

# Quality Assurance Project Plan

## **Whole Effluent Toxicity (WET) Testing of Aquatic Pesticides of Concern for the Reissuance of the Aquatic Pesticide and Algae Management Permit (APAM)**



August 2025

Publication No. 25-10-068

## Publication Information

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Each study conducted by the Washington State Department of Ecology must have an approved Quality Assurance Project Plan (QAPP). The plan describes the objectives of the study and the procedures to be followed to achieve those objectives. After completing the study, Ecology will post the final report of the study to the Internet.

This QAPP was approved to begin work in July of 2025. It was finalized and approved for publication in September 2025.

The final QAPP is available on Ecology's website at

<https://apps.ecology.wa.gov/publications/SummaryPages/251006.html>

### **Suggested Citation:**

Ultican, S., M. Bennett, A. Gillen, C. Dudenhoeffer, J. DiToro, D. Mulligan. 2025. Quality Assurance Project Plan: Whole Effluent Toxicity (WET) Testing of Aquatic Pesticides of Concern for the Reissuance of the Aquatic Pesticide and Algae Management Permit (APAM). Publication 25-10-068. Washington State Department of Ecology, Olympia.

<https://apps.ecology.wa.gov/publications/SummaryPages/251006.html>

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**Quality Assurance Project Plan  
Whole Effluent Toxicity (WET) Testing of Aquatic Pesticides of Concern for the  
Reissuance of the Aquatic Pesticide and Algae Management Permit (APAM)**

By Amanda Gillen, Chris Dudenhoeffer, and Shawn Ultican

September 2025

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## 1.0 Abstract

The Washington State Department of Ecology issues coverages under the Aquatic Plant and Algae Management general permit (APAM permit), which authorizes permittees to use various products to control aquatic plants, algae, and phosphorus. Seven of the aquatic herbicides approved for use under the APAM permit have the potential to harm fish. To allow treatments under the permit while minimizing risk to fish species, the Washington Department of Fish and Wildlife (WDFW) identified treatment timing windows for lakes and rivers around Washington.

In some cases, the treatment timing window restrictions make it difficult to effectively control invasive plants in those waterways. For example, if the timing window starts in July but the aquatic plants have already reproduced by then, the plants will continue to spread. In other situations, the WDFW treatment timing windows entirely prohibit the use of products containing the seven active ingredients.

This project will help provide information on what concentrations of the most commonly used aquatic herbicide products containing these seven active ingredients could be used while posing minimal risk to sensitive fish species and aquatic invertebrates. A Whole Effluent Toxicity (WET) testing approach is intended to scientifically inform treatment timing windows more accurately and precisely, which can allow for both protecting vulnerable species and the effective management of invasive aquatic species.

Conducting WET testing with these products on aquatic species in a laboratory environment that mimics real-world pesticide application conditions can provide detailed toxicological data. WET testing will allow Ecology to assess the toxicity of these pesticides within the scope of their intended use and maximum application rate allowed by the product label. This information will help Ecology determine safe dose concentrations and make changes to the permit if necessary.

## 2.0 Background

### 2.1 *Introduction and problem statement*

The Washington State Department of Fish and Wildlife (WDFW) has expressed concern about seven pesticide active ingredients that are authorized for use in the APAM permit. In some cases, WDFW limits when products with any of the 7 active ingredients can be used in waterways that contain sensitive or priority species, such as migrating waterfowl and many trout species. The restrictions within the treatment timing windows make it difficult to treat invasive species in those waterways. Ecology's Water Quality Program is seeking more detailed data on the toxicity of these pesticides. We believe this detailed toxicological data can be obtained by conducting WET testing on the aquatic pesticide products in a lab environment that can mimic the real-world conditions in which these pesticides are used. WET testing will allow Ecology to assess the toxicity of these pesticides within the scope of their intended use and product label. Many aquatic herbicide products contain one or more of the active ingredients that could potentially harm fish. We intend to test 6 of the most used pesticide products, some of which have the same active ingredients, but the formulas may have different modes of action. WET testing will allow us to determine safe dose concentrations and make changes to the permit if necessary. This data can inform treatment timing windows more accurately and precisely, which can allow for both protecting vulnerable species and allow for the effective management of invasive aquatic species.

### 2.2 *Parameters of interest and potential sources*

The aquatic herbicide products proposed for testing each contain at least one of the following active ingredients; 2,4-D (amine), diquat dibromide, dipotassium salt of endothall, mono salt of endothall, and Flumioxazin. The product brand names include Tribune, Aquathol K, Hydrothol 191, Flumigard SC, Sculpin G, and Alligare 2,4-D amine. These are also listed in Table 5.

### 2.3 *Regulatory criteria or standards*

Standard EPA methods for WET testing for acute Rainbow trout (*Oncorhynchus mykiss*), chronic Fathead Minnow (*pimephales promelas*), and Daphnid (*Ceriodaphnia dubia*) with acute dual endpoints.

## 3.0 Project Description

The purpose of this project is to identify concentrations of several aquatic pesticides to effectively manage invasive aquatic plants, without unintended impacts on fish and other native aquatic organisms. We will identify these concentrations by conducting Whole Effluent Toxicity (WET) testing. This study will provide the necessary data for Ecology to enhance our scientifically based approach in developing language and best practices in our aquatic permits and clarify policies with WDFW to protect fish and wildlife, while allowing the control of invasive aquatic plant species under Ecology's Aquatic Plant and Algae Management general permit (APAM).

Without more specific toxicity data, there is risk of WDFW prohibiting treatments using these six aquatic herbicides in up to 66% of lakes in Washington. Removing the option to treat with the herbicides in question would potentially result in the spread of invasive aquatic plants, damage to native aquatic habitats, areas of increased temperatures and lower dissolved oxygen, and reduced beneficial recreational uses by the public.

This project is important since aquatic invasive plants have garnered public attention. The lack of specific toxicity data has created uncertainty around when some types of aquatic herbicide treatments will be allowed by WDFW. This uncertainty has caused increasing levels of frustration for local governments, lake residents, and other types of permittees. While low, there is a risk that this frustration could result in public scrutiny and legal or legislative action. It also increases the risk that frustrated lake residents will treat their shoreline with herbicide products, which are not safe for use in aquatic environments.

