



Quality Assurance Project Plan: Effects of 6PPD, 6PPD-Quinone, and Potential Alternatives on Freshwater Mussels

by

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COVER PHOTO: Freshwater mussel shells. PHOTO BY M.C. BARNHART

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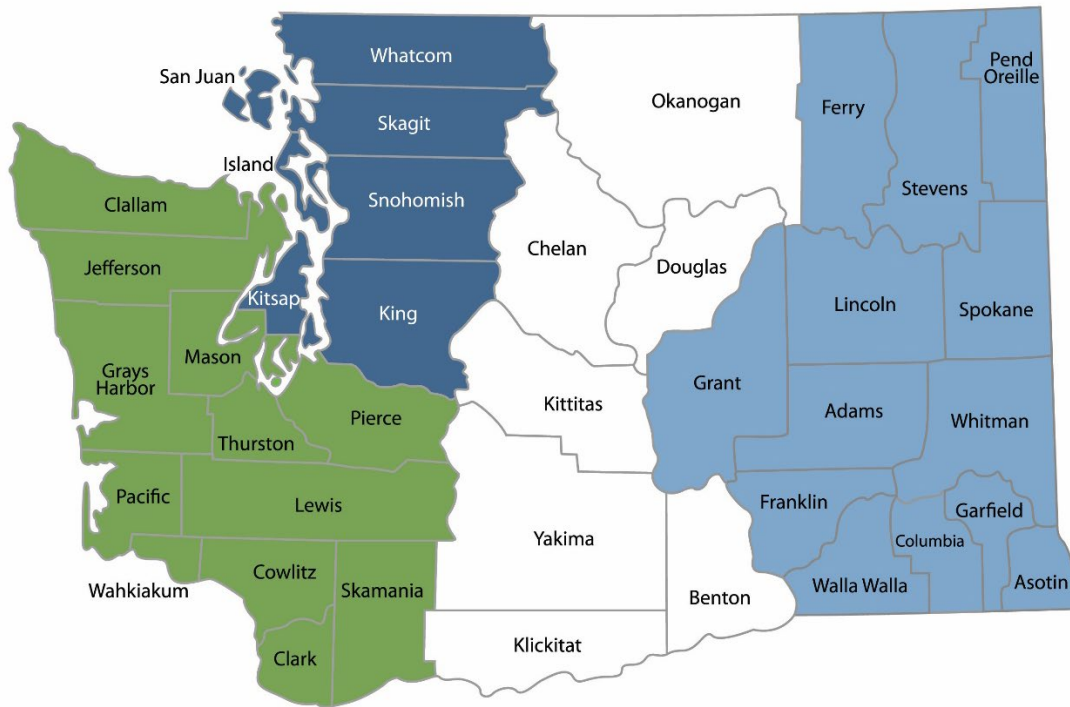
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By Stephanie Gill and David Soucek
January 2026

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2.0 Abstract

N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is an antioxidant and antiozonant widely used in tires. When 6PPD reacts with ozone, it transforms into a breakdown product called 6PPD-quinone (6PPDQ). Both compounds are currently being studied because of their high aquatic toxicity to fish, particularly coho salmon (*Oncorhynchus kisutch*).

The Washington State Legislature directed Ecology to perform an Alternatives Assessment on 6PPD in motor vehicle tires. The goal is to identify and evaluate potential replacement compounds for these products. As part of this work, Ecology is studying how potential alternatives interact with species of concern and compare those effects with what is already known about 6PPD and 6PPDQ. One of those species of concern is freshwater mussels.

Freshwater mussels are an imperiled group of organisms with high cultural and ecological significance. However, there are major data gaps concerning how 6PPD affects aquatic species other than salmonids. Mussels are of particular concern due to their unique parasitic life cycle where a fish host is required to fully develop into a juvenile. If their fish host is sensitive to 6PPD, mussel populations could also be at risk.

This project aims to determine lethal and sublethal toxicity of 6PPD, 6PPDQ, and any potential alternatives to freshwater mussels. This objective will be accomplished by conducting acute toxicity tests with the compounds to determine median lethal concentrations (LC₅₀s) of the tested compounds to freshwater mussels. Sublethal toxicity will be studied by then determining if the presence of 6PPD, 6PPDQ, and potential alternatives impacts the mussel's ability to use a fish host for the important parasitic stage of its life cycle.

