79	0.40	1.74	0.87	0.44

Table F-7. Environmental Introduction Concentrations s based on degradation of Formaldehyde administered at a dose of 68.5 mg/L.

	Typical Average Concentrations			Worst-Case Average Concentrations		
	EICs over time 1/2 - life 30-hr increments					
	30-hr	60-hr	90-hr	30-hr	60-hr	90-hr
mean	0.78	0.39	0.19	0.90	0.45	0.23
median	0.85	0.43	0.21	1.10	0.55	0.28
75%ile	1.40	0.70	0.35	1.40	0.70	0.35
95%ile	1.45	0.73	0.36	1.45	0.73	0.36
95% UCL	1.04	0.52	0.26	1.15	0.57	0.29

FDA suggests that the concentrations of effluent from treatment tanks or raceways when discharged into the receiving waterbody be no greater than 1 ppm (FDA, 1995). In the finding of no significant impact for Parasite-S®, FDA requires a 10-fold dilution of finfish and penaeid shrimp treatment water and a 100-fold dilution of finfish egg treatment water, which should lead to a discharge concentration of no more than 25 ppm. FDA contends that additional in-stream dilution, infrequent use, and rapid degradation would render the discharged formalin below a level that causes significant environmental effects on aquatic animals (FDA 1995; EPA 2004).[18]

Measures of Effect

Acute Toxicity of Formalin and Formaldehyde – Fish

As previously stated, concentrations up to 250 mg/L are used to treat external parasites on salmon and trout. It stands to reason that the short-term therapeutic dose would not result in mortality of the fish being treated, which is supported by the data presented in Tables F-10 and F-11. Formalin toxicity is inversely correlated with exposure time; rainbow trout LC_{50}'s range from 1,407 to 2,400 ppm and decrease

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http://water.epa.gov/scitech/wastetech/guide/aquaculture/upload/2005_09_01_guide_aquaculture_EEBA_EEBA-Chapter-7.pdf

to 100 ppm over 96 hours. Not unexpectedly, there are cases where the therapeutic concentration has resulted in latent mortality (2 to 4 hour after exposure) of some individuals (4 to 6 % of the test population, but this is within the acceptable control mortality according to standard toxicity testing. The 96 hour LC_{50} values for rainbow trout, coho salmon and Chinook salmon from Taylor and Glenn (2008) were calculated from a logistic response function. The equation used was that given below:

$$Y_i = \frac{e^{(\beta_0 + \beta_1 x_j)}}{1 + e^{(\beta_0 + \beta_1 x_j)}}$$

Where: Y_i = mortality probability (= 0.50)

β_0 = logistic regression intercept

β_1 = logistic regression slope

x_j = chemical concentration (mg/L)

Calculated 96 hour LC_{50} values are given in Table F-10. Taylor and Glenn (2008) did not report confidence intervals around their LC_{50} values.

Table F-10. Juvenile rainbow trout (steelhead) mortality data and confidence intervals for varying static test durations.

Exposure Duration (hr)	Formalin LC_{50} (95% CI) (µl/L)	Adjusted LC_{50}/EC_{50} mg/L Formaldehyde	Water Temp. (^oC)	Reference
1	2310 (1959-2724	930.8 (789.3 – 1097.6)	12	(Bills et al. 1977)
	1407 (mg/L) (NR)	520.6	13	Taylor and Glenn (2008)
3	1230 (957 – 1581	495.6 (385 – 637)	12	Bills et al. (1977)
6	>400	>161.2	12	(Howe et al. 1995)
	655 (580-740	264 (233.7 – 298.2)	12	(Bills et al. 1977)
24	220 (198-245)	88.6 (79.8 – 98.7)	12	(Howe et al. 1995)
	300 (237 – 380	120.9 (95.5 – 153.1)	12	(Bills et al. 1977)
96	117 (100 – 136)	47.1 (40.3 – 54.8)	12	(Marking et al. 1984)
	121 (101 -144)	48.7 (40.7 – 58.0)	12	(Howe et al. 1995)
	122(102 – 146)	49.2 (41.1 – 58.8)	12	(Marking et al. 1984)
	118 (99.7 – 140)	47.5 (40.3 – 56.4)	12	(Bills et al. 1977)

Data are compiled from literature searches conducted in 2008 and 2014.

Assumed that the Howe et al. 1984 was reporting formalin with 37% formaldehyde not reported in the paper

NR: Not reported

Table F-11. 96-hour static acute toxicity data for tests with other Salmonid species and varying exposure durations.

Species	Effect	Formalin Concentration mg/L	Exposure treatment vs test duration	Reference
Chinook	LD_2	304	1 hour treatment over 96-hr test duration	(Taylor and Glenn 2008)
	LD_{50}	563		
Coho	LD_2	653	1 hour treatment over 96-hr test duration	(Taylor and Glenn 2008)
	LD_{50}	840 mg/L		
Atlantic Salmon	LD_{50}	173 (149 -201)	96-hours	(Bills et al. 1977)
Lake Trout	LC_{50}	100 (78.2 – 128)	96-hours	(Bills et al. 1977)

The toxicity of formalin is primarily attributed to the formaldehyde, as methanol is practically non-toxic. During their derivation of the acute and chronic ambient water quality criteria for formaldehyde, Hohreiter and Rigg (2001) conducted an ECOTOX search for methanol because many of the studies they considered were testing formalin, which contains 10% to 15% methanol. According to these studies, the 96-hour LC_{50} of methanol alone was greater than 10,000 mg/L.

Hohreiter and Rigg (2001) used the EPA methodology (Stephan et al. 1985) to derive the ambient water quality criteria for formaldehyde. They considered data for 12 species of fish species, three species of amphibians, and 11 species of invertebrates. Although chronic data were limited, they were able to calculate an acute-to-chronic ratio using invertebrate and amphibian data and a decision rule for missing fish data presented in Stephan et al. (1985). They focused on the 96-hour LC_{50} data for 12 species of fish, and where the toxicity data were reported on a volume to volume basis for formalin they adjusted the results to report the formaldehyde results on a weight to volume basis (Table F-12).

Table F-12. Formaldehyde acute toxicity data including species and genus mean acute values from Hohreiter and Rigg(2001) .

Common Name	Scientific Name	96-hr LC_{50}/EC_{50} µL/L	Adjusted 96-hr LC_{50}/EC_{50} (mg/L)	Species Mean Acute Value (mg/L)	Genus Mean Acute Value (mg/L)
Striped Bass	*Morone saxatilis*	10 -75 mg/L[a]	10.0 - 27.8	16.9	16.9
Rainbow Trout	*Oncorhynchus mykiss*	118 - 245	47.6 - 98.8	58.7	58.7
Lake Trout	*Salvelinus namaycush*	100	40.33	40.3	40.3
Atlantic salmon	*Salmo salar*	173	69.8	69.8	69.8
Bluegill	*Lepomis macrochirus*	73.5 – 125	29.7 – 50.4	39.2	
Green Sunfish	*L. cyanellus*	173	69.8	69.8	52.3
Channel Catfish	*Ictalurus punctatus*	35 – 69.9	14.1 -28.2	22.3	22.3
Fathead Minnow	*Pimephales promelas*	NR	24.1 – 27.2	25.6	25.6
Smallmouth Bass	*Micropterus dolomieui*	136	54.8	54.8	

Largemouth Bass	*M. salmoides*	143	57.7	57.7	56.2

[a]: These data are from Bills et al. (Bills et al. 1977) and Hughes (1973, cited in Hohreiter and Rigg 2001) who have adjusted the concentrations for the density of formalin in water.

When studies presented concentrations on a volume by volume (µl/L) basis, the authors converted to a weight-basis (mg/L) by multiplying the concentrations by the density of formalin (1.089 g/ml). This density is also used along with the percent formaldehyde in the formulation (37%) to convert formalin to formaldehyde. Using these data, Hohreiter and Rigg (2001) calculated the following aquatic life criteria for formaldehyde:

Final Acute Value (FAV): 9.15 mg/L

Acute aquatic life water quality criterion (one-half the FAV): 4.58 mg/L

Final Chronic Value (FCV): 1.61 mg/L

Acute to Chronic Ratio (ACR): 5.69

It should be noted that these values are not regulatory criteria and do not carry the same weight. They do contribute to our understanding of the risk to species exposed to levels exceeding these criteria, and are used in the weight of evidence approach for this assessment. Additionally, because Hohreiter and Rigg (2001) developed these acute and chronic values using the same approach that EPA uses to derive aquatic life criteria, we used the ACR they developed rather than the default ACR reported in Raimondo et al. (2007). See Appendix G for ECOTOX results.

Interspecies Correlation Models

WEB-based interspecies correlation models (ICE models) estimate the acute toxicity (LC_{50}/LD_{50}) of a chemical to a species, genus, or family with no test data (the predicted taxon) from the known toxicity of the chemical to a species with test data (the surrogate species). ICE models are least square regressions of the relationship between surrogate and predicted taxon based on a database of acute toxicity values: median effect or lethal water concentrations for aquatic species (EC/LC_{50} µg/L). EPA used the WEB-ICE Model to calculate acute LC_{50}'s for most ESA- listed salmonids (see Appendix H).

A significant amount (60 records) of rainbow trout 96-hr LC_{50} data are available from three sources (Bills et al. 1977), Marking et al.(1994), and Howe et al. (1995). The only species-specific data available for other salmonids was presented in Taylor and Glenn (2008). However, fish in this study were exposed to formalin for only one hour and while appropriate for examining realistic exposures, was not appropriate for use in the ICE models which require 96 hour exposure periods.

We compiled the 60 records of formalin data, some of which included the upper and lower 95% confidence intervals (UCI, LCI), and calculated the geometric means for rainbow trout. In addition to using the dataset for rainbow trout, Bills et al. (1988) and Marking et al. (1994) reported 96-hr LC_{50} data for a

number of other species of Salmonids, Centrarcids and Ictalurids. While there was only one data point for these species, we included them as surrogates in the ICE models for formalin.

Table F-13 presents the geometric means of the 96-hour LC_{50}s and 95% LCI for each data set and the three combined data sets which were compiled for use in the ICE models. We calculated the geometric means for the 96-hour LC_{50}, and the associated 95% LCI and 95% UCI for each data set. Finally, we calculated the arithmetic means of each of these parameters for all data sets and ultimately used the minimum 95% LCI of the 96-hour LC_{50} as the input to the ICE model to represent toxicity of formalin to rainbow trout; we repeated these calculations for formaldehyde. The remaining ICE models, with poorer predictive ability and which were not selected as the source, are also presented in Appendix H.

Table F-13. Summary of the geometric means and the lower 95% confidence intervals for formalin and formaldehyde in rainbow trout.

Statistic	**Bills et al. 1977**		**Howe et al. 1995**		**Marking et al. 1984**	
	Formalin mg/L	Formaldehyde mg/L	Formalin mg/L	Formaldehyde mg/L	Formalin mg/L	Formaldehyde mg/L
GeoMean 96-hr LC_{50}	157.23	58.17	131.77	48.75	134.72	49.85
GeoMean 95% LCI 96-hr LC_{50}	119.78	44.32	113.22	41.89	113.56	42.02
Minimum 96-hr LC_{50}	108.90	40.29	131.77	48.75	127.41	47.14
Minimum 95% LCL 96-hr LC_{50}	85.92	31.79	109.99	40.70	108.9	40.29

We evaluated the results of the ICE models to select the most robust outcome with the least amount of uncertainty. In doing so we followed the "rules of thumb" (ROT) presented in Raimondo et al. (2013). These rules are as follows:

1) Relatively low mean square error (<0.22)
2) Close taxonomic distance (<3)
3) High cross-validation success rate (>85%)
4) High degrees of freedom (df>8, N>10)
5) High r^2 value (>0.6)
6) Low p- value (0.01)
7) Narrow confidence bands on the graph

All the models except the rainbow trout, Atlantic salmon, and lake trout violated at least one of the ROT. Interestingly, the model output for the bluegill as surrogate had the highest df (312) with a robust R^2 (0.88), cross validation success (91.1) and narrow confidence bands (± 2,024; range 9,127 to 11,151). However, the taxonomic distance exceeds the <3 ROT with a value of 4. Aside from taxonomic similarity, this model has high power and acceptable mean square error. The next most robust model devoid of ROT violations uses the rainbow trout as surrogate. The models utilizing other salmonids contained warnings

that the input toxicity data were greater than the maximums, indicating that the predicted values were generated for a surrogate species toxicity value that was outside the range of the toxicity values used to generate the model. Therefore, we did not consider the results of these models in the generation of the Chronic NOECs, and we relied on the output of the rainbow trout model alone.

The chronic data needed to calculate ACRs and generate chronic NOECs was very limited. EPA had two options for selecting the most appropriate ACR: 1) the national default ACR of 8.3 (Raimondo et al. 2007), 2) the ACR developed by Hohreiter and Rigg (2001) in their derivation of formaldehyde ambient water quality criteria using EPA guidelines (5.69). The Stephan et al. (1985) guidelines do not specify how ACRs for the various taxa should be combined to calculate the final ACR, therefore Hohreiter and Rigg (2001) calculated a geometric mean to come up with the final ACR of 5.69. As recommended by EPA, they used the default value of 20 for fish, because the fish data did not meet the minimum requirements set out in Stephan et al. (1985) (Stephan et al. 1985) for development of the final ACR. We used the formaldehyde-specific ACR of 5.69 rather than the national default value to calculate the chronic NOECs.

EPA generated the final chronic value for using the ICE model and the ACR from Hohreiter and Rigg (2001) to calculate both formaldehyde and formalin chronic NOECs, as formaldehyde is the active ingredient in formalin, and methanol is not contributing to the toxicity of the formalin. The chronic NOEC values are presented in Table F-14.

Table F-14. Chronic no observed effect concentrations (NOEC) for formalin, formaldehyde and ESA-listed salmonids.

Species	Formalin (mg/L)	Formaldehyde (mg/L)	Source of chronic NOEC
Bull trout	10.6	4.4	ICE model – Family level
Chinook salmon	181.9	67.3	Empirical data (NOEC for growth and seawater challenge)
Sockeye salmon	10.6	4.4	ICE model – Family level
Steelhead	15.1	7.6	Empirical data
Chum Salmon	10.6	4.4	ICE model –Family level

EPA ran the ICE model for all ESA-listed salmonids except Chinook salmon and steelhead. Since steelhead and rainbow trout are the same species, we used the empirical (LC_{50}) data to directly calculate the Chronic NOEC for steelhead. The literature contained a chronic NOEC for Chinook salmon based on a seawater challenge test and while the exposure period was not a constant 96 hours, it was repeated three times (likely a more realistic use scenario) over a period of six weeks.

Smith et al. (1987) measured gill ATPase (enzyme activity correlated with seawater tolerance), growth, and survival in a seawater challenge test using Chinook to evaluate a particularly sensitive life stage of anadromous salmonids. Fish were exposed to formalin for one hour every two weeks for six weeks and then subjected to the seawater challenge test. The authors found no significant difference in survival and growth in between the formalin treated (167µl/L) fish and the control group. The authors concluded that formalin at a concentration of 167 µl/L (182 mg/L) is safe for smolts and pre-smolts; this value was used as the chronic NOEC for Chinook salmon.

Use of the ICE model and the ACR resulted in lower (i.e., more conservative) NOEC values than reliance on empirical data. Bull trout, chum and sockeye salmon all have lower NOEC values than steelhead and Chinook.

Sublethal Toxicity of Formalin - Fish

The use of therapeutic chemicals often results sublethal effects (or side-effects in the case of human pharmaceuticals) that impact the homeostatic functions of the organism undergoing treatment (Table F-15). The presumption is that the condition being treated would be more deleterious if left unchecked than the sublethal effects from the use of the drug.

Most of the formalin studies tested the standard therapeutic doses (Table F-1); these are the concentrations to which fish are routinely exposed, so there is interest in determining whether sublethal effects are occurring, and if treatment modifications would lessen any of these effects (Wedemeyer and Yasutake 1974; Bills et al. 1977; Smith 1984; Smith et al. 1987). Exposure periods often mimic standard dosing periods of 30 min to 1 hour; other exposure periods were extended up to 6 hours (Wedemeyer 1971). Some studies included a single short-term exposure period coupled with longer term monitoring (Wedemeyer and Yasutake 1974; Williams and Wootten 1981; Taylor and Glenn 2008). Nieminen et al. (1983) tested multiple exposures separated by 24 hour periods simulating repeated treatment to control parasites.

In order to attribute a measurable effect to a sublethal endpoint, it is necessary to understand how the endpoint affects the organism in a way that reduces its fitness or survival. The interpretation of meaningful biological consequences of immunological, histopathological and hematological responses to formalin exposure is necessary in order to predict a measurable effect in the organism. Unless explicitly tested, it is difficult to predict the reduction in an organism's fitness in a meaningful way, particularly when the affect elicits a short-term response and has a low ecological relevance (Adams et al. 1989).

Adams et al. (1989) recommended the use of bioindicators, which include a suite of identified stress responses representing various levels of biological significance to evaluate the sublethal effects on fish from exposure to environmental contaminants. The authors identified levels of biological response along gradients of response time and toxicological and ecological significance. Homeostatic indices including detoxification enzymes, immunological, and histopathological measures are considered short-term responses at lower ecological significance; while condition, reproductive competence and population and community indices are long-term responses with greater ecological relevance.

Various blood parameters and liver histopathology indicative of fish health and chemotherapeutic stress are routinely evaluated as secondary effects fish physiology and on metabolism following formalin treatment (Wedemeyer 1971; Smith and Piper 1972; Wedemeyer and Yasutake 1974; Williams and Wootten 1981; Nieminen et al. 1983; Smith et al. 1987) Table F-15). These measures conform primarily to short-term responses of low ecological relevance, with gill and liver pathology representing conditions of greater ecological significance (Adams et al. 1989).

Table F-15. Documented no effect and sublethal effects from the use of formalin on Salmonid species.

Species	Effect	Concentration and Exposure	Endpoint	Reference
Chinook	NOEC	167 (µl/L)/182 mg/L (1x/2 weeks for 6 weeks)	Growth or survival during seawater challenge/smoltification progress	(Smith et al. 1987)
	NOEC	200 (µl/L)/217 mg/L(up to 1 hour exposure)	Hematological parameters; gill pathology	(Wedemeyer and Yasutake 1974)
Rainbow Trout	NOEC	200 (µl/L)/ 217 mg/L (up to 1 hour exposure)	Hematological parameters; gill pathology	(Wedemeyer and Yasutake 1974)
	EC	250 and 1250 (µl/L)[1] / 272 and 1361 mg/L (2X/30 min/24hrs)	Chemotherapeutic stress	(Nieminen et al. 1983)
	EC	200 (µl/L)/217 mg/L (1 hour bath treatments)	Hematological and hepatic responses indicative of regulatory stress	(Williams and Wootten 1981)
Atlantic Salmon	EC	250 and 1250 (mg/L) 272 and 1361 mg/L (2X/30 min/24hrs)	Chemotherapeutic stress	(Nieminen et al. 1983)
Coho	NOEC	200 (mg/L)	Hematological parameters	(Wedemeyer 1971)

[1]: Assumed the ppb (reported in the paper) is in µl/L

Exposure to formalin at therapeutic concentrations results in a stress response in salmonids that is more pronounced in rainbow trout. The stress response is measured through changes in blood chemistry that influence the homeostatic balance in fish. A second notable marker of exposure commonly measured includes gill and liver pathology (Wedemeyer 1971; Smith and Piper 1972; Wedemeyer and Yasutake 1974; Williams and Wootten 1981; Nieminen et al. 1983). Rainbow trout experience greater effects on the gill epithelial layer when exposed to formalin, this may be the noted difference in response between this species and other salmonids tested (Wedemeyer 1971; Nieminen et al. 1983).

Various authors have demonstrated recovery in salmon (Coho and Chinook) and steelhead after 24 hours from 1 hour exposures to therapeutic concentrations of formalin. They concluded that the stress of a 1 hour treatment was not great enough to result in changes in the blood resulting in respiratory alkalosis (increased respiration increasing the pH of blood) or liver pathology; repeated exposures resulted in significant sublethal effects (Wedemeyer and Yasutake 1974; Williams and Wootten 1981).

Nieminen et al. (1983) exposed Atlantic salmon and rainbow trout for two and four 30 minute treatments to 272 and 1,361 mg/L formalin, respectively (in excess of the therapeutic concentration). They found no significant difference blood glucose levels (an indicator of stress) between Atlantic salmon and rainbow trout and control fish after a single 30 min exposure to 272 mg/L formalin. Response continued to be insignificant in the salmon upon a second 30 min exposure and only a slight significance in rainbow trout after this second treatment. Mortality occurred when salmon and trout were exposed four times for 30 minutes to 272 and 1,361 mg/L at 24 hour intervals (Nieminen et al. 1983). Formalin is not permitted for use at these concentrations and exposure frequencies in standard aquaculture practices.