An inability to legally treat invasive aquatic plants around swimming beaches, docks, public boat launches, and other public access points could increase the risk of entanglement and drowning for people, pets, and wildlife. Ecology's aquatic pesticide permits must protect native habitats and species, as well as public access points, and allow for the effective control of invasive aquatic plants based on sound science.

### 3.1 Project goals

This study will provide the necessary data for Ecology to enhance our scientifically based approach in developing language and best practices in our aquatic pesticide permits and clarify policies with WDFW to protect fish and wildlife while allowing the control of invasive aquatic plant species under Ecology's Aquatic Plant and Algae Management general permit (APAM).

- Identify scientifically based Best Management Practices (BMPs) for the use of aquatic pesticides in surface waters.

- Characterize the level of toxicity to aquatic organisms for the pesticides of concern.
- Allow more flexibility around when treatments can happen based on the science produced by this study.

### *3.2 Project objectives*

- Analyze 6 of the most commonly used aquatic herbicide products for toxicity using standard EPA WET testing lab methods and surrogate species that mimic real-world conditions.
- Identify concentrations of pesticides that can be safely used in surface waters to manage invasive and noxious aquatic plant species while avoiding unintended consequences to fish and other aquatic species.

### *3.3 Information needed and sources*

We need information on the toxicity of these 6 pesticide formulations to inform the APAM permit. We will achieve this by working with the EcoAnalysts Laboratory in Port Gamble, WA, to run a series of standardized tests that will determine the toxicity and safe level of use for these pesticides of concern on aquatic species.

### *3.4 Tasks required*

- Purchase and deliver the aquatic pesticide mixtures to the lab.
- Development of the Quality Assurance Project Plan (QAPP) by EcoAnalysts with Ecology staff (Chris Dudenhoeffer, Shawn Ultican, and Amanda Gillen).
- Laboratory toxicity testing (equipment, supplies, and labor).
- Report of the results.
- Sharing raw data with Ecology's WET testing lead (Chris Dudenhoeffer) APAM general permit writer Shawn Ultican and Environmental Planner Amanda Gillen.

## 4.0 Organization and Schedule

**Table 1: Organization of project staff and responsibilities.**

Staff <sup>1</sup>	Title	Responsibilities
<p><b>Shawn Ultican</b></p> <p>Phone: (360) 870-3492</p> <p>Email: <a href="mailto:shawn.ultican@ecy.wa.gov">shawn.ultican@ecy.wa.gov</a></p>	<p>Aquatic Pesticide Permit Specialist</p>	<p>QAPP development and review. Raw data review, report review, and managing questions and concerns from the lab regarding the testing, relevant to the needs of permit development.</p>
<p><b>Amanda Gillen</b></p> <p>Phone: (360) 480-2389</p> <p>Email: <a href="mailto:amanda.gillen@ecy.wa.gov">amanda.gillen@ecy.wa.gov</a></p>	<p>Environmental Planner 3</p>	<p>Contract and schedule management. QAPP development and review. Data and report organization. Additional support as needed.</p>
<p><b>Michelle Bennett</b></p> <p>Phone: (707) 480-0912</p> <p>Email: <a href="mailto:michelle.bennett@spherosenvironmental.com">michelle.bennett@spherosenvironmental.com</a></p>	<p>Project Manager</p>	<p>Develops draft QAPP, coordinates with QA Coordinator on final QAPP, conducts bioassay testing, and submits final report</p>
<p><b>Chris Dudenhoeffer</b></p> <p>Phone: (360) 870-8409</p> <p>Email: <a href="mailto:cdud461@ecy.wa.gov">cdud461@ecy.wa.gov</a></p>	<p>Ecology Quality Assurance Coordinator</p>	<p>Reviews and approves the draft QAPP and the final QAPP. Review the final report. Raw data review to make sure lab results match the needs of the permit.</p>
<p><b>Marisa Seibert</b></p> <p>Phone: (360) 297-6040 ext. 6052</p> <p>Email: <a href="mailto:marisa.seibert@spherosenvironmental.com">marisa.seibert@spherosenvironmental.com</a></p>	<p>Laboratory Manager</p>	<p>Oversees laboratory operations. Conducts laboratory testing. Responsible for daily review of data while testing is in progress.</p>

Staff <sup>1</sup>	Title	Responsibilities
<b>Danielle Mulligan</b> <b>Phone: (360) 440-8537</b> <b>Email:</b> <b><a href="mailto:dani.mulligan@spherosenvironmental.com">dani.mulligan@spherosenvironmental.com</a></b>	Assistant Quality Systems Director	Assists in developing and maintaining the laboratory QA program. Coordinates and tracks management of change and corrective action records. Ensures testing is conducted in accordance with the QAPP.
<b>Jessica DiToro</b> <b>Phone : (802) 793-8545</b> <b>Email : <a href="mailto:Jessica.DiToro@LREwater.com">Jessica.DiToro@LREwater.com</a></b>	Senior Project Manager	Develops draft QAPP. Review and consult with the chemical components.

#### 4.1 *Special training and certifications*

The laboratory is accredited by the Washington State Department of Ecology (ECY) and also holds a National Environmental Laboratory Accreditation Program (NELAP) accreditation under the Oregon Environmental Laboratory Accreditation Program (Certificate No. 4165-006). The table below lists the laboratory's SOPs associated with the project's scope of work.

The *O. mykiss* has a chronic test method, but it is not an EPA-approved method. Procedures are based on the Rainbow Trout (*Oncorhynchus mykiss*) Survival and Growth Test Method (Lazorchak and Smith 2007). The test acceptability criteria for the growth endpoint are difficult to achieve, and this test is not a common method.

**Table 2: Laboratory Standard Operating Procedures (SOPs)**

Test Type	Species	SOP ID
Acute	<i>Oncorhynchus mykiss</i> Rainbow Trout	SOP TOX016.08
Chronic	<i>Ceriodaphnia dubia</i> Water Flea*	SOP TOX003.11
	<i>Pimephales promelas</i> Fathead Minnow*	SOP TOX018.11

\*Acute data will be taken on Day 2 from the *C. dubia* chronic tests and Day 4 from the *P. promelas* chronic tests (acute dual endpoint).