3.0 Background

3.1 Introduction and problem statement

For more than 30 years, mass pre-spawn mortality of coho salmon in urban streams has been documented in the Pacific Northwest. This condition is known as urban runoff mortality syndrome. Research shows that this mortality is directly influenced by urbanization density, specifically traffic levels and proximity of waterways to roads (Feist et al. 2011, 2017). Roadway runoff affects both pre-spawn and juvenile salmon and harms many other species of aquatic organisms (Skinner et al. 1999; Kayhanian et al. 2008; McIntyre et al. 2014, 2015). Specifically, however, urban runoff mortality syndrome is responsible for mass die-offs of coho salmon. Using a high-resolution mass spectrometry approach, Tian et al. (2020, 2022) identified 6PPDQ as the toxicant leading to acute toxicity for coho.

6PPDQ is a transformation product of 6PPD, a rubber anti-degradant found in car tires worldwide. 6PPDQ is highly toxic to coho salmon, with lethal effects at concentrations below 1 part per billion (Tian et al. 2020). Monitoring results show that concentrations of 6PPDQ in urban streams in cities such as Seattle, San Francisco, and Los Angeles frequently exceed this level, particularly during storm events (Tian et al. 2022). Recently, 6PPDQ has been shown to be

harmful to rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) but not chum salmon (*Oncorhynchus keta*) or at least seven other salmonids, thus showing varying species-specific sensitivity (McIntyre et al. 2021; Brinkmann et al. 2022).

Freshwater mussels hold enormous cultural significance for Tribal Nations and are officially recognized as a First Food of the Confederated Tribes of the Umatilla Indian Reservation. They also fill an important ecological niche. They help to filter contaminants out of water, stabilize sediments, and are a key food source for terrestrial and aquatic organisms (Haag 2012).

Freshwater mussels also rely on fish as part of their life cycle when they are in their parasitic glochidial stage. Glochidia will typically attach to the blood vessels of a fish's gills and use them as a source of nourishment while they transform into juvenile mussels. If fish hosts are harmed by 6PPDQ, this life cycle stage could be interrupted, leading to population loss.

Currently Ecology is working to identify alternatives to 6PPD in tires to reduce lethality to coho salmon. However, we need to understand how these alternatives may affect other species of cultural and ecological significance, like freshwater mussels, to avoid regrettable substitutions. This includes establishing baseline median lethal concentrations (LC₅₀s) for 6PPD, 6PPDQ, and potential alternatives, as well as assessing their impacts on the freshwater mussel life cycle. This project will follow current work supported by Ecology and test alternatives that have been previously identified as potentially viable. As this work is ongoing, a definitive list has not been finalized.

3.2 Study area and surroundings

This project will take place in a controlled laboratory at the U.S. Geological Survey (USGS) Columbia Environmental Research Center (CERC) in Columbia, Missouri. There will be no outdoor access needed for this project.

3.2.1 History of study area

Not applicable. This project is in a controlled laboratory environment, and the history of the facility is not relevant to the project.

3.2.2 Summary of previous studies and existing data

Not applicable. There are no other studies that test the effects of these alternatives on freshwater mussels.

3.2.3 Parameters of interest and potential sources

The primary contaminants of concern are 6PPD and 6PPDQ. Current research identifies 6PPDQ as the second most toxic chemical to aquatic life ever identified (ITRC 2024). However, research is still ongoing to determine its toxicity to species beyond coho salmon, including potential effects on human health. Preliminary studies in mice have shown that 6PPDQ can accumulate in the liver (Fang et al. 2023) and may alter liver and kidney function after exposure (Zhao et al. 2023). Other contaminants of concern have not yet been finalized and will be determined based on ongoing research from Ecology's 6PPD Alternatives Assessment.