Exposure to formalin at therapeutic concentrations triggers a stress response in treated fish only after repeated short-term (30 min) exposures at elevated concentrations (272 and 1,361 mg/L). The frequency of exposure and allowable recovery period influence the magnitude of the sublethal response. After one hour, exposure gill pathology ranges from limited biological significance (217 mg/L) to severe (272 mg/L), with no effect on growth, survival or osmoregulation at the lowest therapeutic concentration tested (181 mg/L). Fish generally recover from homeostatic stress responses after a 24 hour period, however, repeated exposures at or exposures in excess of treatment levels can result in damage to gills. It is unlikely that the concentration of formalin in receiving waters would exceed 181 mg/L to 217 mg/L given that these are the therapeutic dosages, the label requires a 100x dilution prior to discharge, the EIC is set at the end-of-pipe, and dilution of the treatment water will occur after discharge. Additionally, the FDA recommends that the discharge concentration of formalin not exceed 25 mg/L and the receiving water concentration not exceed 1 mg/L after dilution. Finally, it is important to note that the EICs will be compared to the chronic NOEC values for ESA-listed species to determine the potential for adverse effect.

Both lethal and sublethal endpoints were reported from numerous literature sources. Lethality (LC_{50}) was measured more frequently than sublethal effects, but biochemical and histological parameters and growth were reported as well. Although we discuss homeostatic measures representing sublethal endpoints, EPA did not rely on these modes of action (e.g. change in blood chemistry) if they could not be attributed to a measurable effect in the ESA-listed, non-target, or surrogate species. Instead, we relied on endpoints representative of long-term, ecologically relevant responses in fish including survival, growth and reproduction; where available these data were used to estimate chronic NOECs.

Toxicity of Formalin - Other Aquatic Organisms

There is a paucity of formalin toxicity data for aquatic plants and invertebrates. Again, in most cases, EPA used the results of the 96-hr LC_{50} and EC_{50} toxicity tests on aquatic invertebrates reported in Bills et al. (1977) and Hohreiter and Rigg (2001) (Table F-16) to evaluate effects. Limited studies were available for both *Daphnia* and algae from other sources (Chen et al. 2005).

In addition to 10 species of fish, Bills et al. (1977) exposed five species of invertebrates to formalin administered at the maximum therapeutic use concentrations. These species included ostracod (Cypridopsis sp[19].), freshwater prawns (*Palaemonetes kadiakensis*), bivalves (*Corbicula cyanellus*), snails (*Helisoma sp.*) and the backswimmer (*Notonecta sp.*). The test temperature and pH ranged from 7 to 22

[19] Hohreiter and Rigg (2001) report that the Bills et al. (1977) study of Cypridopsis sp was anomalous.

°C, and 7.5 to 9.5, respectively depending on species. Hohreiter and Rigg (2001) also reported these studies and others which are presented in Tables F-15 and F-16. Ostracods or seed shrimp are the most sensitive species by orders of magnitude compared to other invertebrates as reported by Bills et al. (1977).

Table F-16. Toxicity (LC_{50}) of formalin to invertebrates at various time intervals in soft water at 16 °C.

Organism Type	Concentration (mg/L) (95% CI)				
	1-hr	3-hr	6-hr	24-hr	96-hr
Ostracods *Cypridopsis sp*[a].	9.8 7.4-12.9	7 5.3-9.0	1.3 0.7-2.5	1.2 0.7-2.1	1.1 0.6-2.0
Freshwater prawn *Palaemonetes kadiakensis*	ND	2341 2121.4-2584.2	2069 1729-2478.6	1203.3 975-1483.2	506.4 400.7-640.3
Bivalve *Corbicula*[b]	ND	ND	ND	800 638-1003	126 80.9-196
Snail *Helisoma sp.*[c]	3838.7 3485.9-4226.4	1459.2 1037.8-2050.6	849.4 640.3-1053	773.2 592.4-1007.3	101.3 75.7-135
Backswimmer *Notonecta*	ND	ND	ND	4900 3273.5-7334.4	909.3 710-1164

[a] : toxicity based on immobility

[b]: resist attempts or open valves and respond to tactile stimulus

[c]: toxicity based on ability to respond to tactile stimulus

ND – No data

Note: These data have been adjusted from µl/L to mg/L using the density of formalin.

In their derivation of an ambient water quality criteria for formaldehyde Hohreiter and Rigg (2001) compiled data (in addition to the fish species) for 3 species of amphibians and 11 species of invertebrates (Table F-17).

Table F-17. Compilation of 48-hr EC_{50}s and 96-hr LC_{50}s Species Mean Acute Values and No Observed Effect Concentrations for snails and prey species of ESA-listed salmonids.

Organism Type	*Genus species*	Formaldehyde		Formalin	
		SMAV (mg/L)	NOEC (mg/L)	SMAV (mg/L)	NOEC (mg/L)
Amphibians	*R. pipens*	8.7	1.5	22.9	4.0
	Rana catesbeiana	9.5	1.7	25.0	4.4
	Bufo sp.	18.6	3.3	49.0	8.6
Crustaceans	*D. pulex*	10.1	1.8	10.9	1.9
	Ceriodaphnia dubia	11.0	1.9	11.4	2.0
	Daphnia magna	16.4	2.9	18.9	3.3

	Bosmina sp.	20.0	3.5	20.0	3.5
	Cyclops sp.	20.0	3.5	20.0	3.5
	Cypridopsis.	63.4	11.1	63.7	11.2
Molluscs	*Helisoma sp.*	37.5	6.6	101.3	17.8
	Corbicula sp.	43.7	7.7	119.4	21.0
Insects	*Notonecta sp.*	336.8	59.2	909.3	159.8
	Chironomus sp.	450.0	79.1	450.0	79.1
	Palaemonetes	187.6	33.0	506.4	89.0

Formalin has been adjusted from a v:v basis t a w:v basis using the density of formalin (1.089 g/cm3)
Where possible we have calculated the GMAC or SMAC see Table 2 in Hohreiter and Rigg 2001

Chen et al. (2005) exposed the algae *Raphidocelis subcapitata* (formerly known as *Selenastrum capricornutum*) to formaldehyde. Two chronic endpoints were used to assess the toxicity of formaldehyde, dissolved oxygen production and the algal growth rate measured by cell density (number of cells per unit volume). The median effect concentration (EC_{50}) was defined as the formaldehyde concentration that reduced the final growth rate or the DO production to half of that observed in the control group. The median EC_{50} of 4.2 mg/L and 2.6 mg/L represent a decrease in DO production and growth, respectively.

Risk Characterization

Risks to ESA-listed fish species for which toxic concentrations of formalin and formaldehyde can be identified from the literature were calculated using a standard ecological risk assessment hazard quotient approach. In general, using this risk-based approach, the end-of-pipe EIC is divided by the chronic NOEC for each species to calculate a hazard quotient (HQ). Hazard quotients less than 1.0 are indicative of acceptable levels of ecological risk. In the context of this BA, an acceptable ecological risk is represented as an EIC which, if not exceeded, results in no discernable effect on the survival, reproduction and growth of an ESA-listed species.

As discussed in the Chloramine-T section of this Biological Evaluation, EPA used the model and data set provided by USGS to calculate EICs for time periods ranging from 24 hours to 96 hours for facilities without holding ponds. This model generated a range of EICs depending on whether the typical or worst-case facility flows were used. Additionally, the model was used to calculate ranges in EIC values from the mean to the 95% UCL to represent the central tendency of the data for use as the exposure point concentrations. We also incorporated the degradation half-life of formalin in 30-hour increments.

As described previously, EPA calculated HQs for the ESA-listed fish species for both formalin and formaldehyde dose regimes (Table F-18). In most cases, surrogate species were used to calculate the chronic NOEC using 96-hour LC50 data, as species-specific data were lacking for bull trout, sockeye salmon, and chum salmon.

The HQs for ESA-listed salmonids and other fish species generated using: 1) the 95th % UCL for both formalin and formaldehyde dose regimes, 2) the ACR developed by Hohreither and Rigg (2001) in their ambient water quality criteria and, 3) EICs at the formalin/formaldehyde half-life (30 hours). All EICs were less than 1.0 (Tables F-18 to F - 22).

Table F-18. Hazard Quotients for ESA-listed salmonids exposed to Formalin and Formaldehyde at worst-Case Environmental Introduction Concentrations discharged by Facilities without ponds for 24 hour and 96 hour exposure periods.

	Formaldehyde				Formalin			
Treatment Concentrations	**68.5 mg/L**		**100.7 mg/L**		**170 mg/L**		**250 mg/L**	
Species	**24-hr**	**96-hr**	**24-hr**	**96-hr**	**24-hr**	**96-hr**	**24-hr**	**96-hr**
Bull trout	0.50	0.54	0.80	0.80	0.55	0.55	0.81	0.81
Chinook salmon	0.50	0.54	0.80	0.80	0.55	0.55	0.81	0.81
Sockeye salmon	0.50	0.54	0.80	0.80	0.55	0.55	0.81	0.81
Steelhead	0.39	0.43	0.62	0.62	0.39	0.39	0.57	0.57
Chum salmon	0.50	0.54	0.80	0.80	0.55	0.55	0.81	0.81
Coho	0.50	0.54	0.80	0.80	0.55	0.55	0.81	0.81

Table 19. Hazard Quotients for ESA-listed salmonids exposed to Formalin administered at 250 mg/L over 30 hour increments representing ½ life degradation discharged by Facilities without ponds.

Species	**Typical Average EIC**			**Worst-Case EIC**		
	EICs over time 1/2 - life 30 hour increments					
	30 hrs	**60 hrs**	**90 hrs**	**30 hrs**	**60 hrs**	**90 hrs**
Bull trout	0.36	0.18	0.09	0.40	0.20	0.10
Chinook salmon	0.36	0.18	0.09	0.40	0.20	0.10
Sockeye salmon	0.36	0.18	0.09	0.40	0.20	0.10
Steelhead	0.25	0.13	0.06	0.28	0.14	0.07
Chum salmon	0.36	0.18	0.09	0.40	0.20	0.10
Coho	0.36	0.18	0.09	0.40	0.20	0.10

Table 20. Hazard Quotients for ESA-listed salmonids exposed to Formaldehyde administered at 100.7 mg/L over 30 hour increments representing ½ life degradation discharged by Facilities without ponds.

Species	**Typical Average EIC**			**Worst-Case EIC**		
	EICs over time 1/2 - life 30 hour increments					
	30 hrs	**60 hrs**	**90 hrs**	**30 hrs**	**60 hrs**	**90 hrs**
Bull trout	0.36	0.18	0.09	0.40	0.20	0.10

Chinook salmon	0.36	0.18	0.09	0.40	0.20	0.10
Sockeye salmon	0.36	0.18	0.09	0.40	0.20	0.10
Steelhead	0.28	0.14	0.07	0.31	0.16	0.08
Chum salmon	0.36	0.18	0.09	0.40	0.20	0.10
Coho	0.36	0.18	0.09	0.40	0.20	0.10

Table 21. Hazard Quotients for other fish species exposed to Formalin and Formaldehyde at Worst-Case Environmental Introduction Concentrations discharged by Facilities without ponds for 24 hour and 96 hour exposure periods.

	Formaldehyde				**Formalin**			
Treatment Concentrations	**68.5 mg/L**		**100.7 mg/L**		**170 mg/L**		**250 mg/L**	
Species	**24 hr**	**96 hr**	**24 hr**	**96 hr**	**24 hr**	**96 hr**	**24 hr**	**96 hr**
Atlantic Salmon	0.08	0.08	0.12	0.12	0.21	0.206	0.30	0.30
Lake Trout	0.15	0.16	0.23	0.23	0.39	0.39	0.58	0.58
Black Bullhead	0.22	0.24	0.36	0.36	0.60	0.60	0.89	0.89
Channel Catfish	0.20	0.21	0.31	0.31	0.53	0.53	0.78	0.78
Green Sunfish	0.09	0.10	0.15	0.15	0.25	0.25	0.37	0.37
Bluegill	0.14	0.16	0.23	0.23	0.38	0.38	0.56	0.56
Smallmouth Bass	0.13	0.14	0.20	0.20	0.34	0.34	0.50	0.50
Largemouth Bass	0.09	0.10	0.14	0.14	0.24	0.24	0.35	0.35

Table 22. Hazard Quotients for other fish species exposed to Formalin administered at 250 mg/L over 30 hour increments representing ½ life degradation discharged by Facilities without ponds.

Species	**Typical Average EIC**			**Worst-Case EIC**		
	EICs over time 1/2 - life 30 hour increments					
	30 hrs	**60 hrs**	**90 hrs**	**30 hrs**	**60 hrs**	**90 hrs**
Atlantic Salmon	0.20	0.10	0.05	0.22	0.11	0.05
Lake Trout	0.37	0.19	0.09	0.41	0.21	0.10
Black Bullhead	0.58	0.29	0.14	0.64	0.32	0.16
Channel Catfish	0.50	0.25	0.13	0.56	0.28	0.14
Green Sunfish	0.24	0.12	0.06	0.26	0.13	0.07
Bluegill	0.37	0.18	0.09	0.40	0.20	0.10
Smallmouth Bass	0.32	0.16	0.08	0.36	0.18	0.09
Largemouth Bass	0.23	0.11	0.06	0.25	0.13	0.06

Table 23. Hazard Quotients for other fish species exposed to Formaldehyde administered at 100.7 mg/L over 30 hour increments representing ½ life degradation discharged by Facilities without ponds.

Species	Typical Average EIC			Worst-Case EIC		
	EICs over time 1/2 - life 30 hour increments					
Atlantic Salmon	**30 hrs**	**60 hrs**	**90 hrs**	**30 hrs**	**60 hrs**	**90 hrs**
Lake Trout	0.08	0.04	0.02	0.01	0.04	0.02
Black Bullhead	0.15	0.17	0.04	0.02	0.08	0.04
Channel Catfish	0.24	0.26	0.06	0.03	0.13	0.07
Green Sunfish	0.21	0.23	0.05	0.03	0.11	0.06
Bluegill	0.10	0.11	0.02	0.01	0.05	0.03
Smallmouth Bass	0.15	0.17	0.04	0.02	0.08	0.04
Largemouth Bass	0.13	0.15	0.03	0.02	0.07	0.04
Atlantic Salmon	0.09	0.10	0.02	0.01	0.05	0.03

As discussed earlier, the toxicity of formalin increases with exposure duration, which is no doubt one reason why the fish are treated with the drug for up to one hour and the frequency of treatment is separated by a 24 hour periods. This treatment regime brings into question the likelihood of the continuous exposure of fish to formalin at EICs that could result in adverse effect.

Because formalin is used as a therapeutic agent in aquaculture (where the goal is to produce and maintain healthy fish), conditions indicative of chemotherapeutic stress are routinely evaluated as secondary effects on fish physiology and metabolism following formalin treatment. EPA has discussed these and identified concentrations of formalin that are associated with these stress responses (Table F-15). Because formalin is administered for a short periods of time, the evaluations of secondary toxicity have also been designed for short (30 minutes to 1 hour) exposures. As presented in Table F-15, the concentrations resulting in these sublethal effects range from 200 to 1400 mg/L - orders of magnitude greater than the EICs for formalin and formaldehyde. The EICs predicted from the USGS survey data and model are not expected to result in sublethal effects to listed salmonids and other fish.

Lethal effects to listed salmonids are also not likely to occur. The chronic NOEC (which was calculated using the lowest LC_{50} from the WebICE model and the formaldehyde-specific ACR, along with the 95% UCL of the worst-case EICs) results in HQs below 1.0 (Table F-18).

Other lines of evidence considered include the final chronic formaldehyde ambient water quality criteria (1.61 mg/L) developed by Hohreiter and Rigg (2001). When comparing this chronic value to the worst case formaldehyde EIC (3.48 mg/L), the maximum HQ is 2.16. The EICs represent the end-of-pipe concentrations and do not incorporate receiving water dilution. The level of dilution will depend on the amount of flow in the water body, which will fluctuate seasonally. We anticipate that dilution would reduce the formaldehyde concentration of 3.48 mg/L to a receiving water formaldehyde concentration below the final chronic value of 1.61 mg/L.

The chronic NOEC is generated using a 96 hour LC_{50}. Use of this chronic NOEC assumes exposure of fish to formalin and formaldehyde for a 96 hour period. In order for this to occur, the formalin discharge would need to be continuous for 96 hours, and the fish would need to remain in the plume for 96 hours. This scenario is extremely unlikely because formalin treatments do not exceed 1.0 hour, and fish are not expected to remain in the plume for 96 hours continuously. Additionally, the presence of formalin will be episodic and concentrations will fluctuate because of dilution and degradation in receiving water, and the 30 hour half-life will further reduce the receiving water concentrations.

Risk to Other Aquatic Organisms from Formalin and Formaldehyde

Exposure of formalin to non-target species is limited to a small subset of studies. Hohreiter and Rigg (2001) have summarized studies on insects, crustaceans, molluscs and amphibians that meet EPA guidelines for development of ambient water quality criteria for formaldehyde. The majority of these studies are 96-hour static LC_{50} tests. EPA converted the formaldehyde data reported in Hohreiter and Rigg (2001) to formalin concentrations as described previously (Table F-17).

The seed shrimp *Cypridopsis* was the most sensitive with the LC/EC_{50} at 1.0 mg/L. Daphnia were the most common invertebrate species tested, all were sensitive to formaldehyde with SMAV's ranging from 10.14 to 16.4 mg/L (numerous authors as cited in Hohreiter and Rigg (2001). Amphibians were examined as well: two species of *Rana, R. catesbeian*a and R. *pipens* and *Bufo sp*, LC_{50}s ranged from 8.7 to 18.6 for *Rana sp.* and *Bufo*, respectively (Helms 1967 as cited in Hohreiter and Rigg (2001).

When the NOECs are compared to the EIC, HQs exceed 1.0 for all species evaluated except the Crustacean *Cypridopsis*, molluscs (*Helisoma* and *Corbicula*) and the insects *Notonecta sp*. and *Chironomus.* One important thing to note is that none of the HQs for molluscs (*Helisoma* or *Corbicula*) considered surrogates for ESA-listed snails (snails are ESA-listed in Idaho, not in Washington) exceeds an HQ of 1.0 based on the worst-case exposure to formalin and formaldehyde (Tables F-24 to F-28).

Table F-24. Hazard Quotients for other aquatic organisms exposed to Formalin and Formaldehyde at worst-Case Environmental Introduction Concentrations discharged by Facilities without ponds for 24 hour and 96 hour exposure periods.

		Formaldehyde				Formalin			
	Treatment Concentrations	**68.5 mg/L**		**100.7 mg/L**		**170 mg/L**		**250 mg/L**	
Organism	**Species**	**24 hr**	**96 hr**	**24 hr**	**96 hr**	**24 hr**	**96 hr**	**24 hr**	**96 hr**
Amphibians	R. pipens	**1.42**	**1.55**	**2.28**	**2.28**	**1.46**	**1.46**	**2.15**	**2.15**
	Rana catesbeiana	**1.30**	**1.42**	**2.09**	**2.09**	**1.33**	**1.33**	**1.96**	**1.96**
	Bufo sp.	0.67	0.73	**1.07**	**1.07**	0.68	0.68	1.00	1.00
Crustaceans	D. pulex	**1.22**	**1.33**	**1.95**	**1.95**	**3.07**	**3.07**	**4.50**	**4.51**
	Ceriodaphnia dubia	**1.13**	**1.23**	**1.80**	**1.80**	**2.93**	**2.93**	**4.31**	**4.31**
	Daphnia magna	0.75	0.82	**1.21**	**1.21**	**1.77**	**1.77**	**2.60**	**2.60**
	Bosmina sp.	0.62	0.68	0.99	0.99	**1.67**	**1.67**	**2.45**	**2.46**
	Cyclops sp.	0.62	0.68	0.99	0.99	**1.67**	**1.67**	**2.45**	**2.46**
	Cypridopsis.	0.20	0.21	0.31	0.31	0.52	0.52	0.77	0.77
Molluscs	Helisoma sp.	0.33	0.36	0.53	0.53	0.33	0.33	0.48	0.49
	Corbicula sp.	0.28	0.31	0.45	0.45	0.28	0.28	0.41	0.41
Insects	Notonecta sp.	0.04	0.04	0.06	0.06	0.04	0.04	0.05	0.05
	Chironomus sp.	0.03	0.03	0.04	0.04	0.07	0.07	0.11	0.11
	Palaemonetes	0.07	0.07	0.11	0.11	0.07	0.07	0.10	0.10

Table F-25. Hazard Quotients for other aquatic organisms exposed to Formalin administered at 250 mg/L over 30 hour increments representing ½ life degradation discharged by Facilities without ponds.

		Typical Average EIC			**Worst-Case EIC**		
		EICs over time 1/2 - life 30 hour increments					
Organism	**Species**	**30 hrs**	**60 hrs**	**90 hrs**	**30 hrs**	**60 hrs**	**90 hrs**
Amphibians	R. pipens	0.96	0.48	0.24	**1.06**	0.53	0.26
	Rana catesbeiana	0.87	0.44	0.22	0.97	0.48	0.24
	Bufo sp.	0.45	0.22	0.11	0.49	0.25	0.12
Crustaceans	D. pulex	**2.01**	1.00	0.50	**2.22**	**1.11**	0.55
	Ceriodaphnia dubia	**1.92**	0.96	0.48	**2.12**	**1.06**	0.53
	Daphnia magna	**1.16**	0.58	0.29	**1.28**	0.64	0.32
	Bosmina sp.	**1.09**	0.55	0.27	**1.21**	0.60	0.30
	Cyclops sp.	**1.09**	0.55	0.27	**1.21**	0.60	0.30
	Cypridopsis.	0.34	0.17	0.09	0.38	0.19	0.09
Molluscs	Helisoma sp.	0.22	0.11	0.05	0.24	0.12	0.06
	Corbicula sp.	0.18	0.09	0.05	0.20	0.10	0.05

Insects	Notonecta sp.	0.02	0.01	0.01	0.03	0.01	0.01
	Chironomus sp.	0.05	0.02	0.01	0.05	0.03	0.01
	Palaemonetes	0.04	0.02	0.01	0.05	0.02	0.01

Table F-26. Hazard Quotients for other aquatic organisms exposed to Formalin administered at 170 mg/L over 30 hour increments representing ½ life degradation discharged by Facilities without ponds.