#### 4.2 Proposed project schedule

**Table 3: Proposed schedule for completing field and laboratory work and reports.**

Work type	Due date	Lead staff
<b>laboratory work</b>		
Laboratory analyses completed	August 19, 2025	Marisa Seibert
<b>Final report</b>		
Draft due to Ecology	August 25, 2025	Michelle Bennett
Final report due	September 3, 2025	Michelle Bennett

#### 4.3 Budget and funding

The funding source for this project is general water quality permit fees. Our budget is not to exceed \$200,000.

**Table 4: Project budget and funding.**

<b>Task</b>	<b>Cost</b>
ACUTE: Rainbow trout ( <i>Oncorhynchus mykiss</i> ) 96-hour static-renewal survival test. Dilution series. 6 product tests	\$13,104
CHRONIC: Fathead minnow ( <i>Pimephales promelas</i> ) 7-day survival and growth static-renewal test. Dilution series. Dual Endpoints. 6 product tests	\$16,279
CHRONIC: Daphnid ( <i>Ceriodaphnia dubia</i> ) 6-7-day survival and reproduction static-renewal test. Dilution series. Dual Endpoints. 6 product tests	\$16,440
Sample Manipulation (\$75 Hourly Rate)	\$375
Interpretive Data Report and Quality Assurance Plan Write up	\$1,750
Technical Consulting and Coordination (NTE LRE Water Hourly Rate)	\$3,000
Project Management (NTE 10 hours PM Hourly Rate \$150)	\$1,500
Subcontract Chemistry Laboratory (Analysis, sample kits, and sample shipments to lab)	\$19,360
Lab Grand Total	\$71,808
<b>Project Grand Total</b>	

## 5.0 Quality Objectives

Measurement quality objectives (MQOs) define the acceptable quality (data validity) of laboratory data for this project. Toxicity test MQOs are summarized below.

### **Laboratory Control**

A laboratory control (negative control) is tested with each sample to ensure the quality of the test organisms and dilution water. The organism's performance in the laboratory control determines if the test acceptability criteria (TAC) are met and verifies that the test results are suitable for reporting. Additionally, the laboratory control is used for the statistical comparison of each sample.

### **Reference Toxicant Testing**

Reference toxicant testing (positive control) is conducted to evaluate the sensitivity of the organisms used in testing and to ensure a level of consistency in the testing procedures. Historical control charts are prepared using point estimate data (LC50). The most recent results (5-20 data points) are included in each control chart. The control chart displays the mean  $\pm$  2 standard deviations. Tests exceeding these action levels are examined on a case-by-case basis.

## 6.0 Study Design

The following aquatic herbicide products will be tested using the species of fish and aquatic invertebrates listed in the table below.

**Table 5: Analytes to be Measured**

Product Name	EPA #	Active Ingredient	Formulation	Manufacturer
Tribune	100-1390	Diquat dibromide	Liquid	Syngenta
Aquathol K	70506-176	Dipotassium salt of endothall	Liquid	UPL NA Inc.
Hydrothol 191	70506-175	Mono salt of endothall	Liquid	UPL NA Inc.
Flumigard SC	81927-78	Flumioxazin	Liquid	Alligare
Sculpin G	67690-49	2,4-D amine	Granular	SePro
Alligare 2,4-D amine	81927-38	2,4-D amine	Liquid	Alligare

**Table 6: Species to be Tested**

Test Type	Test Descriptor	Species	Method
Acute	96-hour Survival	<i>Oncorhynchus mykiss</i> Rainbow Trout	WDOE WQ-R-95-80 (2016); EPA-821-R-02-012 Method 2019.0;
Chronic	7-Day Survival and Reproduction	<i>Ceriodaphnia dubia</i> Water Flea	WDOE WQ-R-95-80 (2016); EPA-821-R-02-013; Test Method 1002.0
	7-Day Survival and Growth	<i>Pimephales promelas</i> Fathead Minnow	WDOE WQ-R-95-80 (2016); EPA-821-R-02-013; Test Method 1000.0

### *6.1 Assumptions underlying design*

The range of test concentrations will be determined by ECY and are based off the maximum label rate of each product. Testing will use a 5-concentration definitive test bracketing the values in table 8.

### *6.2 Possible challenges and contingencies*

While challenges with bias and comparability within and among the bioassays are unlikely given the similarity of the test designs and uniform, method-dictated test conditions, one potential source of bias that should be considered is the relative water solubility of the alternative compounds. A compound's solubility can impact its bioavailability in the environment, and subsequently its resulting toxicity to biological life. When evaluating the study data, consideration should be given to the relationship between each compound's solubility and the observed toxicity. Qualitative observations will be made during the production of the test solutions to additionally characterize their solubility. These details will be reported with the biological results to give context to the study findings.

### *6.3 Logistical problems*

Chemicals should be purchased with enough time to account for potential supply-chain complications and shipping delays and arrive close to testing time to decrease the potential for chemical transformation before testing.

All species have specified age ranges within which they must be used for testing. The supplier will need notification far enough in advance to ensure that animals are within the appropriate age range.

All tests have individual test acceptability criteria (TAC) that need to be met for a test to be considered valid. If a test fails TAC, the lab will have to re-test the product, which may delay getting the final results to ECY.

The laboratory houses multiple temperature-controlled water baths, incubators, and testing rooms, but has a limit on the number of tests that can be initiated. The laboratory has issued a tentative schedule to ECY with testing start dates that can meet the deadline if no products need re-tests.

Ecology needs to receive the final report by September 3, 2025, to include the information when the draft Aquatic Plant and Algae Management general permit goes out for public comment.

## 7.0 Laboratory Procedures

### 7.1 Organism Acquisition:

*O. mykiss* will be purchased from Thomas Fish Company in Anderson, California, *P. promelas* will be supplied by EcoAnalysts Florida in Alachua, FL, *C. dubia* are cultured in-house.

#### 7.1.2 Chemical Acquisition

The aquatic herbicides to be tested are being purchased by a licensed pesticide applicator from Wilbur-Ellis Agribusiness in Auburn, WA, and other suppliers as necessary.

These products are being shipped to the EcoAnalysts laboratory in Port Gamble, WA.