4.0 Project Description

4.1 Project goals

- Identify the effects of 6PPD, 6PPDQ, and potential alternatives on freshwater mussels to avoid regrettable substitutions. Because the Ecology alternatives assessment is ongoing, the specific chemicals tested in this project will depend on what is identified in other active Ecology projects as potential alternatives. Potential chemicals for testing, though not yet finalized, are included in Table 1 below.
- Identify a measure of lethal toxicity by determining baseline LC₅₀ values for all chemicals tested on freshwater mussel juveniles.
- Test potential impacts of 6PPD, 6PPDQ, and alternative exposure on the host-parasite interactions to obtain a measure for sublethal toxicity.

Table 1. List of potential compounds to test.

Compound	CAS Number	Purpose
6PPD: N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine	793-24-8	Parent Chemical of Concern
6PPD -Quinone: N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylene diamine-quinone	2754428-18-5	Transformation Product of Concern
CCPD: N, N'-Dicyclohexyl-4-phenylenediamine	4175-38-6	Potential Alternative to 6PPD
77PD: N,N'-Bis (1,4- dimethyl pentyl)-4- phenylenediamine	3081-14-9	Potential Alternative to 6PPD
7PPD: N ¹ -(1,4-dimethylpentyl)-N ⁴ -phenyl-1,4-benzenediamine	3081-01-4	Potential Alternative to 6PPD

4.2 Project objectives

- Is the sensitivity of western juvenile mussels to 6PPD alternatives similar to, greater than, or less than that of 6PPD and 6PPDQ?
- Is the assessment of effects of 6PPD, 6PPDQ, and alternatives on glochidial attachment and development measurable in a reliable and repeatable fashion?

- Is sensitivity of glochidial attachment and development to 6PPD and 6PPDQ similar to, greater than, or less than that of alternatives.

4.3 Information needed and sources

No existing data need to be assembled. New data includes LC₅₀s for all chemicals tested and mortality values for the life cycle study.

4.4 Tasks required

- Task One: Develop a QAPP.
- Task Two: Determine if 6PPD alternatives have greater, similar, or less toxicity to freshwater mussel juveniles than 6PPD and 6PPDQ.
 - Perform LC₅₀ testing on juvenile mussels for 6PPD, 6PPDQ, and all other compounds of interest.
- Task Three: Determine if exposure to 6PPD, 6PPDQ, and alternatives impacts the reproductive cycle of the freshwater mussel.
 - Expose host fish to chemicals while rearing glochidia on them.

4.5 Systematic planning process

Not applicable. This project is straightforward enough to be thoroughly outlined in this QAPP. Therefore, no formalized systematic planning process is needed.

5.0 Organization and Schedule

5.1 Key individuals and their responsibilities

Table 2 shows the responsibilities of those who will be involved in this project.

Table 2. Organization of project staff and responsibilities.

Staff	Title	Responsibilities
Stephanie Gill Department of Ecology Phone: 564-669-1865	Client	Clarifies scope of the project. Provides internal review of the QAPP and approves the final QAPP.
Samuel Iwenofu Department of Ecology Phone: 360-485-5487	Client QA Coordinator and Senior Analytical Chemist	Conduct QA/QAPP review on behalf of the client and approve the final QAPP
David Soucek USGS Phone: 573-876-1818	Project Manager and Principal Investigator	Writes the QAPP. Oversees experimentation. Conducts QA review of data and analyzes and interprets data. Writes the draft report and final report.
Richelle Perez Department of Ecology Phone: 360-742-6794	Client Section Manager	Approve the final QAPP

5.2 Special training and certifications

Not applicable. No field work or specialized certifications are needed to complete this project.

5.3 Organization chart

Not applicable. See Table 2.

5.4 Proposed project schedule

Tables 3 and 4 list key activities, due dates, and lead staff for this project.

Table 3. Schedule for completing field and laboratory work.

Task	Start Date	End Date	Lead Staff
Toxicology testing of juvenile mussels	November 2025	June 2026	David Soucek
Life cycle testing of juvenile mussels	November 2025	June 2026	David Soucek

Table 4. Schedule for final report.

Task	Due Date	Lead Staff
Draft to supervisor	July 2026	David Soucek
Draft to client/ peer reviewer	August 2026	David Soucek
Final report due on web	Sept 2026	Stephanie Gill

5.5 Budget and funding

Table 5. Project budget and funding.