		Typical Average EIC			**Worst-Case EIC**		
		EICs over time 1/2 - life 30 hour increments					
Organism	**Species**	**30 hrs**	**60 hrs**	**90 hrs**	**30 hrs**	**60 hrs**	**90 hrs**
Amphibians	R. pipens	0.67	0.33	0.17	0.73	0.37	0.18
	Rana catesbeiana	0.61	0.30	0.15	0.67	0.33	0.17
	Bufo sp.	0.31	0.16	0.08	0.34	0.17	0.09
Crustaceans	D. pulex	**1.40**	0.70	0.35	**1.53**	0.77	0.38
	Ceriodaphnia dubia	**1.34**	0.67	0.33	**1.47**	0.73	0.37
	Daphnia magna	0.81	0.40	0.20	0.88	0.44	0.22
	Bosmina sp.	0.76	0.38	0.19	0.84	0.42	0.21
	Cyclops sp.	0.76	0.38	0.19	0.84	0.42	0.21
	Cypridopsis.	0.24	0.12	0.06	0.26	0.13	0.07
Molluscs	Helisoma sp.	0.15	0.08	0.04	0.17	0.08	0.04
	Corbicula sp.	0.13	0.06	0.03	0.14	0.07	0.04
Insects	Notonecta sp.	0.02	0.01	0.00	0.02	0.01	0.00
	Chironomus sp.	0.03	0.02	0.01	0.04	0.02	0.01
	Palaemonetes	0.03	0.02	0.01	0.03	0.02	0.01

Table F-27. Hazard Quotients for other aquatic organisms exposed to formaldehyde administered at 100.7 mg/L over 30 hour increments representing ½ life degradation discharged by facilities without ponds.

		Typical Average EIC			**Worst-Case EIC**		
		EICs over time 1/2 - life 30 hour increments					
Organism	**Species**	**30 hrs**	**60 hrs**	**90 hrs**	**30 hrs**	**60 hrs**	**90 hrs**
Amphibians	R. pipens	**1.52**	**1.66**	0.38	0.10	0.83	0.42
	Rana catesbeiana	**1.39**	**1.52**	0.35	0.09	0.76	0.38
	Bufo sp.	0.71	0.78	0.18	0.05	0.39	0.19
Crustaceans	D. pulex	**1.30**	**1.43**	0.33	0.09	0.71	0.36
	Ceriodaphnia dubia	**1.20**	**1.31**	0.30	0.08	0.66	0.33
	Daphnia magna	0.80	0.88	0.20	0.05	0.44	0.22
	Bosmina sp.	0.66	0.72	0.16	0.04	0.36	0.18

	Cyclops sp.	0.66	0.72	0.16	0.04	0.36	0.18
	Cypridopsis.	0.21	0.23	0.05	0.01	0.11	0.06
Molluscs	Helisoma sp.	0.35	0.39	0.09	0.02	0.19	0.10
	Corbicula sp.	0.30	0.33	0.08	0.02	0.17	0.08
Insects	Notonecta sp.	0.04	0.04	0.01	0.00	0.02	0.01
	Chironomus sp.	0.03	0.03	0.01	0.00	0.02	0.01
	Palaemonetes	0.07	0.08	0.02	0.00	0.04	0.02

Table F-28. Hazard Quotients for other aquatic organisms exposed to formaldehyde administered at 68.5 mg/L over 30 hour increments representing ½ life degradation discharged by facilities without ponds.

		Typical Average EIC			**Worst-Case EIC**		
		EICs over time 1/2 - life 30 hour increments					
Organism	**Species**	**30 hrs**	**60 hrs**	**90 hrs**	**30 hrs**	**60 hrs**	**90 hrs**
Amphibians	R. pipens	0.99	0.50	0.25	**1.10**	0.55	0.27
	Rana catesbeiana	0.91	0.45	0.23	1.00	0.50	0.25
	Bufo sp.	0.46	0.23	0.12	0.51	0.26	0.13
Crustaceans	D. pulex	0.85	0.43	0.21	0.94	0.47	0.24
	Ceriodaphnia dubia	0.79	0.39	0.20	0.87	0.43	0.22
	Daphnia magna	0.53	0.26	0.13	0.58	0.29	0.15
	Bosmina sp.	0.43	0.22	0.11	0.48	0.24	0.12
	Cyclops sp.	0.43	0.22	0.11	0.48	0.24	0.12
	Cypridopsis.	0.14	0.07	0.03	0.15	0.08	0.04
Molluscs	Helisoma sp.	0.23	0.12	0.06	0.25	0.13	0.06
	Corbicula sp.	0.20	0.10	0.05	0.22	0.11	0.05
Insects	Notonecta sp.	0.03	0.01	0.01	0.03	0.01	0.01
	Chironomus sp.	0.02	0.01	0.00	0.02	0.01	0.01
	Palaemonetes	0.05	0.02	0.01	0.05	0.03	0.01

Amphibians and daphnids are the most sensitive species according to the magnitude of the HQs for formalin and formaldehyde and dose regimes. As formalin and formaldehyde breakdown in receiving waters, only the HQs for daphnids continue to be greater than 1.0. Since these EICs will be diluted in receiving waters, the likelihood of adverse effects is reduced. This dilution must occur within close proximity to the discharge pipe so that the area where ESA-listed species or their prey is minimized, thereby reducing exposure.

According to the end-of-pipe EICs calculated using the USGS facility survey data and model, there may be a reduction in some amphibian and crustacean populations if dilution in the receiving water does not

reduce exposure point concentrations below effect levels. We do not anticipate adverse effects in molluscs or aquatic invertebrates at these EICs.

Uncertainty Analysis of Formalin Risk Characterization

All four types of uncertainty (variation, model uncertainty, decision rule uncertainty and true unknowns) described in the problem formulation are present in this formalin evaluation. One of the largest sources of uncertainty is the limited toxicity data that would permit a quantitative evaluation of risks to ESA-listed species from formalin and formaldehyde use at aquaculture facilities. Empirical toxicity data are only available for three of the six ESA-listed salmonids (steelhead, coho salmon, Chinook salmon) in Washington. Thus, both model uncertainties from the ICE model and true unknowns are present in the effects analyses and risk characterization for chum salmon, sockeye salmon, and bull trout.

Variation of the EIC in aquaculture discharges and receiving waters is also a large source of uncertainty in this analysis. This is because the use pattern of formalin is short-term and irregular (parasite and fungi control). Formalin is administered in 1.0 hour treatments and so exposure is anticipated to occur for the duration it would take to clear a raceway, the number of raceways treated, and the sequencing of treated raceways.

Because of this use pattern, prediction of exposure duration in receiving waters is confounded and would be expected to be on the order of hours and not days. Using the 96-hour study durations in the ICE model to estimate LC_{50}s for ESA-listed fish species likely overestimates the potential for adverse effects because of the relationship between exposure duration and toxicity of formalin/formaldehyde. The acute toxicity of formalin/formaldehyde is correlated with exposure duration, and organisms would not be exposed in the receiving water at levels resulting in toxicity during a short-term exposure (Table F-1). The toxicity of formalin increases with exposure, as shown in Table F-10. Overestimating the duration of exposure results in a similar overestimation of toxicity. This potential overestimation of exposure uncertainty may affect the risk characterization for several of the amphibian and zooplankton prey species (Tables F-24 through F-27), for which hazard quotients were found to be slightly greater than 1.0. As salmonids are opportunistic predators, the possible adverse effects on some prey species is reduced by the continued available of other prey species which are less sensitive to formalin.

Another source of uncertainty is the rate at which formalin degrades in the environment. According to FDA (1995), which includes anecdotal evidence from USFWS (Appendix I in FDA 1995), 20 mg/L concentrations in ponds begin to decline within 30 to 36 hours. FDA further states that “It is reasonable to conclude that formalin biodegrades within a few days in most natural aquatic environments.” Therefore, the frequency of use affects the presence of formalin in receiving waters.

Lack of chronic toxicity data for standard endpoints such as growth and reproduction adds to the uncertainty of the assessment. The reliance on ACRs generated on studies with limited chronic data results in a likely over estimation of toxicity. The sublethal data included in the analysis shows that sublethal effects occur at elevated concentrations, but that fish often recover. These concentrations are in excess of the calculated NOECs.

Finally, the precise location of the facility discharge pipes is not accounted for in this Biological Evaluation, which confounds the assumption of exposure. The available latitude and longitude data was provided to EPA by the individual facility operators, often these location data are for the office building and not the discharge pipe. Therefore, in many cases, EPA is only able to predict the general location of the discharge in the waterbody.

Next Permit Cycle: Improved Reporting Requirements and a Formalin Field Study

One of the most difficult components of this Biological Evaluation was to estimate the EIC for disease treatment chemicals- largely because of a lack of data. This next permit cycle will address this issue in two ways: 1) by requiring more precise information about the use of disease treatment chemicals, and 2) by conducting a study to collect empirical data on formalin in hatchery effluent.

1) Improved Reporting Requirements

EPA has made significant improvements to the Annual Reports required as part of the proposed draft permit. For each disease treatment chemical used during that calendar year, the permittee will now be required to submit information including: total quantity of formulated product used per treatment and per year; dates of treatment; method of application (e.g. flow through, static bath, medicated feed, or injection); maximum daily volume of treated water; and treatment concentration. Additional information will be required for water-borne treatments, such as those that are evaluated in this Biological Evaluation. Permittees will be required to specify whether treatments were static bath or flow through, the desired concentration, volume of product needed, minimum volume of total water discharged from the facility per day, maximum effluent concentration of solution and active ingredient, and the maximum percent of the facility discharge treated with the chemical. Flow through treatments will also be required to provide the calculated flow rate, the amount of product added initially and during treatment, etc. See the Annual Report template in the draft permit for more detail.

2) Field Study: Formalin in Aquaculture Effluent

EPA is undertaking a study to ascertain the concentrations of formaldehyde in aquaculture effluent. End-of-pipe samples will be collected at regular intervals over the course of a day from federal, tribal, state, and privately owned facilities in Washington and Idaho. EPA will work with facilities to predict maximum effluent concentrations of the chemical, given individual facility retention times. Within Washington State, EPA is partnering with USFWS, tribes, and WDFW to select facilities that present a representative and/or reasonable worst case formalin use. EPA plans to conduct effluent sampling to account for the three formalin use scenarios: egg stacks/hatch houses, juveniles, and returning adults. Sampling will be conducted during the summer and fall seasons, likely in 2016. The EPA Region 10 Laboratory will analyze the samples with a sufficiently sensitive method (e.g. EPA Method 8315).

Effect Determinations:

Based on all chronic NOEC concentrations for six threatened and endangered salmonid species being higher than the estimated environmental concentrations of formalin/formaldehyde released from hatcheries, EPA has made the following effect determinations for formalin:

Bull trout: Not likely to adversely affect

Chinook salmon: Not likely to adversely affect

Chum salmon: Not likely to adversely affect

Coho salmon: Not likely to adversely affect

Sockeye salmon: Not likely to adversely affect

Steelhead: Not likely to adversely affect

The above determinations are all based on the estimated environmental concentrations from hatchery releases being lower than the chronic NOECs for the above six species.

Based on the lack of current discharges from any Washington hatchery directly into estuarine or marine waters, the following species are not exposed to formalin releases from Washington hatcheries. Therefore, a no effect determination from formalin released by hatcheries is warranted for the following species.

Eulachon: No effect

Bocaccio: No effect

Canary rockfish: No effect

Yelloweye rockfish: No effect

These no effect determinations would need to be revisited if hatcheries which discharge directly into estuarine or marine systems would begin to use formalin in their operations at some future date.

5.5 HYDROGEN PEROXIDE

CAS ID: 7722-84-1

Chemical formula: H_2O_2

Synonyms / Trade names: Peroxide, hydrogen dioxide, Perox-Aid®

Chemical composition: Hydrogen peroxide is the simplest peroxide, which are compounds with a single bond between two oxygen atoms. It is a liquid at room temperature, with a melting point of approximately 0.43° C, and decomposes between 150 – 152° C. Hydrogen peroxide is slightly denser (density of 1.44 g/cm^3) and more viscous than water. Concentrated solutions appear light blue in color. Its molecular weight is 34.015. Commercial hydrogen peroxide solutions used at fish hatcheries contain 35% hydrogen peroxide, with the remainder being water. The 35% solution is then diluted to the desired exposure concentration.

Hatchery use: Primary use is as a bath treatment to control fungal diseases in fish, as well as in fish eggs prior to hatch. The commercially available 35% hydrogen peroxide solution is diluted before use in disinfection. The diluted solution to which fish and fish eggs are exposed contains 50 – 1000 mg/L hydrogen peroxide. Exposure durations at hatcheries range between 15 – 60 minutes/day, with the higher concentrations used in conjunction with the shortest exposure durations. Depending on the specific fungal infection, treatments can be repeated on multiple days, or on alternating days up to a total of three treatments/fish. Hydrogen peroxide is also believed to be effective against many bacterial and viral infections. It is not normally used to treat bacterial and viral infections in fish hatcheries, although it is beginning to be used to treat bacterial infestations of fish gills. The only two hatcheries in Washington currently reporting use of hydrogen peroxide are the Quilcene and Little White Salmon National Fish Hatcheries, both of which discharge to freshwater systems.

Measures of Exposure:

Hydrogen peroxide is classified as a low regulatory priority aquaculture drug by the FDA (2006). Its use in hatcheries is generally for the control of external fungal infestations. It is also beginning to be used to treat bacterial infections of fish gills. Application is generally at a concentration between 50 – 1000 mg/L to fish and fish eggs. Both the Quilcene and Little White Salmon National Fish Hatcheries report using H_2O_2 at a concentration of up to 1000 mg/L for 15 minutes/day. This use rate and concentration is in keeping with AFS (2011) recommendations for exposure concentration and duration to treat external fungal infections.

Quilcene and Little White Salmon Hatcheries provided additional information to EPA regarding the daily volume of H2O2 use and the number of days per year H2O2 is used. This information allowed us to calculate the concentration of hydrogen peroxide in hatchery discharges. These calculations are presented in the Expected Environmental Concentration (EEC) portion of this Measures of Exposure section.

In addition to its potential discharge from hatcheries, hydrogen peroxide is a naturally occurring chemical, produced by both biochemical and photochemical processes. It is found in freshwater at concentrations between 0.001 – 0.109 mg/L, and in marine waters at concentrations between 0.001 – 0.0136 mg/L (FDA 2006). Most organisms produce hydrogen peroxide under aerobic metabolism, which is then metabolically transformed into water and elemental oxygen (O_2), primarily by the enzyme catalase. Hydrogen peroxide is freely soluble in water. Its estimated log octanol-water partition coefficient (log K_{OW}) of -1.5, combined with the ability of organisms to rapidly metabolically transform hydrogen peroxide into water and oxygen are all indicative of a chemical with little ability to bioaccumulate.

The remainder of this measures of exposure assessment will evaluate two aspects that combined define the exposure of ESA listed species to hydrogen peroxide in the environment: its environmental fate once released into the environment, and its expected environmental concentration.

Environmental Fate of Hydrogen Peroxide

This section will describe the expected environmental fate of hydrogen peroxide.

Under non-sterile conditions in aerobic surface waters, the half-life of hydrogen peroxide is 1.1 – 5.3 hours (Breithaupt 2007). These are the conditions found in nearly all surface waters except for highly oligotrophic systems containing little in the way of organic matter and bacterial populations.

The two hatcheries covered by this permit that currently use hydrogen peroxide (Quilcene National Fish Hatchery and Little White Salmon National Fish Hatchery) both treat fish for fungal and gill bacterial issues using an initial concentration of up to 1000 mg/L H_2O_2. Using the range of half-lives given in Breithaupt (2007), the concentration of H_2O_2 remaining in water, assuming no dilution, after any given time period after the initial exposure can be estimated assuming first order degradation kinetics with the following two equations.

$$\lambda = \frac{\ln 2}{t_{½}}$$

Where: λ = Degradation rate (hour^{-1})

$t_{½}$ = Half-life of the chemical in the environment (hours), and

$$C_t = C_0 e^{-\lambda t}$$

Where: C_t = Chemical concentration in water at time t (mg/L)

C_0 = Initial chemical concentration in water (mg/L)

λ = degradation rate (hour^{-1})

t = Time elapsed after initial addition of chemical to water (hours)

Table HP-1 shows estimated residual hydrogen peroxide concentrations in water after an initial addition of 1000 mg/L H_2O_2, using both the shortest (1.1 hours) and longest (5.3 hours) half-lives given by Breithaupt (2007) for H_2O_2 in surface waters.

Table HP-1. Hydrogen peroxide residual concentrations (mg/L) in surface water at different time periods after an initial concentration of 1000 mg/L, based on two different half-lives in water. Residual concentrations assume no dilution by additional water.

Time after initial dose (hours)	Half-life = 1.1 hours	Half-life = 5.3 hours
0	1000	1000
1	533	877
2	284	770
3	151	675
4	80.4	593
6	22.8	456
12	0.52	208
18	0.012	95.0
24	0.00027	43.3
48	7.31×10^{-11}	1.88
72	1.98×10^{-18}	0.081

Under sterile conditions, and particularly sterile conditions in the absence of light, hydrogen peroxide solutions can remain stable for months, with only minimal reductions (approximately 2% reduction in H_2O_2 / year) in the concentration of hydrogen peroxide. This is the reason commercially available solutions of hydrogen peroxide can be sold.

The primary reactions of hydrogen peroxide in surface water include the following:

$$2H_2O_2 \leftrightarrow 2H_2O + O_2 \ (metabolic\ transformation\ by\ catalase, other\ peroxidases)$$

$$Fe^{+2} + H_2O_2 \leftrightarrow OH^- + \cdot OH + Fe^{+3} \ (hydroxyl\ ion\ and\ free\ radical\ formation)$$

$$R + \cdot OH \leftrightarrow ROH \ (oxidation\ of\ organic\ matter\ (R)\ by\ hydroxyl\ free\ radicals)$$

Although ferrous iron (Fe^{+2}) is shown in the above reaction, other metals, including manganese and several divalent cations can also serve as catalysts for the production of hydroxyl ions and hydroxyl free radicals ($\cdot OH$). Most organic matter, including cell membranes and viral envelopes, is quickly oxidized by the hydroxyl free radicals released during the breakdown of H_2O_2 in surface water. This oxidation of organic matter with hydroxyl free radicals is the primary mechanism of toxic action by which hydrogen peroxide serves as a disinfectant.

Expected Environmental Concentration (EEC) of Hydrogen Peroxide

The highest treatment concentration of hydrogen peroxide at the two hatcheries that currently report its use is 1000 mg/L. Quilcene National Fish Hatchery and Little White Salmon National Fish Hatchery have

provided EPA with information that permits us to calculate the expected environmental concentration (EEC) of hydrogen peroxide in water at the point where the hatchery discharges into a receiving water (i.e. the end of pipe hydrogen peroxide concentration). This end of pipe concentration is used as a conservative estimate of the hydrogen peroxide concentration in receiving waters prior to any dilution of hatchery discharges by the receiving body of water. This EEC calculation also does not take into account the degradation of hydrogen peroxide described in the environmental fate portion of this Measures of Exposure section.

As described in the Problem Formulation section of the methodology used in this Biological Evaluation, the EEC is calculated as follows, based on procedures described in Schmidt et al. (2007).

$$EEC = \frac{C \times V}{F + E}$$

Where: EEC = Expected environmental concentration (mg/L or µg/L)

C = Treatment concentration of chemical in the hatchery (mg/L or µg/L)

V = Volume of chemical used (gallons/day)

F = Volume of water discharged from hatchery to receiving water (gallons/day)

E = Effluent pond volume (gallons)

For the purposes of calculating the hydrogen peroxide EEC for Quilcene National Fish Hatchery, EPA assumed that the effluent pond volume is zero. The Quilcene hatchery hydrogen peroxide use volume, concentration, and the hatchery low, average and maximum daily discharges to receiving water are presented in Table HP-2, along with the calculated EEC for each of the three hatchery discharge volumes.

Table HP-2. Expected environmental concentration of hydrogen peroxide under low, average and high water volume daily discharges from the Quilcene National Fish Hatchery.