### 7.2 Laboratory Methods

**Table 7: Methods (laboratory).**

Test Type	Test Descriptor	Species	Method
Acute	96-hour Survival	<i>Oncorhynchus mykiss</i> Rainbow Trout	WDOE WQ-R-95-80 (2016); EPA-821-R-02-012 Method 2019.0; SOP TOX016.08
Chronic	7-Day Survival and Reproduction	<i>Ceriodaphnia dubia</i> Water Flea	WDOE WQ-R-95-80 (2016); EPA-821-R-02-013; Test Method 1002.0; SOP TOX003.11
	7-Day Survival and Growth	<i>Pimephales promelas</i> Fathead Minnow	WDOE WQ-R-95-80 (2016); EPA-821-R-02-013; Test Method 1000.0; SOP TOX018.11

### 7.3 Sample preparation method(s)

The samples used for testing will be prepared by making stock solutions for each test concentration on Day 0 of the tests. These stock solutions will be used for all renewals and stored in the laboratory at room temperature.

Due to the potential of the Tribune and Flumigard SC products adhering to plastic, the lab will collect subsamples of each test concentration at test initiation and test termination to see if any of the product is lost throughout the duration of the test.

The range of test concentrations are calculated based on the maximum application rates listed on the FIFRA label for each product and shown in the table below.

**Table 8: Maximum Label Rates of Analytes**

Product Name	EPA #	Active Ingredient	Max Label Rate
Tribune	100-1390	Diquat dibromide	686 ppb
Aquathol K	70506-176	Dipotassium salt of endothall	5.0 ppm
Hydrothol 191	70506-175	Mono salt of endothall	5.0 ppm
Flumigard SC	81927-78	Flumioxazin	0.4 mg a.i./L
Sculpin G	67690-49	2,4-D amine	4.0 ppm
Alligare 2,4-D amine	81927-38	2,4-D amine	4.0 ppm

These maximum application rates are then multiplied by the factors below to determine the concentration used in each test.

- 2.0 max label rate
- 1.0 max label rate
- 0.5 max label rate
- 0.25 max label rate
- 0.10 max label rate
- 0 (control)

For example, the test solution concentrations used for Aquathol K follows:

**Table 9: Example Test Solution Concentrations**

<b>Product Name</b>	<b>Max Label Rate</b>	<b>Multiplier</b>	<b>Concentration of test solution</b>
Aquathol K	5.0 ppm	2.0 max label rate	10.0 ppm
		1.0 max label rate	5.0 ppm
		0.5 max label rate	2.5 ppm
		0.25 max label rate	1.25 ppm
		0.10 max label rate	0.5 ppm
		0 (control)	0.0 ppm

#### *7.4 Laboratories accredited for methods*

All testing conducted under this QAPP must be done by a laboratory accredited for the specific method used in the analysis.

EcoAnalysts is accredited by WDOE and holds a National Environmental Laboratory Accreditation Program (NELAP) accreditation under the Oregon Environmental Laboratory Accreditation Program (Certificate No. 4165-006).

Pacific Agricultural Labs will be analyzing the pesticide compounds for Tribune and Flumigard SC. The laboratory is accredited to analyze pesticides by ANAB but not certified by WDOE.

**Table 10: Test Specification Summary**

<b>Parameter</b>	<b>Rainbow Trout (Oncorhynchus mykiss)</b>	<b>Ceriodaphnia dubia</b>	<b>Fathead Minnow (Pimephales promelas)</b>
<b>Test Type</b>	96-hour survival	7-Day survival & reproduction (acute endpoints at 48 hrs)	7-Day survival & growth (acute endpoints at 96 hrs)
<b>Test Chamber</b>	8-L Chamber	30-mL Plastic Chamber	20 oz. Plastic Chamber
<b>Organism Age</b>	15–30 days post swim-up	<24 hours (within an 8-hour range)	<48 hours
<b>Organisms per Chamber</b>	10	1	10
<b>Test Volume per Chamber</b>	4 L	15 mL	250 mL
<b>Number of Replicates</b>	4	10	4
<b>Temperature</b>	12 ± 1°C	25 ± 1°C	25 ± 1°C
<b>pH Range</b>	6 – 9	6 – 9	6 – 9
<b>Dissolved Oxygen (DO)</b>	≥ 6.0 mg/L	≥ 4.0 mg/L	≥ 4.0 mg/L
<b>Reference Toxicant</b>	Sodium Dodecyl Sulfate (SDS)	Copper	Copper
<b>Photoperiod</b>	16 hr light / 8 hr dark	16 hr light / 8 hr dark	16 hr light / 8 hr dark
<b>Water Renewal</b>	At 48 hours	Daily	Daily
<b>Feeding</b>	None	1:1 mix of YCT & Selenastrum daily	1500 Artemia nauplii twice daily (Days 1–6)

<b>Endpoints</b>	Survival	Survival, Reproduction	Survival, Growth
<b>Test Acceptability Criteria</b>	Control survival ≥ 90%	Acute survival ≥ 90% Chronic survival ≥ 80% Reproduction: Avg. ≥15 neonates/survivor; ≥60% females ≥3 broods; PMSD ≤ 47%	Acute survival ≥ 90% Chronic survival ≥ 80% Dry weight ≥ 0.25 mg PMSD biomass: 12–30%

## 8.0 Quality Control Procedures

The laboratory has a Quality Manual (QM) based on Quality Management Systems (QMS) derived from both The NELAC Institute (TNI) and WDOE guidance for performing bioassays. Standard operating procedures for the exposures as well as support procedures including equipment calibration, organism receipt and acclimation, data QA/QC, and report production and review, will be used in conjunction with a project-specific study plan to complete the work and ensure a high level of data quality.

### *8.1 Reference Toxicant Testing*

Reference toxicant testing will be performed on each batch of organisms used for testing. Results will be used to determine the sensitivity of the test organisms and the reproducibility of the laboratory. Results will be included in the final report.

### *8.2 Laboratory Control*

A laboratory control, made with laboratory water, will be tested with each chemical. The laboratory controls will be used for statistical comparisons and to calculate median lethal concentrations (LC50). In addition, the laboratory controls will be used to determine test acceptability criteria (TAC) and to verify that the organisms are healthy and suitable for testing.