Item	Description	Year 1	Year 2**	Total
1	Project Management and QAPP	10,461.00	10,774.83	21,235.83
2	Mussel Toxicity Investigation of 6PPD and Alternative Tire Anti-Ozonants	59,000.00	0.00	59,000.00
2a	<i>Funding dedicated to analytical chemistry 6PPD/6PPDQ</i>	<i>9,000.00</i>	<i>0.00</i>	<i>9,000.00</i>
3	Mussel Reproductive Cycle Investigation of 6PPD and Alternative Tire Anti-Ozonants	40,000.00	20,600.00	60,600.00
	Total Direct Charges	109,461.00	31,374.83	140,835.83
	Indirect Charges *	54,982.26	15,759.58	70,741.84
	Total Project Cost (Sum of Items 1, 2, and 3 plus indirect charges)	164,443.26	47,134.41	211,577.67

* Annually established indirect cost rates (overhead) applies of 50.23%

** Contingent on further funding from Ecology

6.0 Quality Objectives

6.1 Data quality objectives

Currently, significant data gaps exist regarding the sensitivity of early life stages of western species of freshwater mussels to 6PPD, 6PPDQ, and alternative tire anti-ozonants and their transformation products.

The main data quality objective (DQO)³ for this project is to successfully: 1) conduct toxicity experiments with juvenile western freshwater mussels (likely *Margaritifera falcata*, Western Pearlshell) with 6PPD, 6PPDQ, and up to eight alternative tire anti-ozonants (“alternatives”) identified from the results of existing Ecology projects; 2) develop a method to assess the effects of 6PPD, 6PPDQ, and alternatives on attachment and development of freshwater mussel glochidia on fish hosts.

This study aims to answer the following questions:

- Is the sensitivity of western juvenile mussels to 6PPD alternatives similar to, greater than, or less than that of 6PPD and 6PPDQ?
- Is the assessment of effects of 6PPD, 6PPDQ, and alternatives on glochidial attachment and development measurable in a reliable and repeatable fashion?
- Is sensitivity of glochidial attachment and development to 6PPD and 6PPDQ similar to, greater than, or less than that of alternatives?

6.2 Measurement quality objectives

Laboratory Control:

A laboratory control is tested with each sample to ensure the quality of the test organisms. The test organism performance in the laboratory control determines if 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. Study data will be considered valid if test organism survival in laboratory controls (test water without added toxicant) is at least 90%, as recommended by ASTM (2023).

³ DQO can also refer to **Decision** Quality Objectives. The need to identify Decision Quality Objectives during the planning phase of a project is less common. For projects that do lead to important decisions, DQOs are often expressed as tolerable limits on the probability or chance (risk) of the collected data leading to an erroneous decision. And for projects that intend to estimate present or future conditions, DQOs are often expressed in terms of acceptable uncertainty (e.g., width of an uncertainty band or interval) associated with a point estimate at a desired level of statistical confidence.

6.2.1 Targets for precision, bias, and sensitivity

6.2.1.1 Precision

Precision is a measure of mutual agreement among individual measurements of the same property, usually under prescribed similar conditions. For this project, measures of analytical precision will be determined by the analysis of laboratory duplicates. Laboratory duplicates are conducted for all analyses, including analytical chemistry, water quality measures, and bioassay replicates, following CERC internal protocols or in standard methods. Laboratory duplicates will be prepared by splitting a sample in the laboratory and carrying the subsamples through the entire analytical process. Precision will be expressed in terms of the relative percent difference (RPD). For all analysis where duplicates are performed, RPD will be calculated as follows:

$$\text{RPD} = \frac{(C1 - C2)}{(C1 + C2)/2} \times 100$$

C1 = larger measured value
C2 = smaller measured value

6.2.1.2 Bias/Accuracy

Accuracy is the closeness of agreement between an observed value and an accepted value. For analytical chemistry, accuracy measurements can be made by testing stock and test solutions. For toxicity tests, no true accuracy measurements are possible because responses to toxicants vary among tests due to many factors such as species, life stage, water quality, and test conditions. Test acceptability will be determined using performance-based criteria outlined by ASTM (2023). Performance of organisms in negative controls (test water without added toxicant) is commonly used as a performance indicator, with ASTM (2023) recommending a minimum survival of 90% in acute toxicity tests and 80% survival in chronic toxicity tests.