Parameter	Value	EEC (µg/L)
Chemical use concentration, mg/L	1000	
Daily volume used, gallons	7.94	
Total volume used/year, gallons	286	
Days/year chemical used	36	
Low hatchery discharge, gallons/day	59,305	134
Average hatchery discharge, gallons/day	9,217,390	0.862
High hatchery discharge, gallons/day	31,966,747	0.249

EPA also calculated a simple, dilution-based EEC for Little White Salmon National Fish Hatchery, using treatment information provided by the hatchery manager. Little White Salmon's hydrogen peroxide use during the previous cycle is as follows: during a one hour flow through treatment at 75 ppm, there were two raceways treated. 26,100 mL hydrogen peroxide used per raceway or 52,200 total in one hour. The

hatchery used hydrogen peroxide for ten total days (only the highest treatment amounts were considered in this analysis). Little White Salmon National Fish Hatchery only used hydrogen peroxide in 2010 (not during any other years). The total chemical used over the ten days was 115 gallons. The hatchery effluent at the time of use was: 13,160 gpm (August), 11,797 gpm (September) and 9,705 gpm (October). EPA used the October 2010 low flow (9,705 gpm) in its calculations to represent a reasonable worst case scenario. EPA calculated Little White Salmon National Fish Hatchery's EEC for hydrogen peroxide to be 150 µg/L end of pipe concentration.

EEC values for Quilcene National Fish Hatchery and Little White Salmon National Fish Hatchery do not take into account any degradation of hydrogen peroxide that occurs during the time between hatchery fish were exposed to H_2O_2 and the time at which the exposure water was discharged into a receiving water. Because degradation of H_2O_2 was not considered in the EEC calculations, the EEC values presented are likely overestimates of the concentrations that would be discharged into surface waters. The EEC concentrations will be compared to the chronic NOEC estimates calculated in the Measures of Effect section. This comparison will take place in the Risk Characterization section to estimate ecological risks to threatened and endangered species exposed to hydrogen peroxide discharges from hatcheries in Washington.

Measures of Effect:

For fully aquatic species, the available toxicity data was identified from a search in EPA's ECOTOX database (http://cfpub.epa.gov/ecotox/).

A combined total of 321 toxicity records were identified from the search. These results are presented in Appendix I. Of these records, only 10 exposed animals to hydrogen peroxide under flow through conditions: 9 records for *Daphnia magna* and one for rainbow trout. The one flow through exposure with rainbow trout (Powell and Perry 1997) only exposed the fish to hydrogen peroxide for one hour, not the 96 hour exposure called for by EPA in its data quality guidelines for a study to be useable in the derivation of EPA water quality criteria. Powell and Perry (1997) observed 100% mortality of rainbow trout in one hour when exposed to 1500 mg/L H_2O_2. Both the H_2O_2 concentration and exposure duration in Powell and Perry (1997) are higher than the 15 minute exposure to 1000 mg/L H_2O_2 used by hatcheries to treat fungal and bacterial infections.

The remaining available toxicity data for aquatic species was performed under static, static renewal or pulsed exposures. Taxa for which hydrogen peroxide toxicity data are available that does not meet EPA requirements for use in deriving water quality criteria are as follows:

- Freshwater algae: 13 species
- Freshwater macrophytes: 4 species
- Aquatic insects: 1 species
- Freshwater crustaceans: 4 species
- Freshwater zooplankton: 1 species
- Freshwater molluscs: 2 species
- Other freshwater invertebrate taxa (e.g. oligochaetes): 1 species

- Freshwater fish: 23 species
- Marine algae: 7 species
- Marine macrophytes: None
- Marine insects: None
- Marine crustaceans: 4 species
- Marine zooplankton: 4 species
- Marine molluscs: 4 species
- Other marine invertebrate taxa (e.g. polychaetes): 1 species
- Marine amphibians: None
- Marine fish: 7 species

Of the available toxicity data, some information on a threatened and endangered species under evaluation in this Biological Evaluation is for rainbow trout (steelhead), Chinook salmon, and coho salmon. We have used the available 96 hour LC_{50} data under static exposure conditions for rainbow trout, coho salmon and Chinook salmon to estimate the toxicity of hydrogen peroxide to the remaining ESA listed salmonid species in Washington. We have used the methodologies described under the problem formulation section of this Biological Evaluation, specifically using ICE models. We have done this even though the rainbow trout, coho salmon and Chinook salmon 96 hour LC_{50} studies were performed under static exposure conditions, not flow through conditions. Flow through conditions are particularly important for maintaining the desired exposure concentrations of chemicals such as hydrogen peroxide that degrade quickly under environmental conditions. Exposing organisms to chemicals that rapidly degrade under flow through conditions provides a greater likelihood that the exposure concentrations are as intended throughout the study, relative to the chemical degradation and subsequent reduction in exposure concentration that occurs over time during static or static renewal exposure conditions.

Toxicity of Hydrogen Peroxide

No toxicity studies with fish meeting EPA requirements for use in developing aquatic life criteria are available for hydrogen peroxide. Of the available data, the most useful in evaluating potential hydrogen peroxide toxicity to threatened and endangered species in receiving waters is a series of 96 hour LC_{50} studies performed under static exposure conditions on two size classes of rainbow trout, coho salmon and Chinook salmon (Taylor and Glenn 2008). The Taylor and Glenn (2008) studies were performed at the Abernathy Fish Technology Center of the U.S. Fish and Wildlife Service (Longview, WA) using fish stocks native to Washington (rainbow trout, Chinook salmon) or Oregon (coho salmon).

Taylor and Glenn (2008) exposed two different size classes of fish to hydrogen peroxide. Their 'small' group of fish had a target body weight of 2 grams, while their 'large' group of fish had a target body weight of 10 grams. The 96 hour LC_{50} values for rainbow trout, coho salmon and Chinook salmon from Taylor and Glenn (2008) are given in Table HP3. Taylor and Glenn (2008) did not report confidence intervals around their LC_{50} values.

Table HP-3. Empirical 96 hour LC_{50} values for three salmonid species as reported by Taylor and Glenn (2008).

Species	Size Class	LC_{50} (mg/L)
Rainbow trout	2 gram body weight	373
Rainbow trout	10 gram body weight	196
Chinook salmon	2 gram body weight	200
Chinook salmon	10 gram body weight	106
Coho salmon	2 gram body weight	231
Coho salmon	10 gram body weight	225

No empirical chronic toxicity data with hydrogen peroxide are available for rainbow trout, Chinook salmon or coho salmon. Therefore, the procedures given in the Problem Formulation are used to convert the empirical 96 hour LC_{50} values in Table HP-3 to chronic NOEC concentrations. This calculation involves dividing the lower of the two available LC_{50} values for each of the salmonid species in Table HP-3 by 2.27 to first derive a 'LC_{LOW}' concentration. The LC_{LOW} is then divided by a default national acute-chronic ratio of 8.3 to calculate the chronic NOEC concentrations for rainbow trout, Chinook salmon and coho salmon. These calculated chronic NOEC values are presented in Table HP-4.

Output of all ICE models run with hydrogen peroxide for the three remaining threatened and endangered species (bull trout, chum salmon and sockeye salmon), genera or family with available data in ICE is shown in Appendix J. Using the ICE model selection guidelines set forth in the problem formulation, models used to estimate chronic NOEC's for salmonid species are highlighted in green and bolded in Appendix J.

A family level ICE model using the empirical rainbow trout LC_{50} data was used as the starting point to derive chronic NOEC values for bull trout, chum salmon and sockeye salmon. The genus and family level ICE models using empirical coho salmon toxicity data as input could not be used to estimate toxicity to bull trout, chum and sockeye salmon, because the empirical toxicity data was outside of the useable range of the ICE regression between coho salmon and bull trout, chum and sockeye salmon. The empirical genus level Chinook salmon – bull trout also could not be used to estimate hydrogen peroxide toxicity to bull trout, chum and sockeye salmon, again because the empirical Chinook salmon toxicity data was outside of the useable range of the ICE regression. The family level ICE model between rainbow trout and bull trout, chum and sockeye salmon was selected from the remaining ICE models because of the large number of data pairs in the regression, and high r^2 and cross-validation scores.

The remaining ICE models, with poorer predictive ability and which were not selected as the source of chronic NOEC's are shown in red in Appendix J. As described in the problem formulation, the lower 95% confidence interval of the predicted chronic NOEC, if available, is used as the chronic NOEC in this Biological Evaluation. All ICE models used for hydrogen peroxide generated lower 95% confidence intervals of the chronic NOEC, and are shown in this section.

No information is available in ICE for eulachon or any of the threatened and endangered rockfish species, genera or families in Washington (bocaccio, canary rockfish, yelloweye rockfish). Therefore, hydrogen

peroxide effects on eulachon and the rockfish species cannot be quantitatively evaluated, and must be considered as a toxicological uncertainty in this Biological Evaluation. However, as neither the Quilcene nor Little White Salmon National Fish Hatcheries directly discharge to marine or estuarine waters, it is unlikely that hydrogen peroxide discharges from these two hatcheries would impact saltwater species such as eulachon or rockfish.

The final selected chronic NOEC values for bull trout, Chinook salmon, chum salmon, coho salmon, sockeye salmon and steelhead that were compared to the expected environmental concentration of hydrogen peroxide in receiving water environments are summarized in Table HP-4.

Table HP-4. Chronic no effect concentrations (NOEC) for threatened and endangered salmonid species exposed to hydrogen peroxide.

Species	Chronic NOEC (mg/L)	Source of chronic NOEC
Bull trout	5.09	ICE model – family level
Chinook salmon	5.63	Empirical acute data (Taylor and Glenn 2008)
Chum salmon	5.09	ICE model – family level
Coho salmon	11.9	Empirical acute data (Taylor and Glenn 2008)
Sockeye salmon	5.09	ICE model – family level
Steelhead	10.4	Empirical acute data (Taylor and Glenn 2008)

Risk Characterization: Hydrogen Peroxide

Risks to Threatened and Endangered Fish Species from Hydrogen Peroxide

Risks to threatened and endangered fish species for which toxic concentrations of hydrogen peroxide can be identified from the literature are calculated using a standard ecological risk assessment hazard quotient approach. In the hazard quotient approach, the estimated environmental concentration is divided by the chronic NOEC for each threatened and endangered species to calculate a hazard quotient. Hazard quotients less than 1.0 are indicative of acceptable levels of ecological risk. In the context of this Biological Evaluation, an acceptable ecological risk is represented as an EEC which, if not exceeded, results in no discernable effect on the survival, reproduction and growth of a threatened and endangered species. Note that acceptable EEC values vary between species.

Hazard quotients greater than or equal to 1.0 are indicative of a potential for unacceptable ecological risks to threatened and endangered species. Note that hydrogen peroxide is a naturally occurring chemical, whose sources include aerobic metabolism of fish, and thus ecological risks from hydrogen peroxide cannot be set at zero.

Hazard quotients for the six threatened and endangered salmonid species for which toxicity data is available or could be estimated are presented in Table HP-5. Hazard quotients were calculated using the EEC generated from the lowest and highest daily discharge from Quilcene National Fish Hatchery, combined with the EEC from Little White Salmon National Fish Hatchery (.15 mg/L). This resulted in the EEC range to which threatened and endangered species could be exposed.

Table HP-5. Hazard quotients (HQ) for threatened and endangered species exposed to the range of estimated environmental concentrations (EEC) of hydrogen peroxide discharged by hatcheries.

Species	EEC range (mg/L)	Chronic NOEC (mg/L)	Hazard quotient range
Bull trout	0.000249 – 0.150	5.09	0.000049 – 0.029
Chinook salmon	0.000249 – 0.150	5.63	0.000044 – 0.027
Chum salmon	0.000249 – 0.150	5.09	0.000049 – 0.029
Coho salmon	0.000249 – 0.150	11.9	0.000021 – 0.013
Sockeye salmon	0.000249 – 0.150	5.09	0.000049 – 0.029
Steelhead	0.000249 – 0.150	10.4	0.000024 – 0.014

All hazard quotients in Table HP-5 are substantially lower than 1.0, indicative of acceptable levels of ecological risk to the species under all hatchery discharge scenarios. Note that the EEC values do not take into account the rapid degradation of environmental concentrations of hydrogen peroxide. This is discussed more fully in the uncertainty analysis portion of risk characterization, as it is likely the major uncertainty in this Biological Evaluation which overestimates potential ecological risks to threatened and endangered species.

Risks to Potential Freshwater Prey of Threatened and Endangered Species from Hydrogen Peroxide

Although not of a data quality useful for deriving EPA water quality criteria, a fairly substantial number of species have some hydrogen peroxide toxicity data available for them (Appendix I). The only toxicity study with hydrogen peroxide that appears to be of a suitable quality for use in EPA water quality criteria derivation is that of Meinertz et al. (2008), who performed a 21 day chronic flow through exposure of the cladoceran *Daphnia magna* to hydrogen peroxide. Endpoints evaluated by Meinertz et al. (2008) included survival, reproductive output, growth and population sex ratio. Growth was the most sensitive endpoint for *D. magna*, with growth reductions occurring within 21 days at H_2O_2 concentrations ≥ 0.32 mg/L. *D. magna* reproductive output was unaffected at concentrations ≤ 0.63 mg/L, survival was unaffected at concentrations ≤ 1.25 mg/L, while sex ratio was unaffected at concentrations as high as 5.0 mg/L.

In addition to the Meinertz et al. (2008) study on the crustacean zooplankter *Daphnia magna*, empirical adverse effect toxicity data for hydrogen peroxide exists for 13 freshwater algal species, four aquatic macrophyte species, one aquatic insect, three crustaceans, two molluscs, one worm, one amphibian, and 23 freshwater fish species.

Despite the lack of studies of a quality that could be used to develop EPA water quality criteria, we have used the procedures outlined in the Problem Formulation (i.e. divide the acute toxicity value by 2.27, then dividing the LC_{LOW} by a default acute-chronic ratio of 8.3 to obtain a chronic NOEC) to estimate chronic NOEC concentrations for prey of threatened and endangered fish species. Chronic NOEC concentrations of hydrogen peroxide to prey of threatened and endangered species is summarized in Table HP-6.

Table HP-6. Toxicity of Hydrogen Peroxide to Freshwater Prey of Threatened and Endangered Listed Species.

Organism Type	Chronic NOEC range (mg/L)
Algae	0.086 – 55.7
Aquatic macrophytes	1.8 – 12.6
Aquatic invertebrates	0.20 – 53.1
Aquatic insects	20.5
Crustaceans	0.20 – 53.1
Zooplankton	0.32 – 1.25
Molluscs	0.53 – 0.83
Others (e.g. oligochaetes, etc.)	5.31
Amphibians	0.97
Fish	0.53 - 164

The most sensitive freshwater species to hydrogen peroxide appears to be the cyanobacterium (blue-green alga) *Microcystis pulverea*, with a three day EC_{50} for reduction in population abundance of 0.71 mg/L under static exposure conditions (Drabkova et al. 2007). For algae, a three day exposure is considered a chronic exposure period, as multiple algal generations are produced during a three day period. Conversion of this empirical EC_{50} to a chronic NOEC yielded a value of 0.086 mg/L, the only chronic NOEC for a prey species lower than the highest calculated EEC of 0.134 mg/L. The *Microcystis* chronic NOEC is higher than both the average and maximum hatchery discharge EECs.

Fish species appear to have the widest range of sensitivity to hydrogen peroxide among the taxa for which empirical toxicity information is available. The most sensitive freshwater fish appears to be the northern pikeminnow, with a calculated chronic NOEC of 0.53 mg/L. The most tolerant fish species is sea lamprey exposed in freshwater, with a chronic NOEC of 164 mg/L. The chronic NOEC values for most fish species falls between 1 – 15 mg/L, with salmonids as a group among the more tolerant species of hydrogen peroxide exposures (salmonid chronic NOECs between 5.63 and 26.5 mg/L).

As all other prey species chronic NOECs are higher than the highest EEC for hydrogen peroxide, we conclude that hydrogen peroxide is not likely to adversely affect prey species of threatened and endangered fish species in Washington.

Uncertainty Analysis of Hydrogen Peroxide Risk Characterization

All four types of uncertainty (variation, model uncertainty, decision rule uncertainty and true unknowns) described in the problem formulation are present in this hydrogen peroxide evaluation. By far the largest uncertainty in this evaluation is the complete absence of toxicity data in the literature that would permit a quantitative evaluation of risks to threatened and endangered rockfish species from hydrogen peroxide use at fish hatcheries. This type of uncertainty is a true unknown in this Biological Evaluation. However, as the only two Washington hatcheries currently using hydrogen peroxide both discharge to freshwater streams, not marine or estuarine systems, eulachon and rockfish species are not currently exposed to any hydrogen peroxide releases from permitted hatcheries.

Variation of expected environmental concentrations in hatchery discharges and receiving waters is also a large source of uncertainty in this analysis. This is because the use pattern of hydrogen peroxide occurs only during a small portion of a year. This use pattern means that during much of the year, hydrogen peroxide is not released from a hatchery. Variation also is expressed in the confidence limits surrounding statistically reduced expressions of the empirical toxicity data (e.g. LC_{50}, EC_{50}, etc.). Confidence limits describe random variation around the central tendency response of laboratory organisms exposed to chemicals in toxicity tests.

The rapid environmental degradation rates of hydrogen peroxide in aquatic systems also introduce variation in exposure concentrations and EECs over time. Variation in hydrogen peroxide concentrations due to its environmental degradation is a unidirectional process, with the environmental concentration constantly declining. Without consideration of the degradation rate of H_2O_2 in surface water, the EEC values used to describe exposure of threatened and endangered species to H_2O_2 overestimate the concentrations threatened and endangered species are actually exposed to in the environment. Not attempting to estimate the effect on hydrogen peroxide EECs of dilution of hatchery discharges by receiving waters also serves to overestimate the actual EEC to which threatened and endangered species are exposed. Although we have estimated EECs and degradation rates separately in this Biological Evaluation, given the already low hazard quotients calculated from our EECs, we have chosen not to modify our EECs by inclusion of a degradation rate term.

Model uncertainty in the ICE models is described by the percent cross-validation success statistic. According to Raimondo et al. (2013), the percent cross-validation success rate for each model is the proportion of data points that are predicted within 5-fold of the actual LC_{50} value. There is a strong relationship between taxonomic distance and cross-validation success rate, with uncertainty generally, although not always increasing with larger taxonomic distance. Maximizing the value of the cross-validation statistic was a primary determinant of which of multiple ICE models were used to estimate toxicity values in this Biological Evaluation for species where no empirical toxicity data exists for a chemical-species pair.

Effect Determinations of Hydrogen Peroxide on Threatened and Endangered Species

Based on all chronic NOEC concentrations for six threatened and endangered salmonid species being substantially higher than the estimated environmental concentrations of hydrogen peroxide released from hatcheries, EPA has made the following effect determinations for hydrogen peroxide:

Bull trout: Not likely to adversely affect

Chinook salmon: Not likely to adversely affect

Chum salmon: Not likely to adversely affect

Coho salmon: Not likely to adversely affect

Sockeye salmon: Not likely to adversely affect

Steelhead: Not likely to adversely affect

The above determinations are all based on the estimated environmental concentrations from hatchery releases being substantially lower than the chronic NOECs for the above six species.

Based on the lack of current discharges from any Washington hatchery directly into estuarine or marine waters, the following species are not exposed to hydrogen peroxide releases from Washington hatcheries. Therefore, a no effect determination from hydrogen peroxide released by hatcheries is warranted for the following species.

Eulachon: No effect

Bocaccio: No effect

Canary rockfish: No effect

Yelloweye rockfish: No effect

These no effect determinations would need to be revisited if hatcheries which discharge directly into estuarine or marine systems would begin to use hydrogen peroxide in their operations at some future date.

5.6 POTASSIUM PERMANGANATE

CAS ID: 7722-64-7

Chemical formula: $KMnO_4$

Synonyms / Trade names: Permanganate of potash, Cairox®, Chameleon mineral, Condy's crystals

Chemical composition: Potassium permanganate is a crystalline inorganic solid which decomposes (it does not actually melt) at 240° C. In its solid form, the crystals appear as a shiny, dark purple to black solid. Its solubility in water is approximately 64,000 mg/L. Concentrated aqueous solutions appear purple in color, while more dilute solutions appear light purple or pink. Its molecular weight is 158.034. Manganese within potassium permanganate is in its +7 valence state, which results in $KMnO_4$ being a strong oxidizing agent due to the reduction of the heptavalent manganese. Potassium permanganate solutions used at fish hatcheries contain between 1 - 10 mg/L $KMnO_4$ in water bath or flow through exposures, with an exposure duration of up to one hour (AFS 2011).

Hatchery use: Potassium permanganate is normally administered in a static bath to control external protozoan and metazoan parasites, and bacterial and fungal infections. Based on the permanganate demand of hatchery water, exposure concentrations range between 2 – 10 mg/L, applied in 2 mg/L increments until an effective concentration is found for the specific hatchery (Francis-Floyd and Klinger 2002). Exposure durations at hatcheries range between 30 – 60 minutes/day. Although fish are normally exposed to only a single $KMnO_4$ exposure, treatments can be safely repeated on multiple days. Potassium permanganate is currently in a deferred regulatory status according to the FDA, meaning that it not a low regulatory priority chemical, however the FDA has deferred regulatory action pending further study. EPA has registered potassium permanganate for use in fish hatcheries as a pesticide. No Washington hatcheries discharging to estuarine or marine waters report using potassium permanganate. Thus, if current use patterns continue, potassium permanganate should have no effect on any estuarine or marine threatened and endangered fish species. The only hatchery in Washington currently reporting use of potassium permanganate is the Keta Creek Fish Hatchery in Auburn, Washington, which discharges to freshwater systems.