### *8.3 Corrective action processes*

In the event there is a problem observed with any data or lab operation, it is the laboratory staff's responsibility to inform their supervisor, who will, in turn, alert the Lab Manager and/or the QA Manager immediately. Together, the staff will identify an appropriate course of action to remedy the problem. Minor protocol deviations or errors will be noted on laboratory datasheets using a list of standardized qualifier codes. Other errors affecting data quality require the use of a Corrective Action Record (CAR). These CARs are reviewed monthly by the QA Manager to ensure they are being appropriately utilized and to identify recurring problems or trends. The QA Manager will discuss trends and ways to correct them with the Lab Manager and the Operations Director, if necessary. If appropriate, a copy of the CAR will be included with the affected data file. testing.

### *8.4 Equipment calibration and maintenance*

All pertinent lab monitoring records are kept in bound logbooks and maintained securely for a minimum of five years. Records of field collections, organism receipt, shipping, culture and holding, chemical receipt, chemical stock preparation, laboratory water quality, instrument calibration, oven and refrigerator temperatures, and environmental chamber temperatures are maintained.

A master list of laboratory equipment is maintained. All lab instruments and equipment have regular service schedules according to specific manufacturer requirements. Service files are maintained for each piece of equipment, including all invoices and certifications, as appropriate. The service schedules are tracked on an annual calendar. The QA Manager reviews the equipment maintenance system to ensure that the Lab Technician in charge of the program is maintaining files and servicing equipment in an appropriate and timely manner.

## 9.0 Data Management Procedures

### 9.1 *Laboratory data package requirements*

Survival, reproduction, and growth counts and observations will be recorded on electronic datasheets by EcoAnalysts laboratory staff. In addition, supplemental water quality will be measured from each test concentration daily. Should a recording error occur, the laboratory staff will place a note in the cell, initial it, date it, and note the reason for the correction. All electronic datasheets will be in shared mode, which enables the tracking of all value changes over time. A printout of all value changes will be kept on file. EcoAnalysts maintains a standardized list of qualifier codes that references common laboratory errors and issues. Daily review of the data will be conducted by the Laboratory Manager or other trained staff.

Once testing is complete, the Project Manager will verify, review, and analyze the data. The Assistant Quality Systems Director will perform an additional review of all the data, including the statistical analysis reports. The data review process is performed to ensure that defined procedures were followed, and that reported data is reasonable and consistent with associated QA/QC results.

The Project Manager will write the final report. The report will include a description of the purpose of the study and test design, the test results, reference toxicant results, and an explanation of any QA/QC anomalies in the laboratory data. The Assistant Quality Systems Director will review the final report to ensure completeness and accuracy.

The final report will include a written narrative, statistical analysis reports, copies of all raw data sheets used in the laboratory, sample receipt information, and chain of custody forms. All the test results for this project will be reported under one cover.

### 9.2 *Electronic transfer requirements*

Preliminary results will be shared via Microsoft Excel files as a summary. Final results will be shared in the final report, via Microsoft Excel files, and CETIS files.

## 10.0 Reports

### *10.1 Frequency and distribution of reports*

Preliminary results will be issued to ECY when the test ends and has statistical data to report.

A single final report will include results, analysis of all data, and copies of all laboratory datasheets. The final report will be submitted to Ecology no later than September 3, 2025.

### *10.2 Responsibility for reports*

The final report will be authored by the Project Manager (Michelle Bennett) and reviewed by the Assistant Quality Systems Director (Danielle Mulligan).

## 11.0 Data Verification and Project Objectives

### *11.1 Laboratory data verification*

Data will be verified by evaluating the completeness, correctness, and conformance/compliance of the specific data set against the method, procedural, or contractual requirements. The primary goal of verification is to document that applicable method, procedural, and contractual requirements were met in the laboratory analysis. Verification checks to see if the data is complete, if the analysis matched QAPP requirements, and if Standard Operating Procedures (SOPs) were followed. Verification of data is the responsibility of the Project Manager.

### *11.2 Process for determining whether project objectives were met*

Processes and data quality will be evaluated for usability by ensuring methods are followed and quality objectives described in the QAPP are met. The study will be considered successful if testing of all compounds is completed as outlined and the lab controls meet applicable test acceptability criteria. Results will be rejected or qualified if the objectives are not met.

### *11.3 Data analysis and presentation methods*

Statistical analyses will be conducted using flowchart specifications outlined in USEPA test guidance manuals. Test results will be statistically compared to the corresponding laboratory control. The statistical analyses will be performed using the Comprehensive Environmental Toxicity Information System™ (CETIS), version 2.1.4.6 by Tidepool Scientific Software. Results will be used to calculate the no observed effect concentrations (NOEC), the lowest observed effect concentrations (LOEC) the 50 percent effect concentrations (LC50).

### *11.4 Documentation of assessment*

The data usability assessment will be included in the final report.

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## 13.0 Appendices

### *Appendix A Glossaries, Acronyms, and Abbreviations*

#### *Glossary of General Terms*

**Accreditation:** A certification process for laboratories, designed to evaluate and document a lab's ability to perform analytical methods and produce acceptable data.

**Ambient:** Background or away from point sources of contamination. Surrounding environmental conditions.

**Anthropogenic:** Human-caused.

**Chronic critical effluent concentration:** The maximum concentration of effluent during critical conditions at the boundary of the mixing zone assigned in accordance with WAC 173-201A-100. The boundary may be based on distance or a percentage of flow. Where no mixing zone is allowed, the chronic critical effluent concentration shall be 100% effluent.

**Control chart:** A graphical representation of quality control results demonstrating the performance of an aspect of a measurement system.

**Critical condition:** When the physical, chemical, and biological characteristics of the receiving water environment interact with the effluent to produce the greatest potential adverse impact on aquatic biota and existing or designated water uses. For steady-state discharges to riverine systems, the critical condition may be assumed to be equal to the 7Q10 flow event unless determined otherwise by the department.

**Designated uses:** Those uses specified in Chapter 173-201A WAC (Water Quality Standards for Surface Waters of the State of Washington) for each water body or segment, regardless of whether or not the uses are currently attained.

**Dissolved oxygen (DO):** A measure of the amount of oxygen dissolved in water.

**Dilution factor:** The relative proportion of effluent to stream (receiving water) flows occurring at the edge of a mixing zone during critical discharge conditions as authorized in accordance with the state's mixing zone regulations at WAC 173-201A-100.  
<http://apps.leg.wa.gov/WAC/default.aspx?cite=173-201A-020>

**Diurnal:** Of, or pertaining to, a day or each day; daily. (1) Occurring during the daytime only, as different from nocturnal or crepuscular, or (2) Daily; related to actions which are completed in a

calendar day, and which typically recur every calendar day (e.g., diurnal temperature rises during the day and falls during the night).