6.2.1.3 Sensitivity

Sensitivity is the capability of methodology or instrumentation to discriminate among measurement responses for quantitative differences of a parameter of interest. For chemical analyses, sensitivity will be described by the method detection limit, which is the minimum concentration of a substance that can be measured and reported. Target detection limits for the parameters of interest are based on the applicable methods.

6.2.2 Targets for comparability, representativeness, and completeness

6.2.2.1 Comparability

Comparability expresses the confidence with which one data set can be compared to another. For this project, the comparability of data generated by USGS will be evaluated using test acceptability criteria, such as control survival and growth of organisms, as outlined in standard bioassay method guidance (ASTM 2023). Laboratory data will be compared across existing data sets for species where that data is available (e.g., Prosser et al. 2023; Soucek et al., in press).

The comparability of water chemistry data will be assured through the use of consistent, standardized laboratory methods.

6.2.2.2 Representativeness

Representativeness expresses the degree to which data accurately and precisely represents a characteristic of a population, parameter variations at a sampling point, a process condition, or an environmental condition. For this project, representativeness will be addressed primarily in the experimental design and selection of appropriate procedures, as detailed in ASTM (2023). Representativeness will also be ensured by proper sample handling and storage, and analysis within the accepted holding times, so that the material analyzed reflects the material collected as accurately as possible. When appropriate, the representativeness of the data will be discussed in the draft report developed from all task studies.

6.2.2.3 Completeness

Completeness is a measure of the amount of valid data obtained from a measurement system compared to the amount that was expected to be obtained under normal conditions. Target completeness values are 90% for chemical analyses of water and 100% for ancillary measurements in toxicity tests. Completeness (C) is defined as follows for all measurements:

$$\% C = 100 \times \frac{V}{n}$$

% C = percent completeness;

V = number of measurements judged valid (Section 6.3); and,

n = total number of measurements attempted.

6.3 Acceptance criteria for quality of existing data

Laboratory Control:

A laboratory control is tested with each sample to ensure the quality of the test organisms. The test organism performance in the laboratory control determines if 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. Study data will be considered valid if test organism survival in laboratory controls is at least 90%.

6.4 Model quality objectives

Not applicable. This project does not involve environmental modeling.

7.0 Study Design

7.1 Study boundaries

Not applicable. There is no field work involved in this study. All work will be contained within the controlled laboratory facilities at the USGS Columbia Environmental Research Center.

7.2 Field data collection

7.2.1 Sampling locations and frequency

The study will be completed in the toxicology laboratory (Wet Lab) at CERC. Task 2 study design will follow methods described in ASTM (2023). Task 2 sampling will involve assessment of survival of juvenile mussels exposed to 6PPD, 6PPDQ, and potential alternatives, as well as collecting water samples at the beginning and end of the tests for analytical verification of exposure concentrations.

7.2.2 Field parameters and laboratory analytes to be measured

Some alternatives may not be tested if a suitable supplier cannot be identified.

Potential compounds may include but are not limited to the list in Table 6, identified from the 2021 Ecology Technical Memo and state of current research. Ecology will help source compounds as needed. Compounds tested will depend on availability, stability, and solubility.

Table 6. List of potential compounds to test for Tasks 2 and 3.

Compound	CAS Number	Purpose
6PPD: N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine	793-24-8	Parent Chemical of Concern
6PPD -Quinone: N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylene diamine-quinone	2754428-18-5	Transformation Product of Concern
CCPD: N, N'-Dicyclohexyl-4-phenylenediamine	4175-38-6	Potential Alternative to 6PPD
77PD: N,N'-Bis (1,4- dimethyl pentyl)-4-phenylenediamine	3081-14-9	Potential Alternative to 6PPD

Compound	CAS Number	Purpose
7PPD: N ¹ -(1,4-dimethylpentyl)-N ⁴ -phenyl-1,4-benzenediamine	3081-01-4	Potential Alternative to 6PPD

Test results are based on the endpoint of survival measured 96 hours post-exposure.