According to the facility, Keta Creek Fish Hatchery's use of potassium permanganate is as follows:

Keta Creek Fish Hatchery administers potassium permanganate in a static bath at a target concentration of 2 ppm for one hour. After one hour, the flow to the container is resumed and the treated water is pumped to a holding pond, where it is slowly diluted with other water in the hatchery prior to being discharged to the receiving water. If multiple containers are treated in one day, they are treated one at a time. The decision when to use potassium permanganate is always based on the recommendation of the Northwest Indian Fisheries Commission fish pathologist. In order for the treatment to be effective, it needs to be administered to the sick fish in three consecutive days. In day one of the treatment, the recommended dose is 1 or 1.5 ppm potassium permanganate concentration; in day two 1.5 ppm and in day three 2.0 ppm. The exposure time is 60 minutes for each container.

Other notes regarding Keta Creek Fish Hatchery's potassium permanganate use:

- All treatments are applied using a static bath.
- Potassium permanganate was applied on 14 separate days between March and April of 2012 to fish at the hatchery. No more than two containers were treated in one day.
- The total daily amount used ranged from 3 to 155 grams, and the total (annual) amount used in 2012 was 701.7 grams potassium permanganate.
- The percent of estimated daily effluent flow (i.e., the percent of hatchery water treated with potassium permanganate) ranged from 0.08% to 6.70% during March and April of 2012, the period of peak use.
- According to Keta Creek Fish Hatchery, the maximum concentration of potassium permanganate in the effluent is 83.9 ppb. This is overly conservative because it assumes that the total amount used in one treatment is discharged instantaneously from the treated container into the hatchery effluent stream. Actually, Keta Creek Fish Hatchery's treated water is discharged to an abatement point, from which it is slowly released the next day (personal communication with Hugo Hernandez, 2015). This EEC does not account for degradation of the chemical over time.

Measures of Exposure:

The use of potassium permanganate in hatcheries is generally for the control of external protozoan, metazoan, bacterial and fungal infestations. Application is generally applied at a concentration between 1 – 10 mg/L to fish. The Keta Creek hatchery reports using $KMnO_4$ at a concentration of 2 mg/L.

The remainder of this measures of exposure assessment will evaluate two aspects that combined define the exposure of ESA listed species to potassium permanganate in the environment: its environmental fate once released into the environment, and its expected environmental concentration.

Environmental Fate of Potassium Permanganate

This section will describe the expected environmental fate of potassium permanganate.

Chemically, potassium permanganate is a strong oxidant. Permanganate is not desirable when added to hatchery water in stoichiometric excess, for several reasons. Excess permanganate left over after it has completed disinfection and oxidation of organic matter present in water is stable, and can remain in solution for months in the absence of contact with any additional organic matter. This stoichiometric excess has the potential over time to both continue oxidizing naturally occurring organic matter in hatchery water, and to elicit long term toxicity to fish. Excess permanganate also imparts an undesirable pink or purple color to water.

Oxidizing agents such as $KMnO_4$ release one or more reactive oxygen species (ROS), which are a group of free radical chemical species capable of existing while containing one or more unpaired electrons. The unpaired electron(s) alters the chemical reactivity of the molecule or atom, making it more reactive than the corresponding non-free radical form. The oxygen free radicals include superoxide anion free radical ($\cdot O_2^-$), peroxide free radical ($\cdot O_2^{-2}$), hydroxyl free radical ($\cdot OH$) and perhydroxyl free radical ($\cdot HO_2$). Of these, the hydroxyl free radical is the most reactive, and thus capable of causing the greatest damage to external surfaces of cells and viruses. Singlet oxygen ($^1O_2$) is not, strictly speaking, a free radical, but it is

an electrically excited state of molecular oxygen (O_2) that can also form during permanganate reduction, and is capable of irreversibly damaging cell membranes.

Free radicals and singlet oxygen irreversibly alter most biological macromolecules, including the proteins and lipids which constitute cell walls, cell membranes and viral envelopes. This irreversible alteration of the structure and function of biological macromolecules is responsible for the disinfecting properties of potassium permanganate, and is also why $KMnO_4$ acts as an external toxicant, not requiring uptake into the organism before eliciting toxicity.

Potassium permanganate undergoes violent combustion reactions with several classes of organic compounds, including alcohols, glycols and aldehydes (including formalin, which contains both formaldehyde and methanol).

The primary reactions of potassium permanganate in surface water include the following:

$$KMnO_4 + 2H_2O \leftrightarrow MnO_2 + KOH + 3OH^- \ (occurs\ between\ pH\ 3.5\ to\ 12)$$

$$2KMnO_4 \leftrightarrow K_2MnO_4 + MnO_2 + O_2 \ (photodecomposition)$$

$$2KMnO_4 + 2H_2O \leftrightarrow K_2MnO_4 + 2H^+ + \cdot OH \ (hydroxyl\ free\ radical\ production)$$

$$R + \cdot OH \leftrightarrow ROH \ (oxidation\ of\ organic\ matter\ (R)\ by\ hydroxyl\ free\ radicals)$$

A common reaction product of $KMnO_4$ is manganese dioxide (MnO_2), where manganese has been reduced from the +7 valence in $KMnO_4$ to the +4 valence in MnO_2. MnO_2 is a solid, common naturally occurring mineral, generally considered to be nontoxic, which in solution imparts a brown color to water. The above photodecomposition reaction, which forms potassium manganate (K_2MnO_4), MnO_2 and oxygen is the basis for the historical use of adding permanganate to water to increase its oxygen content.

Potassium permanganate has the ability to produce both hydroxyl ions (OH^-) and hydroxyl free radicals ($\cdot OH$) in surface water. Most organic matter, including cell membranes and viral envelopes, is quickly oxidized by the hydroxyl free radicals released during the transformation of potassium permanganate in surface water. This oxidation of organic matter with hydroxyl free radicals is the primary mechanism of toxic action by which $KMnO_4$ serves as a disinfectant.

Although aqueous potassium permanganate solutions are stable in the absence of light and organic matter, they are very reactive when organic matter is present. In the literature, reaction rates and half lives of permanganate reactions with organics are usually expressed in terms of concentrations of the organic compounds being reduced, not the concentration of $KMnO_4$ *per se*. But assuming stoichiometrically equivalent concentrations of organic matter and $KMnO_4$, the half-life of organic matter should be equivalent to the half-life of potassium permanganate.

Marking and Bills (1975) in their study of $KMnO_4$ toxicity to several fish species, also measured the ability of $KMnO_4$ to inactivate the piscicide antimycin. The half-life of the reaction using 1 mg/L $KMnO_4$ ranged between 7 – 11 minutes, depending on the pH of the water. Potassium permanganate oxidized a series

of six chlorophenol compounds with half-lives ranging between 0.41 – 8.25 minutes (Hossain and McLaughlan 2013). With the exception of perchloroethylene (PCE), Huang et al. (2001) observed that potassium permanganate was able to oxidize a series of chlorinated ethylene compounds in half-lives of between 0.13 – 13.5 minutes. PCE was oxidized by permanganate with a half-life between 145 – 350 minutes (Huang et al. 2001). Naturally occurring humic acids in river water are readily oxidized by potassium permanganate (Xia et al. 2005). For many organic compounds in water, the half-life of potassium permanganate used to oxidize the organics would appear to be on the order of minutes. These short half-lives would appear to indicate that any potassium permanganate used in stoichiometric excess of hatchery needs would be quickly reduced to non-oxidizing compounds of low toxicity if released to the environment.

The long term stability of potassium permanganate in aqueous solutions in the absence of organic matter and light leads to a potential for some level of manganese bioaccumulation in aquatic species. This stability is unusual among the chemical oxidants and disinfectants used at hatcheries. Of the hatchery chemicals evaluated in this Biological Evaluation, potassium permanganate is the only one whose chemical structure includes a transition element metal potentially available to be bioaccumulated by aquatic species. A U.S. Fish and Wildlife Service white paper on potassium permanganate use in aquaculture (MacMillan 2009) identified studies where manganese uptake by fish was evaluated during exposure to $KMnO_4$. Studies with both channel catfish and rainbow trout observed no difference in manganese tissue concentrations between Mn-exposed and Mn-unexposed fish.

Expected Environmental Concentration (EEC) of Potassium Permanganate

The desired treatment concentration of potassium permanganate at the Keta Creek hatchery is 2 mg/L. As stated above, the facility calculates the maximum concentration of potassium permanganate in the effluent to be 83.9 ppb. This is overly conservative because it assumes that the total amount used in one treatment is discharged instantaneously from the treated container into the hatchery effluent stream. It does not take into account the rate at which treated water is slowly released from the holding pond. Additionally, this end of pipe concentration is a conservative estimate of the potassium permanganate concentration in receiving waters because it does not account for any dilution of hatchery discharges by the receiving body of water. Finally, the end of pipe concentration estimate is conservative because it does not take into account the reduction of manganese from the +7 to the less toxic +4 valence state.

The EEC concentration of 83.9 ppb will be compared to the chronic NOEC estimates calculated in the Measures of Effect section. This comparison will take place in the Risk Characterization section to estimate ecological risks to threatened and endangered species exposed to potassium permanganate discharges from hatcheries in Washington.

Measures of Effect:

For fully aquatic species, the available toxicity data was identified from a search in EPA's ECOTOX database (http://cfpub.epa.gov/ecotox/).

A combined total of 278 freshwater toxicity records were identified from the above search. These results are presented in Appendix K. As no Washington hatcheries discharging to marine or estuarine systems currently use potassium permanganate, no search was made for toxicity data to marine species. Of the freshwater records, only 12 report results for animals exposed to potassium permanganate under flow through conditions: 1 record for bluegill, 2 records for channel catfish, 3 records for the Asiatic clam *Corbicula manilensis*, and 6 records for the zebra mussel. The bluegill study and one of the *Corbicula manilensis* studies are of suitable quality for use in deriving water quality criteria. The zebra mussel studies are of chronic duration (up to 52 days exposure), but were focused on controlling, eliminating or preventing recolonization of zebra mussel accretions on intake pipes for potable water treatment systems. As such, the zebra mussel studies can be considered to represent lethal concentrations under chronic, long term exposures.

The remaining available toxicity data for aquatic species was performed under static, static renewal or pulsed exposures. Taxa for which potassium permanganate toxicity data are available that does not meet EPA requirements for use in deriving water quality criteria are as follows:

- Freshwater algae: 4 species
- Freshwater macrophytes: None
- Aquatic insects: 1 species
- Freshwater crustaceans: 11 species
- Freshwater zooplankton: 6 species
- Freshwater molluscs: 3 species
- Other freshwater invertebrate taxa (e.g. oligochaetes): 8 species
- Amphibians: None
- Freshwater fish: 21 species

As noted in the previous paragraph, the potassium permanganate toxicity to zebra mussel studies were focused on the use of controlling or eliminating their accretion on pipes or other structures. This type of study with $KMnO_4$ has not been limited to zebra mussels. Most of the potassium permanganate toxicity information on species other than fish has been limited to evaluation of its use as a biocide, where often the only reported endpoint is either a concentration or exposure duration required to elicit 100% mortality of the test species. Fish toxicity studies with potassium permanganate have been limited to evaluating its effects from exposure to its therapeutic dose. The fish studies have either extended the exposure of fish to the therapeutic dose from one hour to 96 hours (the standard test duration for acute lethality studies), or have exposed the fish either to the therapeutic dose or some multiple of the therapeutic dose for one hour, then placed the fish in clean water and monitored any residual toxicity that occurs once the fish is removed from the $KMnO_4$ solution. None of these types of toxicity studies readily lend themselves to evaluation of potassium permanganate toxicity to threatened and endangered species or their prey using the methodologies presented in the Problem Formulation section of this Biological Evaluation. This is because the tests are performed using non-standard test methodologies, report non-standard test endpoints, or both.

Some information is available indicating that the difference in lethal concentrations of $KMnO_4$ and no effect concentrations of $KMnO_4$ is small. This situation generally occurs for chemicals with steep dose-response curves, meaning the difference between adverse and no adverse effect concentrations for a given species may be small. Steep dose-response curves for chemicals acting as oxidants have been empirically identified for fish species (Tsai et al. 1990).

Of the available potassium permanganate toxicity studies, the single study that demonstrates the range of concentrations between lethal and no effect for the same species is that of Hobbs et al. (2006). Hobbs et al. (2006) measured 96 hour LC_{50} values of potassium permanganate for five species: *Daphnia magna*, *Ceriodaphnia dubia*, fathead minnow (*Pimephales promelas*), *Chironomus dilutus* and *Hyalella azteca*. *Daphnia* and *Ceriodaphnia*, as will be discussed in the toxicity to prey species of threatened and endangered species, are both crustacean zooplankton species, the group of species that appears to be more sensitive to potassium permanganate than any other taxonomic grouping of animals. Unlike nearly all other available $KMnO_4$ toxicity studies, the static renewal *Daphnia*, *Ceriodaphnia* and fathead minnow exposures were for a full 96 hours, with renewal of exposure media 48 hours into the test. The *Chironomus* and *Hyalella* tests were static exposures, but the exposure was for the full 96 hours, not a dip into $KMnO_4$ solutions for one hour followed by transfer into clean water. Hobbs et al. (2006) also measured the residual permanganate ion concentration at both test initiation and at the end of the toxicity tests, unlike nearly all other permanganate toxicity tests. This allowed them to evaluate the degradation of permanganate in test solutions during the test. Finally, Hobbs et al. (2006) exposed their species to two types of dilution water: a synthetic laboratory water with low organic carbon content, and an aquaculture pond water with high (34.81 mg/L) organic carbon content. This design feature of their study allowed them to evaluate the reduction in permanganate ion concentration over time in a water that more closely reflected the types of water in hatcheries to which potassium permanganate is added (i.e. a water with a higher level of organic matter which could be oxidized by permanganate relatively to synthetic laboratory dilution water).

The steepness of the dose-response curve for potassium permanganate, as expressed by the difference between the 96 hour LC_{50} values and the 96 hour NOEC observed by Hobbs et al. (2006), as well as the degradation of $KMnO_4$ in the presence and absence of organic matter is summarized in Tables PP-synthetic and PP-pond. These two tables, which combine information from four separate tables in Hobbs et al. (2006), demonstrate both the narrow range between lethal and no effect concentrations of permanganate, and the effects of organic matter on how long permanganate ion remains in solution.

Table PP-synthetic. Response of five species to potassium permanganate in synthetic laboratory water, and the effects of synthetic laboratory water on $KMnO_4$ concentrations throughout the duration of the exposure (Hobbs et al. 2006).

Species	96-h LC_{50} mg/L	NOEC mg/L	LC_{50}:NOEC	LOEC mg/L	% residual $KMnO_4$ at test completion
Daphnia magna	0.053	0.049	1.08	0.071	Not measured
Ceriodaphnia dubia	0.058	0.047	1.23	0.068	86.5 – 107.8

Pimephales promelas	2.13	1.36	1.57	1.68	95.6 – 101.6
Chironomus dilutus	4.43	<3.20	1.38	3.20	103.0 – 104.7
Hyalella azteca	4.74	3.56	1.33	4.31	95.2 – 108.9

Table PP-pond. Response of five species to potassium permanganate in aquaculture pond water, and the effects of pond water on $KMnO_4$ concentrations throughout the duration of the exposure (Hobbs et al. 2006).

Species	96-h LC_{50} mg/L	NOEC mg/L	LC_{50}:NOEC	LOEC mg/L	% residual $KMnO_4$ at test completion
Daphnia magna	1.98	1.75	1.13	2.50	Not measured
Ceriodaphnia dubia	2.39	2.25	1.06	3.22	5.5 – 17.4
Pimephales promelas	11.28	9.45	1.19	13.50	25.5 – 50.6
Chironomus dilutus	13.55	9.45	1.43	13.50	24.3 – 65.0
Hyalella azteca	12.30	7.83	1.57	11.18	23.1 – 65.0

Several observations are immediately apparent from Tables PP-synthetic and PP-pond. The small concentration range between the NOECs and LC_{50}s is obvious, particularly for the two crustacean zooplankton species *Daphnia* and *Ceriodaphnia*. In synthetic laboratory dilution water, the difference between the NOEC and LC_{50} is 0.004 mg/L and 0.011 mg/L for *Daphnia* and *Ceriodaphnia*, respectively. These differences are equal to one standard deviation in the analytical chemistry quantification of $KMnO_4$ in solution in the Hobbs et al. (2006) study, meaning that the LC_{50} and NOEC values are statistically indistinguishable from each other. Further confirmation of this point is provided by the LOEC values for *Daphnia* and *Ceriodaphnia*, which, in both the synthetic laboratory and aquaculture pond waters, the LOECs are higher than the 96-h LC_{50} values. The ratio between the 96-h LC_{50} and NOEC concentrations in Tables PP-synthetic and PP-pond is the calculation of the LC_{LOW} considered to be the acute NOEC as described in the Problem Formulation. The low values of these ratios, all of which are lower than the national default ratio of 2.27 used to convert a LC_{50} to a LC_{LOW} provides evidence that the use of the 2.27 value to convert an acute LC_{50} to a LC_{LOW} is protective of aquatic species.

Tables PP-synthetic and PP-pond also demonstrate the effect of organic matter on the retention of potassium permanganate in solution. For the tests performed in synthetic laboratory dilution water with low organic carbon content, the concentration of potassium permanganate remaining in solution at test termination is close to 100% of the intended concentration at test initiation. However, for tests performed in aquaculture pond water with elevated total organic carbon concentrations, the amount of potassium permanganate remaining in solution at test termination is as low as 5.5% of the intended concentration at test initiation, and at no time exceeded 65% of the original $KMnO_4$ concentration. Hobbs et al. (2006) also observed that the largest percent reduction in potassium permanganate concentrations occurred in solutions with the lowest added amount of $KMnO_4$, while higher nominal concentrations contained a higher percentage of $KMnO_4$ remaining in solution at test termination. This trend is due to potassium permanganate being added in stoichiometric excess relative to the amount of organic matter

in the water at the higher exposure concentrations of $KMnO_4$. This observation, if extended to waters receiving discharges from hatcheries that dilute the hatchery added concentration of $KMnO_4$, leads to a conclusion that permanganate discharges from hatcheries would be reduced in concentration in receiving waters due to $KMnO_4$ oxidizing humic acids and other organic compounds in receiving waters.

Of the available toxicity data, some information on a threatened and endangered species under evaluation in this Biological Evaluation is for rainbow trout (steelhead), Chinook salmon and coho salmon. We have used the available 96 hour LC_{50} data under static exposure conditions for rainbow trout, coho salmon and Chinook salmon from Taylor and Glenn (2008) to estimate the toxicity of potassium permanganate to the remaining ESA listed salmonid species in Washington. We have used the methodologies described under the problem formulation section of this Biological Evaluation, specifically using ICE models. We have done this even though the rainbow trout, coho salmon and Chinook salmon 96 hour LC_{50} studies were performed under static exposure conditions, not flow through conditions. Flow through conditions are particularly important for maintaining the desired exposure concentrations of chemicals such as potassium permanganate whose concentrations are quickly reduced when organic matter is present in water, the norm under the environmental conditions in surface waters where threatened and endangered species are found. Exposing organisms to reactive oxidant chemicals such as $KMnO_4$ under flow through conditions provides a greater likelihood that the exposure concentrations are as intended throughout the study, relative to the chemical degradation and subsequent reduction in exposure concentration that occurs over time during static or static renewal exposure conditions.

Toxicity of Potassium Permanganate

The only toxicity study with fish meeting EPA requirements for use in developing aquatic life criteria available for potassium permanganate is a flow through exposure study with bluegill (*Lepomis macrochirus*) (EPA and OPP 2013), which found a 96 hour LC_{50} range of 2300 - 3600 µg/L in a series of three separate toxicity tests. A second fish toxicity study under flow through conditions (Darwish et al. 2002) with channel catfish (*Ictalurus punctatus*) exposed the fish to $KMnO_4$ for only 36 hours, not the required 96 hour exposure needed for inclusion in a dataset useable by EPA to derive acute water quality criteria. Darwish et al. (2002) found 438 µg/L $KMnO_4$ to be a no effect concentration after 36 hours, while 1315 µg/L and 2190 µg/L $KMnO_4$ resulted in 9.4% and 49.6% mortality, respectively, to channel catfish after a 36 hour exposure. The 36 hour exposure duration used by Darwish et al. (2002) is substantially longer than the one hour exposure duration for therapeutic use currently recommended by AFS (2011).

All other fish toxicity data for $KMnO_4$ was performed under static, static renewal or pulsed exposures. Of the available data, the most useful in evaluating potential potassium permanganate toxicity to threatened and endangered species in receiving waters is a series of 96 hour LC_{50} studies performed under static exposure conditions on two size classes of rainbow trout, coho salmon and Chinook salmon (Taylor and Glenn 2008). The Taylor and Glenn (2008) toxicity tests were performed at the Abernathy Fish Technology Center of the U.S. Fish and Wildlife Service (Longview, WA) using fish stocks native to Washington (rainbow trout, Chinook salmon) or Oregon (coho salmon), and are part of the same study whose results were used to evaluate toxicity of hydrogen peroxide to threatened and endangered salmonid species in this Biological Evaluation.