**Effluent:** An outflowing of water from a natural body of water or a human-made structure. For example, the treated outflow from a wastewater treatment plant.

**Existing uses:** Those uses attained in fresh and marine waters on or after November 28, 1975, whether they are designated uses. Introduced species that are not native to Washington and put-and-take fisheries comprised of non-self-replicating introduced native species do not need to receive full support as an existing use.

**Geometric mean:** A mathematical expression of the central tendency (an average) of multiple sample values. A geometric mean, unlike an arithmetic mean, tends to dampen the effect of very high or low values, which might bias the mean if a straight average (arithmetic mean) were calculated. This is helpful when analyzing bacteria concentrations, because levels may vary anywhere from 10 to 10,000-fold over a given period. The calculation is performed by either: (1) taking the  $n$ th root of a product of  $n$  factors, or (2) taking the antilogarithm of the arithmetic mean of the logarithms of the individual values.

**Lowest Observed Effect Concentration (LOEC):** the lowest tested concentration of a substance at which adverse effects are observed to test organisms.

**Margin of safety:** Required component of TMDLs that accounts for uncertainty about the relationship between pollutant loads and quality of the receiving water body.

**Median Lethal Concentration (LC50):** the concentration of substance at which it will kill 50% of test organisms.

**National Pollutant Discharge Elimination System (NPDES):** National program for issuing, modifying, revoking and reissuing, terminating, monitoring, and enforcing permits, and imposing and enforcing pretreatment requirements under the Clean Water Act. The NPDES program regulates discharges from wastewater treatment plants, large factories, and other facilities that use, process, and discharge water back into lakes, streams, rivers, bays, and oceans.

**No Observed Effect Concentrations (NOEC):** the highest tested concentration of a substance at which no adverse effects are observed in test organisms.

**pH:** A measure of the acidity or alkalinity of water. A low pH value (0 to 7) indicates that an acidic condition is present, while a high pH (7 to 14) indicates a basic or alkaline condition. A pH

of 7 is considered to be neutral. Since the pH scale is logarithmic, a water sample with a pH of 8 is ten times more basic than one with a pH of 7.

**Point source:** A Source of pollution that discharges at a specific location from pipes, outfalls, and conveyance channels to the surface water. Examples of point source discharges include municipal wastewater treatment plants, municipal stormwater systems, industrial waste treatment facilities, and construction sites where more than 5 acres of land have been cleared.

**Pollution:** Contamination or other alteration of the physical, chemical, or biological properties of any waters of the state. This includes changes in temperature, taste, color, turbidity, or odor of the waters. It also includes the discharge of any liquid, gaseous, solid, radioactive, or other substance into any waters of the state. This definition assumes that these changes will, or are likely to create a nuisance or render such waters harmful, detrimental, or injurious to (1) public health, safety, or welfare, or (2) domestic, commercial, industrial, agricultural, recreational, or other legitimate beneficial uses, or (3) livestock, wild animals, birds, fish, or other aquatic life.

**Primary contact recreation:** Activities where a person would have direct contact with water to the point of complete submergence, including, but not limited to, skin diving, swimming, and water skiing.

**Reference Toxicant:** a chemical used in toxicity tests to provide results that can be compared within a laboratory or among laboratories. Additionally, reference toxicants can be used to determine organism sensitivity.

**Salmonid:** Fish that belong to the family *Salmonidae*. Species of salmon, trout, or char.

**Standard Operating Procedure (SOP):** A document that describes in detail a reproducible and repeatable organized activity.

**Streamflow:** Discharge of water in a surface stream (river or creek).

**Surface waters of the state:** Lakes, rivers, ponds, streams, inland waters, salt waters, wetlands, and all other surface waters and water courses within the jurisdiction of Washington State.

**Test Acceptability Criteria (TAC):** the criteria listed in the EPA manual that a test must meet in order to be considered valid.

**1-D Max or 1-day maximum temperature:** The highest water temperature reached on any given day. This measure can be obtained using calibrated maximum/minimum thermometers or continuous monitoring probes having sampling intervals of thirty minutes or less.

**303(d) list:** Section 303(d) of the federal Clean Water Act, requiring Washington State to periodically prepare a list of all surface waters in the state for which beneficial uses of the water – such as for drinking, recreation, aquatic habitat, and industrial use – are impaired by pollutants. These are water quality-limited estuaries, lakes, and streams that fall short of state surface water quality standards and are not expected to improve within the next two years.

**7-DADMax or 7-day average of the daily maximum temperatures:** The arithmetic average of seven consecutive measures of daily maximum temperatures. The 7-DADMax for any individual day is calculated by averaging that day's daily maximum temperature with the daily maximum temperatures of the three days before and the three days after that date.

**90<sup>th</sup> percentile:** An estimated portion of a sample population based on a statistical determination of distribution characteristics. The 90<sup>th</sup> percentile value is a statistically derived estimate of the division between 90% of samples, which should be less than the value, and 10% of samples, which are expected to exceed the value.

### *Acronyms and Abbreviations*

BMP	Best management practice
DO	(see Glossary above)
DOC	Dissolved organic carbon
e.g.	For example
Ecology	Washington State Department of Ecology
ECY	Washington State Department of Ecology
EIM	Environmental Information Management database
EPA	U.S. Environmental Protection Agency
et al.	And others
FC	(see Glossary above)
GIS	Geographic Information System software
GPS	Global Positioning System
i.e.	In other words
LC <sub>xx</sub>	Median Lethal Concentration to XX percent of organisms
LOEC	(see Glossary above)
MEL	Manchester Environmental Laboratory
MQO	Measurement quality objective
NAF	New Approximation Flow
NOEC	(see Glossary above)
NPDES	(See Glossary above)
NSDZ	Near-stream disturbance zones
NTR	National Toxics Rule
PBDE	Polybrominated diphenyl ethers

PBT	Persistent, bioaccumulative, and toxic substance
PCB	Polychlorinated biphenyls
QA	Quality assurance
QC	Quality control
RM	River mile
RPD	Relative percent difference
RSD	Relative standard deviation
SOP	Standard operating procedures
SRM	Standard reference materials
TAC	Test acceptability criteria
TIR	Thermal infrared radiation
TMDL	(see Glossary above)
TOC	Total organic carbon
TSS	(see Glossary above)
USFS	United States Forest Service
USGS	United States Geological Survey
WAC	Washington Administrative Code
WDFW	Washington Department of Fish and Wildlife
WQA	Water Quality Assessment
WRIA	Water Resource Inventory Area
WSTMP	Washington State Toxics Monitoring Program
WWTP	Wastewater treatment plant

### *Units of Measurement*

°C	degrees centigrade
Cfs	cubic feet per second
Cfu	colony forming units
Cms	cubic meters per second, a unit of flow
Dw	dry weight
Ft	feet
G	gram, a unit of mass
Kcfs	1000 cubic feet per second
Kg	kilograms, a unit of mass equal to 1,000 grams
kg/d	kilograms per day
km	kilometer, a unit of length equal to 1,000 meters
l/s	liters per second (0.03531 cubic foot per second)
m	meter
mm	millimeter
mg	milligram
mgd	million gallons per day
mg/d	milligrams per day
mg/kg	milligrams per kilogram (parts per million)
mg/L	milligrams per liter (parts per million)
mg/L/hr	milligrams per liter per hour
mL	milliliter
mmol	millimole or one-thousandth of a mole
mole	an International System of Units (IS) unit of matter

ng/g	nanograms per gram (parts per billion)
ng/kg	nanograms per kilogram (parts per trillion)
ng/L	nanograms per liter (parts per trillion)
NTU	nephelometric turbidity units
pg/g	picograms per gram (parts per trillion)
pg/L	picograms per liter (parts per quadrillion)
psu	practical salinity units
s.u.	standard units
µg/g	micrograms per gram (parts per million)
µg/kg	micrograms per kilogram (parts per billion)
µg/L	micrograms per liter (parts per billion)
µm	micrometer
µM	micromolar (a chemistry unit)
µmhos/cm	micromhos per centimeter
µS/cm	microsiemens per centimeter, a unit of conductivity
ww	wet weight

## *Quality Assurance Glossary*

**Accreditation:** A certification process for laboratories, designed to evaluate and document a lab's ability to perform analytical methods and produce acceptable data. For Ecology, it is "Formal recognition by (Ecology)...that an environmental laboratory is capable of producing accurate analytical data." [WAC 173-50-040] (Kammin, 2010)

**Accuracy:** The degree to which a measured value agrees with the true value of the measured property. USEPA recommends that this term not be used, and that the terms *precision* and *bias* be used to convey the information associated with the term *accuracy* (USGS, 1998).

**Analyte:** An element, ion, compound, or chemical moiety (pH, alkalinity) which is to be determined. The definition can be expanded to include organisms, e.g., fecal coliform, *Klebsiella* (Kammin, 2010).

**Bias:** The difference between the sample mean and the true value. Bias usually describes a systematic difference reproducible over time and is characteristic of both the measurement system and the analyte(s) being measured. Bias is a commonly used data quality indicator (DQI) (Kammin, 2010; Ecology, 2004).

**Blank:** A synthetic sample, free of the analyte(s) of interest. For example, in water analysis, pure water is used for the blank. In chemical analysis, a blank is used to estimate the analytical response to all factors other than the analyte in the sample. In general, blanks are used to assess possible contamination or inadvertent introduction of analyte during various stages of the sampling and analytical process (USGS, 1998).

**Calibration:** The process of establishing the relationship between the response of a measurement system and the concentration of the parameter being measured (Ecology, 2004).

**Check standard:** A substance or reference material obtained from a source independent from the source of the calibration standard; used to assess bias for an analytical method. This is an obsolete term, and its use is highly discouraged. See Calibration Verification Standards, Lab Control Samples (LCS), Certified Reference Materials (CRM), and/or spiked blanks. These are all check standards but should be referred to by their actual designator, e.g., CRM, LCS (Kammin, 2010; Ecology, 2004).

**Comparability:** The degree to which different methods, data sets and/or decisions agree or can be represented as similar; a data quality indicator (USEPA, 1997).

**Completeness:** The amount of valid data obtained from a project compared to the planned amount. Usually expressed as a percentage. A data quality indicator (USEPA, 1997).

**Continuing Calibration Verification Standard (CCV):** A quality control (QC) sample analyzed with samples to check for acceptable bias in the measurement system. The CCV is usually a midpoint calibration standard that is re-run at an established frequency during the course of an analytical run (Kammin, 2010).

**Control chart:** A graphical representation of quality control results demonstrating the performance of an aspect of a measurement system (Kammin, 2010; Ecology 2004).

**Control limits:** Statistical warning and action limits calculated based on control charts. Warning limits are generally set at +/- 2 standard deviations from the mean, action limits at +/- 3 standard deviations from the mean (Kammin, 2010).

**Data integrity:** A qualitative DQI that evaluates the extent to which a data set contains data that is misrepresented, falsified, or deliberately misleading (Kammin, 2010).

**Data quality indicators (DQI):** Commonly used measures of acceptability for environmental data. The principal DQIs are precision, bias, representativeness, comparability, completeness, sensitivity, and integrity (USEPA, 2006).

**Data quality objectives (DQO):** Qualitative and quantitative statements derived from systematic planning processes that clarify study objectives, define the appropriate type of data, and specify tolerable levels of potential decision errors that will be used as the basis for establishing the quality and quantity of data needed to support decisions (USEPA, 2006).

**Data set:** A grouping of samples organized by date, time, analyte, etc. (Kammin, 2010).

**Data validation:** An analyte-specific and sample-specific process that extends the evaluation of data beyond data verification to determine the usability of a specific data set. It involves a detailed examination of the data package, using both professional judgment and objective criteria, to determine whether the MQOs for precision, bias, and sensitivity have been met. It may also include an assessment of completeness, representativeness, comparability, and integrity, as these criteria relate to the usability of the data set. Ecology considers four key criteria to determine if data validation has actually occurred. These are:

- Use of raw or instrument data for evaluation.
- Use of third-party assessors.
- Data set is complex.

- Use of EPA Functional Guidelines or equivalent for review.

Examples of data types commonly validated would be:

- Gas Chromatography (GC).
- Gas Chromatography-Mass Spectrometry (GC-MS).
- Inductively Coupled Plasma (ICP).