Taylor and Glenn (2008) exposed two different size classes of fish to potassium permanganate. Their 'small' group of fish had a target body weight of 2 grams, while their 'large' group of fish had a target body weight of 10 grams. Fish were exposed to a potassium permanganate bath of various concentrations for one hour, then placed in clean water for an additional 120 hours to identify any residual mortality response from exposure to the $KMnO_4$ bath. Concentrations of $KMnO_4$ used in the one hour bath were 3, 5, 10, 20, 30, 40, and 50 mg/L. The 96 hour LC_{50} values for rainbow trout, coho salmon and Chinook salmon from Taylor and Glenn (2008) were calculated from a logistic response function. The equation used was that given below.

$$Y_i = \frac{e^{(\beta_0 + \beta_1 x_j)}}{1 + e^{(\beta_0 + \beta_1 x_j)}}$$

Where: Y_i = mortality probability (= 0.50)

β_0 = logistic regression intercept

β_1 = logistic regression slope

x_j = chemical concentration (mg/L)

Calculated 96 hour LC_{50} values are given in Table PP-3. Taylor and Glenn (2008) did not report confidence intervals around their LC_{50} values.

Table PP-3. Empirical 96 hour potassium permanganate LC_{50} values for three salmonid species as reported by Taylor and Glenn (2008).

Species	Size Class	96-hour LC_{50} (mg/L)
Rainbow trout	2 gram body weight	23
Rainbow trout	10 gram body weight	34
Chinook salmon	2 gram body weight	33
Chinook salmon	10 gram body weight	27
Coho salmon	2 gram body weight	43
Coho salmon	10 gram body weight	10

No empirical chronic toxicity data with potassium permanganate are available for rainbow trout, Chinook salmon or coho salmon. Therefore, the procedures given in the Problem Formulation are used to convert the empirical 96 hour LC_{50} values in Table PP-3 to chronic NOEC concentrations. This calculation involves dividing the lower of the two available LC_{50} values for each of the salmonid species in Table PP-3 first by a factor of 2.27 to convert the LC_{50} to an LC_{LOW}, which is considered an acute NOEC. The acute NOEC is then divided by the default national acute-chronic ratio of 8.3 to calculate the chronic NOEC concentrations for rainbow trout, Chinook salmon and coho salmon. The factor of 2.27 is used in this instance to account for the toxicity testing methodology employed by Taylor and Glenn (2008), who exposed their fish for one hour to $KMnO_4$, then placed the fish in clean water for the remainder of the 96 hour study. Most toxicity tests expose organisms to a chemical for the full 96 hours, not one out of 96 hours, thus the need for an

additional factor to ensure a conservative, protective estimate of the chronic NOEC. These calculated chronic NOEC values are presented in Table PP-4.

Output of all ICE models run with potassium permanganate for the three remaining threatened and endangered species (bull trout, chum salmon and sockeye salmon), genera or family with available data in ICE is shown in Appendix L. Using the ICE model selection guidelines set forth in the problem formulation, models used to estimate chronic NOEC's for salmonid species are highlighted in green and bolded in Table PP-1

A genus level ICE model using the empirical coho salmon LC_{50} data (10 mg/L $KMnO_4$, equivalent to 10,000 µg/L, Appendix L) was used as the starting point to derive chronic NOEC values for bull trout, chum salmon and sockeye salmon. The genus level ICE model using empirical Chinook salmon toxicity data as input could not be used to estimate toxicity to bull trout because the empirical toxicity data was outside of the useable range of the ICE regression between Chinook salmon and bull trout. The genus level ICE model between coho salmon and bull trout, chum and sockeye salmon was selected from the remaining ICE models because of the large number of data pairs in the regression, taxonomic closeness to the modeled species relative to family level ICE models, and high r^2 and cross-validation scores.

The remaining ICE models, with poorer predictive ability and which were not selected as the source of chronic NOEC's are shown in red in Appendix L. As described in the problem formulation, the lower 95% confidence interval of the predicted chronic NOEC, if available, is used as the chronic NOEC in this Biological Evaluation. All ICE models used for potassium permanganate generated lower 95% confidence intervals of the chronic NOEC, and are shown in this section.

No information is available in ICE for eulachon or any of the threatened and endangered rockfish species, genera or families in Washington (bocaccio, canary rockfish, yelloweye rockfish). Therefore, potassium permanganate effects on eulachon and the rockfish species cannot be quantitatively evaluated, and must be considered as a toxicological uncertainty in this Biological Evaluation. However, as the Keta Creek Fish Hatchery discharges into freshwater (Crisp Creek, a tributary of the Green River), not estuarine or marine water, it is unlikely that potassium permanganate discharges from Keta Creek hatchery would impact saltwater species such as eulachon or rockfish.

The final selected chronic NOEC values for bull trout, Chinook salmon, chum salmon, coho salmon, sockeye salmon and steelhead that were compared to the expected environmental concentration of potassium permanganate in receiving water environments are summarized in Table PP-4.

Table PP-4. Chronic no effect concentrations (NOEC) for threatened and endangered salmonid species exposed to potassium permanganate.

Species	Chronic NOEC (mg/L)	Source of chronic NOEC
Bull trout	0.440	ICE model – genus level
Chinook salmon	1.43	Empirical acute data (Taylor and Glenn 2008)
Chum salmon	0.798	ICE model – genus level
Coho salmon	0.531	Empirical acute data (Taylor and Glenn 2008)

Sockeye salmon	0.798	ICE model – genus level
Steelhead	1.22	Empirical acute data (Taylor and Glenn 2008)

Risk Characterization: Potassium Permanganate

Risks to Threatened and Endangered Fish Species from Potassium Permanganate

Risks to threatened and endangered fish species for which toxic concentrations of potassium permanganate can be identified from the literature are calculated using a standard ecological risk assessment hazard quotient approach. In the hazard quotient approach, the estimated environmental concentration is divided by the chronic NOEC for each threatened and endangered species to calculate a hazard quotient. Hazard quotients less than 1.0 are indicative of acceptable levels of ecological risk. In the context of this Biological Evaluation, an acceptable ecological risk is represented as an EEC which, if not exceeded, results in no discernable effect on the survival, reproduction and growth of a threatened and endangered species. Note that acceptable chronic NOEC values vary between species. Hazard quotients greater than or equal to 1.0 are indicative of a potential for unacceptable ecological risks to threatened and endangered species.

Hazard quotients for the six threatened and endangered salmonid species for which toxicity data is available or could be estimated are presented in Table PP-5. Hazard quotients were calculated using the EEC generated from the lowest and highest daily discharge from the Keta Creek Fish Hatchery, which results in the largest EEC range to which threatened and endangered species could be exposed.

Table PP-5. Hazard quotients (HQ) for threatened and endangered species exposed to the range of expected environmental concentrations (EEC) of potassium permanganate discharged by hatcheries.

Species	EEC range (µg/L)	Chronic NOEC (µg/L)	Hazard quotient range
Bull trout	83.9	440	0.191
Chinook salmon	83.9	1430	0.0587
Chum salmon	83.9	611	0.137
Coho salmon	83.9	531	0.158
Sockeye salmon	83.9	611	0.137
Steelhead	83.9	1220	0.0688

All hazard quotients in Table PP-5 are substantially lower than 1.0, indicative of acceptable levels of ecological risk to the species under all Keta Creek Fish Hatchery discharge scenarios. Note that the EEC values do not take into account the rapid degradation of environmental concentrations of potassium permanganate when it comes into contact with organic matter in surface waters. This is discussed more fully in the uncertainty analysis portion of risk characterization, as it is likely the major uncertainty in this Biological Evaluation, which overestimates potential ecological risks to threatened and endangered species.

Risks to Potential Freshwater Prey of Threatened and Endangered Species from Potassium Permanganate

Although not of a data quality useful for deriving EPA water quality criteria, a fairly substantial number of species have some potassium permanganate toxicity data available for them (Appendix K). The only toxicity studies with potassium permanganate that may be of a suitable quality for use in EPA water quality criteria derivation, in addition to the bluegill study discussed earlier ((EPA and OPP 2013) are several flow through exposure studies with molluscs. Included among the mollusc studies are several chronic duration (up to 56 days of exposure) survival studies (Klerks and Fraleigh 1991) with zebra mussel (*Dreissena polymorpha*).

Klerks and Fraleigh (1991) determined LT_{50} values (LT_{50} is the length of exposure time needed to kill 50% of test organisms) for zebra mussel to be 10.7, 49.8 and 56 days at $KMnO_4$ exposure concentrations of 1250, 530 and 240 µg/L, respectively. A second series of flow through exposures of zebra mussels to $KMnO_4$ by Klerks et al. (1993) reported that a 14 day exposure to 275 µg/L $KMnO_4$ resulted in 17% mortality.

There are also two flow through studies evaluating the effects of potassium permanganate on survival of Asiatic clam (*Corbicula manilensis*). Chandler and Marking (1979) reported a 96 hour LC_{50} for $KMnO_4$ of 112,000 µg/L (95% confidence interval = 101,000 – 125,000 µg/L). Chandler and Marking (1979) also performed a 96-h static LC_{50} test on *Corbicula manilensis* and observed similar results to their flow through results (96 hour LC_{50} of 118,000 µg/L with 95% confidence limits of 103,000 to 136,000 µg/L. They speculated that the ability of the clams to close their valves during the permanganate exposure may be responsible for both the elevated LC_{50}s and the similarity in the static and flow through LC_{50} concentrations. Cameron et al. (1989) observed that a concentration of 1080 µg/L was a 7.9 day LT_{50} for *Corbicula manilensis* under flow through conditions.

The results of the Hobbs et al. (2006) study of potassium permanganate on *Daphnia magn*a, *Ceriodaphnia dubia*, *Chironomus dilutus* and *Hyalella azteca* have been discussed at length in the introduction to the Measure of Effects section, and will not be repeated here, with the exception of how chronic NOEC concentrations were calculated. Unlike all other available crustacean potassium permanganate toxicity studies, the empirical NOEC is reported for the species studied by Hobbs et al. (2006). Therefore, we concluded that the similarity between the 96-h LC50s and 96-h NOECs from the Hobbs et al. (2006) study provides evidence that dividing the LC50 by the default average ACR (8.0) results in a protective estimate of the chronic NOEC. For *Daphnia* and *Ceriodaphnia*, the two most sensitive crustacean zooplankton species, this calculation resulted in chronic NOEC values of 5.9 µg/L and 5.7 µg/L, respectively.

Most other invertebrate toxicity studies report either LC_{100} values, or LT_{100} values. In the absence of any specific guidance on how to convert such endpoints into chronic NOEC values, we have divided the LC_{100} and LT_{100} values by 2.27, then divided that quotient by 8.3 to obtain chronic NOEC values. The uncertainty in chronic NOEC estimates from this assumption is discussed in the decision rule portion of the Uncertainty Analysis.

Despite the lack of studies of a quality that could be used to develop EPA water quality criteria, we have used the procedures outlined in the Problem Formulation (i.e. divide the acute toxicity value by 2.27 if the exposure duration is shorter than 96 hours for fish or 48 hours for an invertebrate to obtain an LC_{LOW}, or using the empirical acute LC_{50} if the test duration was 96 hours for fish or 48 hours for invertebrates, then dividing the LC_{LOW} or acute toxicity LC_{50} value by a default acute-chronic ratio of 8.3 to obtain a chronic NOEC) to estimate chronic NOEC concentrations for prey of threatened and endangered fish species. Chronic NOEC concentrations of potassium permanganate to prey of threatened and endangered species is summarized in Table PP-6.

Table PP-6. Toxicity of Potassium Permanganate to Freshwater Prey of Threatened and Endangered Listed Species.

Organism Type	Chronic NOEC range (µg/L)
Algae	53 - 1698
Aquatic macrophytes	No data
Aquatic invertebrates	3.6 - 5361
Aquatic insects	534
Crustaceans	5.7 - 531
Zooplankton	5.7 - 422
Molluscs	106 - 5361
Others (e.g. oligochaetes, etc.)	3.6 - 955
Amphibians	No data
Fish	41.9 - 1446

Among the taxonomic groups in Table PP-6, crustacean zooplankton appear to be the most sensitive group, with five of the six available toxicity results having chronic NOEC concentrations of 150 µg/L or lower. The most sensitive freshwater species to potassium permanganate, however, appears to be the oligochaete *Branchiura sowerbyi*, with a four day empirical LC_{50} of 30 µg/L under static exposure conditions (Das and Kaviraj 1994) translating to a chronic NOEC of 3.6 µg/L $KMnO_4$. This oligochaete, along with the zooplankton species *Daphnia magna* and *Ceriodaphnia dubia* are the only prey species whose chronic NOEC is lower than any of the EEC values from the Keta Creek Fish Hatchery.

Fish species appear to have an intermediate range of sensitivities to potassium permanganate among the taxa for which empirical toxicity information is available. Algae and molluscs appear to have the widest range of sensitivities to permanganate among taxonomic groups, while the remaining invertebrate species have a narrower range of sensitivities. The most sensitive freshwater fish appears to be the striped bass (*Morone saxatilis*), with a calculated chronic NOEC of 41.9 µg/L. The most tolerant fish species is western mosquitofish (*Gambusia affinis*), with a chronic NOEC of 1446 µg/L.

Only three potential prey species, the crustacean zooplankters *Daphnia magna* and *Ceriodaphnia dubia*, and the oligochaete *Branchiura sowerbyi* has a chronic NOEC lower than any of the EEC values from Keta Creek Fish Hatchery. As all other prey species chronic NOECs are higher than the highest EEC for potassium permanganate, we conclude that the weight of evidence for all potential prey species indicates

that potassium permanganate is not likely to adversely affect prey species of threatened and endangered fish species in Washington.

Uncertainty Analysis of Potassium Permanganate Risk Characterization

All four types of uncertainty (variation, model uncertainty, decision rule uncertainty and true unknowns) described in the problem formulation are present in this potassium permanganate evaluation. By far the largest uncertainty in this evaluation is the complete absence of toxicity data in the literature that would permit a quantitative evaluation of risks to threatened and endangered rockfish species from potassium permanganate use at fish hatcheries. This type of uncertainty is a true unknown in this Biological Evaluation. However, as the only Washington hatchery currently using potassium permanganate discharges to a freshwater stream, not to a marine or estuarine system, eulachon and rockfish species are not currently exposed to any potassium permanganate releases from Washington hatcheries.

Variation of expected environmental concentrations in hatchery discharges and receiving waters is also a large source of uncertainty in this analysis. Variation also is expressed in the confidence limits surrounding statistically reduced expressions of the empirical toxicity data (e.g. LC_{50}, EC_{50}, etc.). Confidence limits describe random variation around the central tendency response of laboratory organisms exposed to chemicals in toxicity tests. This is an uncertainty regarding the true concentration of $KMnO_4$ that elicits a toxic response in aquatic species.

The environmental degradation rates and short half-life of potassium permanganate in aquatic systems containing organic matter also introduce variation in exposure concentrations and EECs over time. Variation in potassium permanganate concentrations due to its environmental degradation is a unidirectional process, with the environmental concentration constantly declining. Without consideration of the reduction of $KMnO_4$ in surface water to MnO_2, the EEC values used to describe exposure of threatened and endangered species to $KMnO_4$ overestimate the concentrations threatened and endangered species are actually exposed to in the environment. Not attempting to estimate the effect on potassium permanganate EECs of dilution of hatchery discharges by receiving waters also serves to overestimate the actual EEC to which threatened and endangered species are exposed. Although we have estimated EECs without the application of half-lives of $KMnO_4$ presented in this Biological Evaluation, given the already low hazard quotients calculated from our EECs, we have chosen not to modify our EECs by inclusion of a degradation rate term.

EPA normally requires fish toxicity studies used to derive water quality criteria to be performed under flow through conditions. Unfortunately, all but one (EPA and OPP 2013) of the available fish studies of potassium permanganate toxicity were performed under static, static renewal or pulsed exposure conditions. Unless chemical concentrations are frequently monitored, uncertainties exist in the chemical concentrations to which test organisms are exposed in static, static renewal or pulsed exposures. This is particularly true for chemicals having the potential to degrade rapidly, which is the case for potassium permanganate coming into contact with organic matter. This uncertainty can be quantitatively evaluated to an extent by comparing the potassium permanganate 96 hour LC_{50} values for bluegills exposed in flow through (EPA and OPP 2013) and static (Marking and Bills 1975) exposures. The range of the three

individual flow through LC_{50} values (2300 – 3600 µg/L) from EPA and OPP (2013) and the single LC_{50} from Marking and Bills (1975) of 2380 µg/L overlap. This overlap in the LC_{50}s obtained under flow through and static exposure conditions provides some level of confidence that static LC_{50}s for $KMnO_4$ may not substantially underestimate the toxicity of potassium permanganate.

Decision rule uncertainty came into play during the evaluation of potassium permanganate risks. This is because nearly all of the toxicity studies were not performed using standardized test protocols. For acute toxicity, standard protocols call for exposing an animal to the test chemical for either 48 hours (invertebrates) or 96 hours (fish). Many permanganate studies exposed organisms to a permanganate bath for a short time period (one hour or less), then transferred the animals into clean water for the duration of the test. While this exposure scenario mimics the use of potassium permanganate at hatcheries, the problem formulation methodology was not designed to evaluate toxicity from this type of non-standard exposure. Therefore, we modified the usual chronic NOEC estimation procedure to account for this type of non-standard exposure scenario. Other permanganate toxicity studies expressed results in terms of the length of time required to kill 100% of test organisms. While an appropriate experimental design for a chemical whose intended use is as a biocide, measurement of a LT_{100} is also a non-standard toxicity test procedure, at least during the derivation of EPA water quality criteria, and thus required a modification to the decision rules on how to convert 48 or 96-h LC_{50} values to chronic NOECs.

Model uncertainty in the ICE models is described by the percent cross-validation success statistic. According to Raimondo et al. (2013), the percent cross-validation success rate for each model is the proportion of data points that are predicted within 5-fold of the actual LC_{50} value. There is a strong relationship between taxonomic distance and cross-validation success rate, with uncertainty generally, although not always increasing with larger taxonomic distance. Maximizing the value of the cross-validation statistic was a primary determinant of which of multiple ICE models were used to estimate toxicity values in this Biological Evaluation for species where no empirical toxicity data exists for a chemical-species pair.

Effect Determinations of Potassium Permanganate on Threatened and Endangered Species

Based on all chronic NOEC concentrations for six threatened and endangered salmonid species being substantially higher than the estimated environmental concentrations of potassium permanganate released from hatcheries, EPA has made the following effect determinations for potassium permanganate:

Bull trout: Not likely to adversely affect

Chinook salmon: Not likely to adversely affect

Chum salmon: Not likely to adversely affect

Coho salmon: Not likely to adversely affect

Sockeye salmon: Not likely to adversely affect

Steelhead: Not likely to adversely affect

The above determinations are all based on the estimated environmental concentrations from hatchery releases being substantially lower than the chronic NOECs for the above six species.

Based on the lack of current discharges from any Washington hatchery directly into estuarine or marine waters, the following species are not exposed to potassium permanganate releases from Washington hatcheries. Therefore, a no effect determination from potassium permanganate released by hatcheries is warranted for the following species.

Eulachon: No effect

Bocaccio: No effect

Canary rockfish: No effect

Yelloweye rockfish: No effect

These no effect determinations would need to be revisited if hatcheries which discharge directly into estuarine or marine systems would begin to use potassium permanganate in their operations at some future date.

5.7 POVIDONE-IODINE (PVP-I)

CAS ID: 25655-41-8 (Povidone-iodine); 9003-39-8 (Povidone); 88-12-0 (1-ethenyl-2-pyrrolidone, 1-vinyl-2-pyrrolidone); 7553-56-2 (Iodine)

Chemical formula: $(C_6H_9I_2NO)_n \cdot I_x$

Synonyms / Trade names: Polyvinylpyrrolidone-iodine, PVP-I, Argentyne, Ovadine, Iodophor, Betadine

Chemical composition: Povidone-iodine is a complex consisting of a synthetic organic polymer (povidone, also called polyvinylpyrrolidone or PVP) that serves to disperse elemental iodine into water. Povidone polymer (EPA 2006) can have molecular weights ranging between 10,000 – 1,000,000 amu (atomic mass units). The monomer used in the synthesis of povidone polymer is 1-ethenyl-2-pyrrolidone, chemical formula C_6H_9NO. Commercial povidone-iodine solutions used at fish hatcheries contain 10% dry weight iodine (range 9 – 12%) dissolved in water, in which the povidone-iodine complex is freely soluble. Povidone-iodine itself is a white solid at room temperature. Commercially available products may contain a small amount of pH buffering material, because the release of elemental iodine can result in acidification of water, as described in the environmental fate section for povidone-iodine.

Hatchery use: Primary use is as a bath treatment to disinfect fish eggs prior to hatch. The commercially available 10% povidone-iodine solution is diluted before use in disinfecting fish eggs. The diluted solution to which fish eggs are exposed contains 50 – 100 mg/L iodine. Povidone-iodine is effective against many bacterial, fungal and viral infections. A secondary, less common use is to disinfect boots and other small pieces of equipment.

Measures of Exposure:

Povidone-iodine (PVP-I) is classified as a low regulatory priority aquaculture drug by the FDA (2011). It is not used in the sections of hatchery facilities containing larval, juvenile or adult fish that discharge to surface waters. With respect to fish, PVP-I is only used to treat fish eggs during or after water hardening of the eggs, after which the solution is discarded, generally to land treatment. Exposure durations of eggs to PVP-I are short, on the order of 10 minutes (AFS 2011). Although the AFS (2011) recommended exposure duration and exposure concentration are 10 minutes at 100 mg/L available iodine for disinfection of fish eggs, the actual exposure durations and concentrations to which fish eggs are exposed, as well as disposal practices vary somewhat among Washington hatcheries.

As the use and disposal practices of PVP-I when used to disinfect small pieces of equipment or gear may also vary among hatcheries, the potential amount of PVP-I discharge from this use is unknown but likely low. Given that the conceptual site model (Figure 4) for this evaluation considers all chemicals used in baths have at least a potential to be released to surface waters, EPA has chosen to evaluate the potential for risks to ESA listed species in surface waters from exposure to PVP-I.

The remainder of this measures of exposure assessment will evaluate two aspects that combined define the exposure of ESA listed species to PVP-I in the environment: its environmental fate once released into the environment, and its expected environmental concentration.

Environmental Fate of Povidone-iodine

This section will describe the expected environmental fate of three chemicals:

1. The parent povidone-iodine complex
2. Povidone, present after it has released its complexed iodine
3. Iodine

Povidone-iodine

The parent compound used in disinfection of fish eggs, povidone-iodine (PVP-I), is a water soluble complex of a synthetic organic polymer (polyvinylpyrrolidone or PVP) which binds a number of triiodide (I_3^-) anions. Triiodide bound within the polymer is converted to free molecular iodine (I_2) and povidone polymer when a 10% solution of povidone-iodine is diluted in water. Molecular iodine is one of the two chemical forms of iodine believed responsible for the disinfecting properties of iodine. The release and conversion of the bound triiodide within povidone-iodine to molecular iodine within water is not instantaneous, but rather occurs over a period of minutes. EPA has been unable to find specific information on the reaction rate for the conversion of triiodide to molecular iodine. But considering the recommended exposure duration of fish eggs to PVP-I is 10 minutes (AFS 2011), and that diluted PVP-I solutions are only used once to treat eggs, then discarded, is evidence that the conversion of bound triiodide to free molecular iodine is essentially complete within 10 minutes.

A detailed description of the reactions of PVP-I in water is given in a review (Gottardi 2001) of the environmental fate of iodine compounds used in disinfection. Povidone-iodine (10%) diluted in water results in an increase (not a decrease as would normally be expected) in the concentration of molecular iodine in the water. The maximum molecular iodine in water concentration is reached at approximately 0.1% PVP-I in water (1000 mg/L), although the amount of molecular iodine present in a 0.01% PVP-I solution (100 mg/L, the recommended exposure concentration for disinfecting fish eggs) is not substantially lower than that in the 0.1% PVP-I dilution. Upon further dilution below 0.01% PVP-I, the amount of molecular iodine in water also begins to decrease.

Povidone

The definitive study of the environmental fate of povidone in surface water appears to be that of Trimpin et al. (2001). Trimpin et al. (2001) dissolved 10 mg/L of povidone in a fixed bed reactor, through which Rhine River water flowed for 30 days. After 30 days, no oxidation of the terminal hydroxyl groups of the polymer was observed. Nor were any changes in the repeating units of the polymer itself observed. Trimpin et al. (2001) concluded that povidone was unlikely to degrade in the environment, and further concluded that its likely ultimate environmental fate would be sorption onto solid products. The recalcitrance of povidone polymer to biodegradation was confirmed by Julinova et al. (2013), who attempted with minimal success to increase the biodegradation of povidone in wastewater treatment plants.

Because povidone does not appear to biodegrade in the environment, it appears unlikely that any of the monomer from which povidone is synthesized (1-ethenyl-2-pyrrolidone) is present in receiving waters due to degradation of PVP-I used at fish hatcheries.

Iodine

Iodine is a chemical element, atomic number 53, atomic weight 126.9. In addition to its disinfectant properties, it is also the heaviest element known to be nutritionally essential for life. Iodine is required for the synthesis of the thyroid hormones thyroxine and triiodothyronine.

As a halogen element, the chemical properties and environmental fate of iodine are similar to those of chlorine (see the chlorine chapter), although apparently not as well studied as chlorine. There are at least 10 chemical forms of iodine that can be present in water, although a number of them are found at only extremely low concentrations under the circumneutral pH conditions between pH 6.5 – 9.0 of most surface waters. Molecular elemental iodine (I_2) and hydriodic acid (HOI) are the only two chemical forms of iodine believed to exhibit disinfecting properties (Gottardi 2001).

At least 10 different chemical species of iodine are present in freshwater, most of which are present to some extent at pH 6 or greater. Chemical forms of iodine known to be present in freshwater include:

$$I_2, I^-, I_3^-, I_5^-, I_6^{-2}, HOI, OI^-, HOI_2^-, OI_2^{-2}, H_2OI^+, and\ IO_3^-$$

These 10 chemical forms of iodine undergo at least nine different chemical equilibrium reactions (Gottardi 2001).

$$I_2 + H_2O \leftrightarrow HOI + I^- + H^+ \ (hydrolysis)$$

$$HOI \leftrightarrow OI^- + H^+ \ (dissociation\ of\ HOI)$$

$$I_2 + I^- \leftrightarrow I_3^- \ (triiodide\ formation)$$

$$HOI + H^+ \leftrightarrow H_2OI^+ \ (protonization\ of\ HOI)$$

$$I_3^- + I_2 \leftrightarrow I_5^- \ (pentaiodide\ formation)$$

$$2I_3^- \leftrightarrow I_6^{-2} \ (dimerization\ of\ I_3^-)$$

$$OI^- + I^- + H_2O \leftrightarrow HI_2O^- + OH^- \ (iodination\ of\ OI^-)$$

$$HI_2O^- \leftrightarrow I_2O^- + H^+ \ (dissociation\ of\ HI_2O^-)$$

$$3HOI \leftrightarrow IO_3^- + 2I^- + 3H^+ \ (disproportionation)$$

Elemental iodine (I_2) itself has relatively poor water solubility, with a maximum solubility of 338.3 mg/L at 25°C at pH 5 (Gottardi 2001). The water solubility of elemental iodine is greatly increased by addition of iodide anion (I^-), the basis for the well known disinfectant known as Lugol's solution. Binding of iodine with povidone polymer provides for the release of elemental iodine at the concentration required to disinfect fish eggs during hatchery operations.

Expected Environmental Concentration (EEC) of Povidone-Iodine

The desired concentration of elemental iodine, the active ingredient used to disinfect fish eggs in trays or other egg rearing devices at hatcheries, is between 50 – 100 mg/L. Iodine constitutes, on average, 10% by weight of the total mass of povidone-iodine added to water as a disinfectant. The total concentration of povidone-iodine, which includes both the povidone polymer and elemental iodine, would therefore be in the range of 500 – 1000 mg/L. Without any dilution, this is the maximum concentration of povidone-iodine that would be found in the receiving water environment where the threatened and endangered species under evaluation are found. Based on communications with Washington hatcheries, a number of hatcheries discharge spent egg disinfecting solutions via land disposal. If spent PVP-I solutions are discharged at all to surface waters, they would first be substantially diluted before discharge.

The desired treatment concentration of iodine at all 13 Washington hatcheries that currently report its use is 100 mg/L as iodine. Since elemental iodine constitutes 10% by weight of povidone-iodine, the desired treatment concentration of povidone-iodine is 10 times the iodine treatment concentration, or 1000 mg/L PVP-I. Four of the 13 hatcheries using PVP-I have provided EPA with the annual and daily use rates, volumes, and hatchery discharge volumes necessary to calculate EECs for both PVP-I and elemental iodine. They are the Carson and Quilcene National Fish Hatcheries, the Skookum Creek Fish Hatchery and the Chief Joseph – Columbia Hatchery. This information permits us to calculate the expected environmental concentration (EEC) of both PVP-I and elemental iodine in water at the point where the hatchery discharges into a receiving water (i.e. the end of pipe chemical concentration). This end of pipe concentration is used as a conservative estimate of the chemical concentration in receiving waters prior to any dilution of hatchery discharges by the receiving body of water. This EEC calculation also does not take into account any degradation of either PVP-I or elemental iodine described in the environmental fate portion of this Measures of Exposure section.

As described in the Problem Formulation section of the methodology used in this Biological Evaluation, the EEC is calculated as follows, based on procedures described in Schmidt et al. (2007).

$$EEC = \frac{C \times V}{F + E}$$

Where: EEC = Expected environmental concentration (mg/L or µg/L)

C = Treatment concentration of chemical in the hatchery (mg/L or µg/L)

V = Volume of chemical used (gallons/day)

F = Volume of water discharged from hatchery to receiving water (gallons/day)

E = Effluent pond volume (gallons)

For the purposes of calculating conservative EECs, EPA has assumed that the effluent pond volume is zero. Under the lowest and highest daily hatchery discharges from the Carson, Skookum, Quilcene and Chief

Joseph-Columbia hatcheries, the ranges of EECs based on their PVP-I use patterns and rates are presented in Table Iodine-1, along with the calculated EEC for each of the hatchery discharge volumes.

Table Iodine-1. Range of EEC values for discharge of povidone-iodine concentrations to receiving waters under lowest and highest daily hatchery discharge volumes to receiving water.

Hatchery	PVP-I Use Gallons/year	PVP-I Use Gallons/day	PVP-I Use Days/year	Discharge Gallons/day	PVP-I Treatment µg/L	EEC µg/L
Carson – low	4	0.98	4	15,399,360	1,000,000	0.064
Carson – high	4	0.98	4	39,566,800	1,000,000	0.025
Skookum – low	45	0.24	188	1,371,184	1,000,000	0.178
Skookum – high	45	0.24	188	8,732,160	1,000,000	0.028
Quilcene - low	26	0.09	289	59,305	1,000,000	1.47
Quilcene - high	26	0.09	289	31,966,747	1,000,000	0.0027
Chief Joseph - low	35	0.19	184	12,417,120	1,000,000	0.015
Chief Joseph - high	35	0.19	184	16,872,720	1,000,000	0.011

The highest and lowest EEC values from Table Iodine-1 (1.47 and 0.0027 µg/L, highlighted in green) are used as the EEC range for the remaining hatcheries that did not provide the information needed to derive EECs. This range of EECs is assumed for the remaining hatcheries regardless of whether their discharge is into freshwater, estuarine or marine systems. No Washington hatcheries currently have empirical data on Iodine concentrations in their discharges to surface waters.

The EEC concentrations from Table Iodine-1 will be compared to the chronic NOEC estimates calculated in the Measures of Effect section. This comparison will take place in the Risk Characterization section to estimate ecological risks to threatened and endangered species exposed to povidone-iodine discharges from hatcheries in Washington.

Expected Environmental Concentration (EEC) of Elemental Iodine

The desired concentration of elemental iodine, the active ingredient used to disinfect fish eggs in trays or other egg rearing devices at hatcheries, is between 50 – 100 mg/L. Without any dilution, this is the maximum concentration of elemental iodine that would be found in the receiving water environment where the threatened and endangered species under evaluation are found. Based on communications with Washington hatcheries, a number of hatcheries discharge spent egg disinfecting solutions via land

disposal. If spent PVP-I solutions are discharged at all to surface waters, they would first be substantially diluted before discharge.

The elemental iodine EECs are calculated in the same manner as were the PVP-I EECs in the previous section, with the exception of a starting iodine concentration of 100 mg/L (= 100,000 µg/L), instead of the 1000 mg/L PVP-I initial treatment concentration. Data from the same four hatcheries (Carson, Skookum, Quilcene and Chief Joseph – Columbia) were used in the calculation of both povidone-iodine and elemental iodine EECs. Elemental iodine EECs are presented in Table Iodine-2.

Table Iodine-2. Range of EEC values for discharge of elemental iodine concentrations to receiving waters under lowest and highest daily hatchery discharge volumes to receiving water.

Hatchery	PVP-I Use Gallons/year	PVP-I Use Gallons/day	PVP-I Use Days/year	Discharge Gallons/day	Iodine Treatment µg/L	EEC µg/L
Carson – low	4	0.98	4	15,399,360	100,000	0.0064
Carson – high	4	0.98	4	39,566,800	100,000	0.0025
Skookum – low	45	0.24	188	1,371,184	100,000	0.0178
Skookum – high	45	0.24	188	8,732,160	100,000	0.0028
Quilcene - low	26	0.09	289	59,305	100,000	0.147
Quilcene - high	26	0.09	289	31,966,747	100,000	0.00027
Chief Joseph - low	35	0.19	184	12,417,120	100,000	0.0015
Chief Joseph - high	35	0.19	184	16,872,720	100,000	0.0011

The highest and lowest EEC values from Table Iodine-2 (0.147 and 0.00027 µg/L, highlighted in green) are used as the iodine EEC range for the remaining hatcheries that did not provide the information needed to derive EECs. This range of iodine EECs is assumed for the remaining hatcheries regardless of whether their discharge is into freshwater, estuarine or marine systems. No Washington hatcheries currently have empirical data on elemental iodine concentrations in their discharges to surface waters.

The iodine EEC concentrations from Table Iodine-2 will be compared to the chronic NOEC estimates calculated in the Measures of Effect section. This comparison will take place in the Risk Characterization section to estimate ecological risks to threatened and endangered species exposed to elemental iodine discharges from hatcheries in Washington.

Measures of Effect:

For fully aquatic species, the available toxicity data was identified from a search in EPA's ECOTOX database (http://cfpub.epa.gov/ecotox/). Searches were performed on the following chemical forms of povidone iodine, povidone and iodine:

- Povidone iodine, CAS ID 25655-41-8
- Povidone, CAS ID 9003-39-8
- 1-ethenyl-2-pyrrolidone, 1-vinyl-2-pyrrolidone (both with CAS ID 88-12-0)
- Iodine, CAS ID 7553-56-2
- Iodide, CAS ID 20461-54-5
- Hydriodic acid, CAS ID 10034-85-2
- Triiodide, CAS ID 14900-04-0
- Potassium iodide (most common inorganic iodide salt), CAS ID 7681-11-0

A combined total of 69 toxicity records were identified from the above search. These results are presented in Appendix K.

- Iodine (N = 35): 7 for *Daphnia magna*, 1 for zebra mussel, 4 for bluegill, 12 for channel catfish, 1 for guppy, 10 for rainbow trout
- 1-ethenyl-2-pyrrolidone (N = 4): 4 for Red Sea bream
- Povidone iodine (N = 19): 2 for *Dunaliella euchlora*, 1 for *Pavlova lutheri*, 1 for *Phaeodactylum tricornutum*, 1 for Asiatic clam, 3 for quahog, 9 for rainbow trout, 2 for largemouth bass
- Potassium iodide (N = 11): 10 for rainbow trout, one for zebra mussel

Of the available toxicity data, the only information on a threatened and endangered species under evaluation in this Biological Evaluation is for rainbow trout. We have evaluated the toxicity of two different chemical forms: the parent povidone iodine, and elemental iodine (I_2). The available rainbow trout data permits us to estimate the toxicity of both povidone iodine and elemental iodine to all ESA listed salmonid species in Washington using the methodologies described under the problem formulation section of this Biological Evaluation, specifically using ICE models.

Toxicity of Povidone Iodine (PVP-I)

No toxicity studies meeting EPA requirements for use in developing aquatic life criteria are available for povidone iodine. Of the available data, the most useful in evaluating potential povidone iodine toxicity to threatened and endangered species in receiving waters is a chronic 35 day exposure of rainbow trout eggs and fry to the commercial povidone iodine product Betadine® (Amend 1974).

Amend (1974) exposed rainbow trout eggs from fertilization to hatch of fry using a pulsed exposure experimental design. Specifically, four weekly 15 minute exposures of fertilized rainbow trout eggs to PVP-I at a concentration of 100 mg/L elemental iodine were performed. This exposure scenario resulted in a much longer exposure time of eggs to PVP-I than is normally employed by hatcheries, and exposure of eggs at developmental stages normally not exposed to PVP-I under standard hatchery operating

conditions. Amend (1974) observed no adverse effect on eggs or fry after hatching from this exposure scenario.

As Amend (1974) evaluated povidone iodine effects over a 35 day period, with exposures of longer duration than normally used at hatcheries, we consider this study to be of chronic duration. Thus, the reported 35-day chronic NOEC concentration of 100,000 µg/L povidone iodine is considered to be the chronic NOEC for steelhead under the pulsed exposure conditions in Amend (1974).

Because no empirical toxicity data for any other threatened and endangered fish species in Washington exists for povidone iodine, we used the rainbow trout chronic NOEC of 100,000 µg/L from Amend (1974) as input to the Interspecies Correlation Estimation (ICE) model to estimate toxicity to the other five threatened and endangered salmonids in Washington.

Output of all ICE models run with povidone-iodine for the threatened and endangered species, genera or family with available data in ICE is shown in Table PI-1. Using the ICE model selection guidelines set forth in the problem formulation, models used to estimate chronic NOEC's for salmonid species are highlighted in green and bolded in Table PI-1 The remaining ICE models, with poorer predictive ability and which were not selected as the source of chronic NOEC's, are shown in red in Table PI-1. As described in the problem formulation, the lower 95% confidence interval of the predicted chronic NOEC, if available, is used as the chronic NOEC in this Biological Evaluation. All ICE models used for povidone-iodine generated lower 95% confidence intervals of the chronic NOEC, and are shown in this section. No information is available in ICE for any of the threatened and endangered rockfish species, genera or families in Washington (bocaccio, canary rockfish, yelloweye rockfish). Therefore, PVP-I effects on rockfish cannot be quantitatively evaluated, and must be considered as an uncertainty in this Biological Evaluation.

The final selected chronic NOEC values for bull trout, Chinook salmon, chum salmon, coho salmon, sockeye salmon and steelhead that were compared to the expected environmental concentration of povidone-iodine in receiving water environments are summarized in Table Iodine-3.

Table Iodine-3. Chronic no effect concentrations (NOEC) for povidone-iodine

Species	Chronic NOEC (µg/L)	Source of chronic NOEC
Bull trout	52,911	ICE model – family level
Chinook salmon	41,731	ICE model – species level
Chum salmon	52,911	ICE model – family level
Coho salmon	52,911	ICE model – family level
Sockeye salmon	52,911	ICE model – family level
Steelhead	100,000	Empirical data (Amend 1974)

Toxicity of Elemental Iodine (I_2)

No toxicity studies meeting EPA requirements for use in developing aquatic life criteria are available for elemental iodine. Of the available data, the most useful in evaluating potential iodine toxicity to threatened and endangered species in receiving waters is a 96 hour LC_{50} survival study of the toxicity of

three iodine chemical species (elemental iodine, iodide anion and iodate anion) stable in water to rainbow trout fry (Laverock et al. 1995). The Laverock et al. (1995) study is well designed, its two primary shortcomings are that rainbow trout were exposed to iodine under static as opposed to flow through exposure conditions, and limitations of the analytical chemistry methods for iodine.

Several 96 hour LC_{50} endpoints are available in Laverock et al. (1995), as they evaluated the effects of water hardness, total organic carbon and chloride concentrations on the toxicity of iodine. The lowest 96 hour LC_{50} of 530 µg/L was used as the starting point for estimating the chronic NOEC value for rainbow trout (steelhead), as well as input into ICE models to estimate chronic NOEC values for the remaining threatened and endangered salmonid species. The 530 µg/L short term acute value for rainbow trout was converted into a chronic NOEC by first dividing 530 µg/L by 2.27 to obtain an LC_{LOW} value of 233 µg/L. In the absence of an iodine specific acute-chronic ratio (ACR) for any species, the default national median ACR of 8.3 (Raimondo et al. 2007) was used to convert the LC_{LOW} to a chronic NOEC value of 28.1 µg/L elemental iodine.

Because no empirical toxicity data for any other threatened and endangered fish species in Washington exists for elemental iodine, we used the rainbow trout estimated chronic NOEC of 28.1 µg/L derived from the LC_{50} of 530 µg/L from Laverock et al. (1995) as input to the Interspecies Correlation Estimation (ICE) model to estimate toxicity to the other five threatened and endangered salmonids in Washington.

Output of all ICE models run with iodine for the threatened and endangered species, genera or family with available data in ICE is shown in Appendices N and O. All estimated effect concentrations are acute toxicity values, not the final chronic NOEC values used in risk characterization to estimate risks to threatened and endangered fish species. Using the ICE model selection guidelines set forth in the problem formulation, models used as the basis to estimate chronic NOEC's for salmonid species are highlighted in green and bolded in Appendices N and O. The remaining ICE models, with poorer predictive ability and which were not selected as the source of LC_{50}s used to estimate chronic NOEC's are shown in red in Appendices N and O. As described in the problem formulation, the lower 95% confidence interval of the predicted LC_{50}, if available, is used to calculate the chronic NOEC in this Biological Evaluation. All ICE models used for iodine generated lower 95% confidence intervals of the LC_{50}, and are shown in this section. No information is available in ICE for any of the threatened and endangered rockfish species in Washington (bocaccio, canary rockfish, yelloweye rockfish). Therefore, elemental iodine effects on rockfish cannot be quantitatively evaluated, and must be considered as an uncertainty in this Biological Evaluation.