The end result of a formal validation process is a determination of usability that assigns qualifiers to indicate usability status for every measurement result. These qualifiers include:

- No qualifier – data are usable for intended purposes.
- J (or a J variant) – data are estimated, may be usable, may be biased high or low.
- REJ – data are rejected, cannot be used for intended purposes.

(Kammin, 2010; Ecology, 2004).

**Data verification:** Examination of a data set for errors or omissions, and assessment of the Data Quality Indicators related to that data set for compliance with acceptance criteria (MQOs).

Verification is a detailed quality review of a data set (Ecology, 2004).

**Detection limit (limit of detection):** The concentration or amount of an analyte which can be determined to a specified level of certainty to be greater than zero (Ecology, 2004).

**Duplicate samples:** Two samples taken from and representative of the same population, and carried through and steps of the sampling and analytical procedures in an identical manner. Duplicate samples are used to assess variability of all method activities including sampling and analysis (USEPA, 1997).

**Field blank:** A blank used to obtain information on contamination introduced during sample collection, storage, and transport (Ecology, 2004).

**Initial Calibration Verification Standard (ICV):** A QC sample prepared independently of calibration standards and analyzed along with the samples to check for acceptable bias in the measurement system. The ICV is analyzed prior to the analysis of any samples (Kammin, 2010).

**Laboratory Control Sample (LCS):** A sample of known composition prepared using contaminant-free water or an inert solid that is spiked with analytes of interest at the midpoint of the calibration curve or at the level of concern. It is prepared and analyzed in the same batch of regular samples using the same sample preparation method, reagents, and analytical methods employed for regular samples (USEPA, 1997).

**Matrix spike:** A QC sample prepared by adding a known amount of the target analyte(s) to an aliquot of a sample to check for bias due to interference or matrix effects (Ecology, 2004).

**Measurement Quality Objectives (MQOs):** Performance or acceptance criteria for individual data quality indicators, usually including precision, bias, sensitivity, completeness, comparability, and representativeness (USEPA, 2006).

**Measurement result:** A value obtained by performing the procedure described in a method (Ecology, 2004).

**Method:** A formalized group of procedures and techniques for performing an activity (e.g., sampling, chemical analysis, data analysis), systematically presented in the order in which they are to be executed (EPA, 1997).

**Method blank:** A blank prepared to represent the sample matrix, prepared and analyzed with a batch of samples. A method blank will contain all reagents used in the preparation of a sample, and the same preparation process is used for the method blank and samples (Ecology, 2004; Kammin, 2010).

**Method Detection Limit (MDL):** This definition for detection was first formally advanced in 40CFR 136, October 26, 1984 edition. MDL is defined there as the minimum concentration of an analyte that, in a given matrix and with a specific method, has a 99% probability of being identified, and reported to be greater than zero (Federal Register, October 26, 1984).

**Percent Relative Standard Deviation (%RSD):** A statistic used to evaluate precision in environmental analysis. It is determined in the following manner:

$$\%RSD = (100 * s)/x$$

where s is the sample standard deviation and x is the mean of results from more than two replicate samples (Kammin, 2010).

**Parameter:** A specified characteristic of a population or sample. Also, an analyte or grouping of analytes. Benzene and nitrate + nitrite are all parameters (Kammin, 2010; Ecology, 2004).

**Population:** The hypothetical set of all possible observations of the type being investigated (Ecology, 2004).

**Precision:** The extent of random variability among replicate measurements of the same property; a data quality indicator (USGS, 1998).

**Quality assurance (QA):** A set of activities designed to establish and document the reliability and usability of measurement data (Kammin, 2010).

**Quality Assurance Project Plan (QAPP):** A document that describes the objectives of a project, and the processes and activities necessary to develop data that will support those objectives (Kammin, 2010; Ecology, 2004).

**Quality control (QC):** The routine application of measurement and statistical procedures to assess the accuracy of measurement data (Ecology, 2004).

**Relative Percent Difference (RPD):** RPD is commonly used to evaluate precision. The following formula is used:

$$[\text{Abs}(a-b)/((a + b)/2)] * 100$$

where “Abs()” is absolute value and a and b are results for the two replicate samples. RPD can be used only with 2 values. Percent Relative Standard Deviation is (%RSD) is used if there are results for more than 2 replicate samples (Ecology, 2004).

**Replicate samples:** Two or more samples taken from the environment at the same time and place, using the same protocols. Replicates are used to estimate the random variability of the material sampled (USGS, 1998).

**Representativeness:** The degree to which a sample reflects the population from which it is taken; a data quality indicator (USGS, 1998).

**Sample (field):** A portion of a population (environmental entity) that is measured and assumed to represent the entire population (USGS, 1998).

**Sample (statistical):** A finite part or subset of a statistical population (USEPA, 1997).

**Sensitivity:** In general, denotes the rate at which the analytical response (e.g., absorbance, volume, meter reading) varies with the concentration of the parameter being determined. In a specialized sense, it has the same meaning as the detection limit (Ecology, 2004).

**Spiked blank:** A specified amount of reagent blank fortified with a known mass of the target analyte(s); usually used to assess the recovery efficiency of the method (USEPA, 1997).

**Spiked sample:** A sample prepared by adding a known mass of target analyte(s) to a specified amount of matrix sample for which an independent estimate of target analyte(s) concentration

is available. Spiked samples can be used to determine the effect of the matrix on a method's recovery efficiency (USEPA, 1997).

**Split sample:** A discrete sample subdivided into portions, usually duplicates (Kammin, 2010).

**Standard Operating Procedure (SOP):** A document which describes in detail a reproducible and repeatable organized activity (Kammin, 2010).

**Surrogate:** For environmental chemistry, a surrogate is a substance with properties similar to those of the target analyte(s). Surrogates are unlikely to be native to environmental samples. They are added to environmental samples for quality control purposes, to track extraction efficiency and/or measure analyte recovery. Deuterated organic compounds are examples of surrogates commonly used in organic compound analysis (Kammin, 2010).

**Systematic planning:** A step-wise process which develops a clear description of the goals and objectives of a project, and produces decisions on the type, quantity, and quality of data that will be needed to meet those goals and objectives. The DQO process is a specialized type of systematic planning (USEPA, 2006).

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