The final selected chronic NOEC values for bull trout, Chinook salmon, chum salmon, coho salmon, sockeye salmon and steelhead that were compared to the expected environmental concentration of povidone-iodine in receiving water environments are summarized in Table Iodine-4.

Table Iodine-4. Chronic no effect concentrations (NOEC) for elemental iodine

Species	Chronic NOEC (µg/L)	Source of chronic NOEC
Bull trout	25.4	ICE model – family level
Chinook salmon	23.1	ICE model – species level
Chum salmon	25.4	ICE model – family level

Coho salmon	34.3	ICE model – species level
Sockeye salmon	25.4	ICE model – family level
Steelhead	28.1	Empirical acute data (Laverock et al. 1995)

Risk Characterization: Povidone-Iodine

Risks to Threatened and Endangered Fish Species from Povidone-Iodine

Risks to threatened and endangered fish species for which toxic concentrations of povidone-iodine can be identified from the literature are calculated using a standard ecological risk assessment hazard quotient approach. In the hazard quotient approach, the estimated environmental concentration is divided by the chronic NOEC for each threatened and endangered species to calculate a hazard quotient. Hazard quotients less than 1.0 are indicative of acceptable levels of ecological risk. In the context of this Biological Evaluation, an acceptable ecological risk is represented as an EEC which, if not exceeded, results in no discernable effect on the survival, reproduction and growth of a threatened and endangered species. Note that acceptable EEC values vary between species.

Hazard quotients greater than or equal to 1.0 are indicative of a potential for unacceptable ecological risks to threatened and endangered species.

Hazard quotients for the six threatened and endangered salmonid species for which toxicity data is available or could be estimated are presented in Table Iodine-5. Hazard quotients were calculated using the EEC generated from the lowest and highest daily discharge from the Quilcene hatchery, which results in the largest EEC range to which threatened and endangered species could be exposed.

Table Iodine-5. Hazard quotients (HQ) for threatened and endangered species exposed to the range of estimated environmental concentrations (EEC) of povidone-iodine discharged by hatcheries.

Species	EEC range (µg/L)	Chronic NOEC (µg/L)	Hazard quotient range
Bull trout	0.0027 – 1.47	52,911	$5.1 \times 10^{-8} – 2.8 \times 10^{-5}$
Chinook salmon	0.0027 – 1.47	41,731	$6.5 \times 10^{-8} – 3.5 \times 10^{-5}$
Chum salmon	0.0027 – 1.47	52,911	$5.1 \times 10^{-8} – 2.8 \times 10^{-5}$
Coho salmon	0.0027 – 1.47	52,911	$5.1 \times 10^{-8} – 2.8 \times 10^{-5}$
Sockeye salmon	0.0027 – 1.47	52,911	$5.1 \times 10^{-8} – 2.8 \times 10^{-5}$
Steelhead	0.0027 – 1.47	100,000	$2.7 \times 10^{-8} – 1.5 \times 10^{-5}$

All hazard quotients in Table PVP-HQ are substantially lower than 1.0, indicative of acceptable levels of ecological risk to the species under all hatchery discharge scenarios. Note that the EEC values do not take into account any degradation of environmental concentrations of povidone-iodine.

Risks to Potential Prey of Threatened and Endangered Species from Povidone-Iodine

Limited information is available on the toxicity of povidone-iodine to other aquatic species (Appendix M). In addition to the empirical rainbow trout toxicity data, empirical adverse effect toxicity data for povidone-

iodine exists for four algal species (Ukeles 1962), two clam species and a second fish species, largemouth bass (Wright and Snow 1975). The algal studies were run for 10 days, and can be considered chronic effect concentrations for algal species with short generation times. The clam studies (Chandler and Marking 1979, Davis and Hidu 1969) were of short duration (2 – 12 days), as was the largemouth bass study (15 minutes), and were treated as short term acute studies. Results of these studies were converted to chronic no effect concentrations using the procedures described in the problem formulation, including the results of the largemouth bass study of Wright and Snow (1975), which reported no effect on egg hatchability after a 15 minute treatment of eggs with Betadine. Chronic NOEC concentrations of povidone-iodine to prey of threatened and endangered species is summarized in Table Iodine-6. See also Appendix O.

Table Iodine-6. Toxicity of Povidone-Iodine to Prey of Threatened and Endangered Listed Species.

Organism Type	Chronic NOEC range (µg/L)
Algae	50,000 – 100,000
Aquatic macrophytes	No data
Aquatic invertebrates	908 - >1,592,272
Aquatic insects	No data
Crustaceans	No data
Zooplankton	No data
Molluscs	908 - >1,592,272
Others (e.g. oligochaetes, etc.)	No data
Amphibians	No data
Fish	24,096 - >102,449

Chronic NOEC concentrations for four algal species appear comparable to those for the two fish species with empirical toxicity data for povidone-iodine. Available data for the two clam species, quahog (*Mercenaria mercenaria*) and Asiatic clam (*Corbicula manilensis*) indicates a wide range of sensitivity between these two species. The low end of the mollusc chronic NOEC range in Table Iodine-6 is derived from a 48 hour EC_{50} concentration of 17,100 µg/L reducing growth and development of quahog (Davis and Hidu 1969). The high end of the mollusc chronic NOEC range is derived from a 96 hour LC_{50} survival study by Chandler and Marking (1979) on the Asiatic clam *Corbicula manilensis*.

Based on the maximum EEC of 1.47 µg/L povidone-iodine, the highest hazard quotient for any prey species for which empirical PVP-I toxicity data is available is a HQ = 0.0016 for quahog, based on the results of the Davis and Hidu (1969) study. As the quahog and all other potential prey species hazard quotient for PVP-I is lower than 1.0, we conclude that povidone-iodine releases from Washington hatcheries is unlikely to adversely affect prey species of threatened and endangered fish species.

Uncertainty Analysis of Povidone-Iodine Risk Characterization

All four types of uncertainty (variation, model uncertainty, decision rule uncertainty and true unknowns) described in the problem formulation are present in this povidone-iodine evaluation. By far the largest uncertainty in this evaluation is the complete absence of toxicity data in the literature that would permit

a quantitative evaluation of risks to threatened and endangered eulachon and rockfish species from povidone-iodine use at fish hatcheries. This type of uncertainty is a true unknown in this Biological Evaluation.

Variation of expected environmental concentrations in hatchery discharges and receiving waters is also a large source of uncertainty in this analysis. This is because the use pattern of PVP-I (disinfection of fish eggs) occurs only during a small portion of a year. The time of year during which PVP-I is potentially discharged to receiving waters varies among hatcheries, as different fish species spawn and produce eggs at different times of the year. This use pattern means that during much of the year, povidone-iodine is not released from a hatchery. Variation also is expressed in the confidence limits surrounding statistically reduced expressions of the empirical toxicity data (e.g. LC_{50}, EC_{50}, etc.). Confidence limits describe random variation around the central tendency response of laboratory organisms exposed to chemicals in toxicity tests.

Model uncertainty in the ICE models is described by the percent cross-validation success statistic. According to Raimondo et al. (2013), the percent cross-validation success rate for each model is the proportion of data points that are predicted within 5-fold of the actual LC_{50} value. There is a strong relationship between taxonomic distance and cross-validation success rate, with uncertainty generally, although not always increasing with larger taxonomic distance. Maximizing the value of the cross-validation statistic was a primary determinant of which of multiple ICE models were used to estimate toxicity values in this Biological Evaluation for species where no empirical toxicity data exists for a chemical-species pair.

Risk Characterization: Elemental Iodine

Risks to Threatened and Endangered Fish Species from Elemental Iodine

Hazard quotients for the six threatened and endangered salmonid species for which toxicity data is available or could be estimated are presented in Table Iodine-7. Hazard quotients were calculated using the EEC generated from the lowest and highest daily discharge from the Quilcene hatchery, which results in the largest EEC range to which threatened and endangered species could be exposed.

Table Iodine-7. Hazard quotients (HQ) for threatened and endangered species exposed to the range of estimated environmental concentrations (EEC) of elemental iodine discharged by hatcheries.

Species	EEC range (µg/L)	Chronic NOEC (µg/L)	Hazard quotient range
Bull trout	0.00027 – 0.147	25.4	0.000011 – 0.0058
Chinook salmon	0.00027 – 0.147	23.1	0.000012 – 0.0064
Chum salmon	0.00027 – 0.147	25.4	0.000011 – 0.0058
Coho salmon	0.00027 – 0.147	34.3	0.0000079 – 0.0043
Sockeye salmon	0.00027 – 0.147	25.4	0.000011 – 0.0058
Steelhead	0.00027 – 0.147	28.1	0.0000096 – 0.0052

All hazard quotients in Table Iodine-7 are substantially lower than 1.0, indicative of acceptable levels of ecological risk to the species under all hatchery discharge scenarios. Note that the EEC values do not take into account any reduction of environmental concentrations of elemental iodine to iodide or other reduced forms.

Risks to Potential Prey of Threatened and Endangered Species from Elemental Iodine

Limited information is available on the toxicity of elemental iodine to other aquatic species (see Appendix M and Appendix N). In addition to the empirical rainbow trout toxicity data, empirical adverse effect on survival data for iodine exists for the zooplankton species *Daphnia magna* (Laverock et al. 1995), and three fish species in addition to rainbow trout: bluegill (EPA 2013), channel catfish (LeValley 1982), and the guppy *Poecilia reticulata* (Yarzhombek et al. 1991). One no effect three day study on survival of the zebra mussel (Waller et al. 1996) exposed to iodine also exists. No chronic growth or reproductive toxicity data was found for elemental iodine for any species.

All of the available elemental iodine toxicity data are considered by EPA to be short term acute studies. All but one of the available iodine toxicity studies had exposure durations of 96 hours (4 days) or less. One rainbow trout study had an exposure duration of 14 days. As the available iodine toxicity data are all short term acute studies, their results were converted into chronic NOEC concentrations by the procedure described in the problem formulation: dividing a concentration causing acutely toxic effects on survival by 2.27 to estimate an LC_{LOW}, then dividing the LC_{LOW} by the default acute-chronic ratio of 8.3 (Raimondo et al. 2007) to estimate a chronic NOEC.

The range of chronic NOEC concentrations for potential prey species of threatened and endangered fish species in Washington is presented in Table Iodine-8.

Table Iodine-8. Toxicity of Elemental Iodine to Prey of Threatened and Endangered Listed Species

Organism Type	Chronic NOEC range (µg/L)
Algae	No data
Aquatic macrophytes	No data
Aquatic invertebrates	8.5 – 200,000
Aquatic insects	No data
Crustaceans	8.5
Zooplankton	8.5
Molluscs	200,000
Others (e.g. oligochaetes, etc.)	No data
Amphibians	No data
Fish	19.3 - 159

The most sensitive prey species for which empirical iodine toxicity data is available is the cladoceran *Daphnia magna*. Assuming *Daphnia* is exposed to the maximum elemental iodine EEC of 0.147 µg/L, the maximum *Daphnia* hazard quotient is 0.017. As the highest prey species hazard quotient is lower than

1.0, we have concluded that elemental iodine releases from Washington hatcheries are unlikely to adversely affect prey species of threatened and endangered fish species.

Uncertainty Analysis of Elemental Iodine Risk Characterization

All four types of uncertainty (variation, model uncertainty, decision rule uncertainty and true unknowns) described in the problem formulation are present in this elemental iodine evaluation. By far the largest uncertainty in this evaluation is the complete absence of toxicity data in the literature that would permit a quantitative evaluation of risks to threatened and endangered eulachon and rockfish species from iodine use at fish hatcheries. This type of uncertainty is a true unknown in this Biological Evaluation.

Variation of expected environmental concentrations in hatchery discharges and receiving waters is also a large source of uncertainty in this analysis. This is because the use pattern of elemental iodine released from PVP-I (disinfection of fish eggs) occurs only during a small portion of a year. The time of year during which iodine is potentially discharged to receiving waters varies among hatcheries, as different fish species spawn and produce eggs at different times of the year. This use pattern means that during much of the year, elemental iodine is not released from a hatchery. Variation also is expressed in the confidence limits surrounding statistically reduced expressions of the empirical toxicity data (e.g. LC_{50}, EC_{50}, etc.). Confidence limits describe random variation around the central tendency response of laboratory organisms exposed to chemicals in toxicity tests.

Model uncertainty in the ICE models is described by the percent cross-validation success statistic. According to Raimondo et al. (2013), the percent cross-validation success rate for each model is the proportion of data points that are predicted within 5-fold of the actual LC_{50} value. There is a strong relationship between taxonomic distance and cross-validation success rate, with uncertainty generally, although not always increasing with larger taxonomic distance between two species. Maximizing the value of the cross-validation statistic was a primary determinant of which of multiple ICE models were used to estimate toxicity values in this BE for species where no empirical toxicity data exists for a chemical-species pair.

Effect Determinations of Povidone-Iodine on Threatened and Endangered Species

Based on all chronic NOEC concentrations for six threatened and endangered salmonid species being substantially higher than the estimated environmental concentrations of povidone-iodine released from hatcheries, EPA has made the following effect determinations for povidone-iodine:

Bull trout: Not likely to adversely affect

Chinook salmon: Not likely to adversely affect

Chum salmon: Not likely to adversely affect

Coho salmon: Not likely to adversely affect

Sockeye salmon: Not likely to adversely affect

Steelhead: Not likely to adversely affect

The above determinations are all based on the estimated environmental concentrations from hatchery releases being substantially lower than the chronic NOECs for the above six species.

We are unaware of any quantitative aquatic toxicological data that would allow us to perform a quantitative ecological risk assessment on povidone-iodine risks to eulachon, bocaccio, canary rockfish, and yelloweye rockfish. This is a true unknown type of uncertainty for the hatcheries that use povidone-iodine and which also discharge into estuarine or marine waters.

Effect Determinations of Elemental Iodine on Threatened and Endangered Species

Based on all chronic NOEC concentrations for six threatened and endangered salmonid species being substantially higher than the estimated environmental concentrations of elemental iodine released from hatcheries, EPA has made the following effect determinations for elemental iodine:

Bull trout: Not likely to adversely affect

Chinook salmon: Not likely to adversely affect

Chum salmon: Not likely to adversely affect

Coho salmon: Not likely to adversely affect

Sockeye salmon: Not likely to adversely affect

Steelhead: Not likely to adversely affect

The above determinations are all based on the estimated environmental concentrations from hatchery releases being substantially lower than the chronic NOECs for the above six species.

As was the case for povidone-iodine, the absence of any quantitative data that allowed us to quantify risks to eulachon and the three threatened and endangered rockfish species is an uncertainty in this Biological Evaluation.

6 CONCLUSION: EFFECTS DETERMINATION

In this Biological Evaluation, EPA performed risk assessments of disease treatment chemicals used by hatcheries covered by this General Permit that are expected to be discharged to surface waters. These risk assessments were based on the best available science and were performed in accordance with EPA risk assessment and toxicological methodologies.

The analyses presented in this Biological Evaluation present a worst-case scenario – and are not reflective of typical hatchery operations. For example, the scenarios presented in this Biological Evaluation assume the lowest facility flow (i.e., least amount of internal dilution) and the greatest amount of the chemical used. These risk assessments assume an end of pipe effluent concentration, and to not allow for mixing or dilution within the receiving water. Further, these risk assessments present chronic (e.g. 96 hour) no effect concentrations, which are unrealistically conservative, since the disease treatment chemicals are used for much shorter spans of time (e.g. 1 hour treatments). Chemicals discharged from hatcheries would not remain present in the same concentrations for any significant length of time – they would be diluted or carried downstream (none of the receiving waters runs dry during the low-flow or summer season). In addition, the risk assessments presented in this Biological Evaluation are overly conservative because they do not generally account for degradation of the chemicals. Many of these chemicals are volatile and degrade rapidly, especially when exposed to organics. Much of the analysis was based on the ICE model, which resulted in more conservative effects concentrations than empirical data from rainbow trout. Finally, EPA consistently erred on the side of caution in this analysis, and selected the higher (more conservative) acute to chronic ratio (ACR).

Thus, EPA has made the following effect determinations for this General Permit:

- Bull trout: May affect, not likely to adversely affect
- Chinook salmon: May affect, not likely to adversely affect
- Chum salmon: May affect, not likely to adversely affect
- Coho salmon: May affect, not likely to adversely affect
- Sockeye salmon: May affect, not likely to adversely affect
- Steelhead: May affect, not likely to adversely affect

Based on the lack of chemical discharges from hatcheries directly into estuarine or marine waters, a no effect determination is warranted for the following species:

Eulachon: No effect

Bocaccio: No effect

Canary rockfish: No effect

Yelloweye rockfish: No effect

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8 ESSENTIAL FISH HABITAT

In this section, Essential Fish Habitat (EFH) is assessed for potential adverse impacts from the issuance by USEPA of NPDES Permit No. WAG130000 for wastewater discharges from federal aquaculture facilities and aquaculture facilities in Indian Country within Washington State.

Background

The Magnuson-Stevens Fishery Conservation and Management Act (MSA), as amended by the Sustainable Fisheries Act of 1996 (Public Law 104-267), requires federal agencies to consult with NOAA Fisheries on activities that may adversely affect EFH. According to the Magnuson-Stevens Fishery Conservation and Management Act (MSA§3), EFH means those waters and substrate necessary to fish for spawning, breeding, feeding, or growth and maturity. For the purpose of interpreting this definition of EFH: "waters" include aquatic areas and their associated physical, chemical, and biological properties that are used by fish; "substrate" includes sediment, hard bottom , structures underlying the waters, and associated biological communities; "necessary" means the habitat required to support a sustainable fishery and the managed species' contribution to a healthy ecosystem; and "spawning, breeding, feeding, and growth to maturity" covers a species' full life cycle (50 CFR 600.01). "Adverse effect" means any impact which reduces quality and/or quantity of EFH, and may include direct (e.g. physical disruption), indirect (e.g. loss of prey), site-specific or habitat-wide impacts, including individual, cumulative, or synergistic consequences of actions (50 CFR 600.810).

Pursuant to the MSA the Pacific Fisheries Management Council (PFMC) has designated EFH for three species of federally-managed Pacific salmon: Chinook (*Oncorhynchus tshawytscha*); coho (*O. kisutch*); and Puget Sound pink salmon (*O. gorbuscha*) (PFMC 1999). Freshwater EFH for Pacific salmon includes all those streams, lakes, ponds, wetlands, and other water bodies currently, or historically accessible to salmon in Washington, Oregon, Idaho, and California, except areas upstream of certain impassable man-made barriers (as identified by PFMC 1999), and longstanding, naturally-impassable barriers (i.e. natural waterfalls in existence for several hundred years).

The objective of this EFH assessment is to determine if the proposed action may "adversely affect" designated EFH for relevant commercially or federally managed fisheries species within the proposed action area. It also describes conservation measures proposed to avoid, minimize or otherwise offset potential adverse effects to designated EFH resulting from the proposed action.

Lummi Bay Fish Hatchery is the only facility covered by the permit that discharged to Puget Sound during the past permit cycle. This facility does not use any disease control chemicals that could be present in the effluent. Since no facilities covered by the permit discharge disease control chemicals to Puget Sound (or other marine waters), the USEPA is not evaluating impacts to Puget Sound EFH.

Description of the Project/Proposed Activity

The USEPA is proposing to reissue the National Pollutant Discharge Elimination System (NPDES) general permit for federal aquaculture facilities and aquaculture facilities in Indian Country within Washington

State. Since this is a general permit, the EPA is considering the Action Area to be the entire state of Washington. The activity under consideration for this EFH assessment is identical to the description contained in the Biological Evaluation (BE) for this permit.

Potential Adverse Effects of Proposed Project

Water quality is an important component of EFH. The potential effects of authorized discharges from federal aquaculture facilities and aquaculture facilities in Indian Country within Washington State on EFH within the action area for this permit are the same as those described in the Biological Evaluation for this general permit. EPA has performed a risk assessment for the disease control chemicals expected to be discharged by permitted facilities. A summary of the determinations made for ESA listed species is found in the Biological Evaluation. Effluent limitations and surface water criteria described in the permit provide restrictions that are sufficient to prevent harm to life stages of threatened and endangered species in the action area. Using the information presented in the Biological Evaluation, USEPA has determined that issuance of the NPDES general permit for federal aquaculture facilities and aquaculture facilities in Indian Country within Washington State is **not likely to adversely affect** EFH in the vicinity of the discharges.

EFH Conservation Measures and Conclusion

Facilities covered by the NPDES general permit for federal aquaculture facilities and aquaculture facilities in Indian Country within Washington State will be required to adhere to the permit limits, monitoring requirements, and best management practices described in the permit.

USEPA concludes that the proposed action is **not likely to adversely affect** EFH for Chinook, coho, or Puget Sound pink salmon.

9 APPENDICES (SUBMITTED ELECTRONICALLY)