7.3 Modeling and analysis design

Dose-response curves will be modelled using USEPA's Toxicity Relationship Analysis Program (TRAP).

7.4 Assumptions underlying design

The purpose of the study is to acquire data on the relative sensitivity of mussels to 6PPD, 6PPDQ, and potential alternative compounds. An underlying assumption of this testing scheme is that because all other factors associated with the testing process except for concentration of chemical added to solution are the same, any observed differences in mortality of the test organisms are due to exposure to the chemical added to the solution.

7.5 Possible challenges and contingencies

7.5.1 Logistical problems

As previously mentioned, the chemicals targeted in this study are not readily available from commercial suppliers and will need to be purchased from specialty providers. Some chemicals may not be available in sufficient quantity, within the timeline, or at a feasible cost for this project. If this occurs, testing for that chemical will be removed from the scope of work. Several providers of chemicals have already been identified, and alternatives are readily available for purchase such that we are confident in being able to get useful information from the experiments, even if we are unable to acquire the ideal chemicals.

Task 3 will involve developing new exposure methods, and it is possible that unforeseen difficulties will emerge. The method as outlined in section 9 below is designed to reduce the potential for error, but important considerations like availability of sufficient diluted well water ("100 Hard" water), and ability to maintain consistent temperature could potentially interfere with this. Staff at CERC have experience managing these kinds of issues.

7.5.2 Practical constraints

In toxicology, it is best practice to verify exposure concentrations of test substances through chemical analysis. However, except for 6PPD and 6PPDQ, the compounds in this study are considered contaminants of emerging concern, and methods for measuring the majority of the chemicals used in this study are not currently available. Due to this limitation, along with budget constraints, analytical chemistry will not be performed on potential alternative

chemicals under this contract. Therefore, LC₅₀ data will be calculated and reported as nominal concentrations. Future amendments may be considered to include analytical chemistry for alternatives if promising results are shown for an alternative to be used in place of 6PPD, and the budget allows.

7.5.3 Schedule limitations

Testing with juvenile life stages of western freshwater mussel species will be dependent on availability of brooding female adults, and the ability to obtain and inoculate appropriate fish species with larvae to produce juveniles.

8.0 Field Procedures

This study will be held in a controlled laboratory environment. There are no field procedures.

8.1 Invasive species evaluation

Not applicable.

8.2 Measurement and sampling procedures

Not applicable.

8.3 Containers, preservation methods, holding times

Not applicable.

8.4 Equipment decontamination

Not applicable.

8.5 Sample ID

Not applicable.

8.6 Chain of custody

Not applicable.

8.7 Field log requirements

Not applicable.

8.8 Other activities

Not applicable.

9.0 Laboratory Procedures

9.1 Lab procedures table

Freshwater mussel rearing methods

Juvenile western freshwater mussels (likely *Margaritifera falcata*, Western Pearlshell) will be produced from females collected by Dr. Alexa Maine of the Confederated Tribes of the Umatilla Indian Reservation. Glochidia will be collected from these females and transformed to juveniles on Rainbow Trout (*Oncorhynchus mykiss*) and propagated for juvenile testing in the laboratory at CERC. Because mussel propagation requires extracting glochidia from gravid adult females, this species must be sourced from the Confederated Tribes of the Umatilla Indian Reservation. This species exists only in the western United States and is of special interest to Ecology because it is one of approximately six species endemic to the region.

Fatmucket (*Lampsilis siliquoidea*) juveniles will be produced from females in CERC culture ponds. Glochidia will be collected from these females and transformed to juveniles on largemouth bass (*Micropterus salmoides*) and propagated for juvenile testing in the laboratory at CERC.

Prior to testing, the adult mussels housed at CERC will be held in well water (hardness ~300 mg/L as CaCO₃) at 10 to 12 °C to prevent the mussels from releasing glochidia. In preparation for testing, the viability of glochidia isolated from each female mussel will be determined as follows: First, glochidia will be gently flushed from the marsupial gills of a female mussel into a 300-mL crystallizing dish using a 1-mm needle and 35-mL syringe filled with well water. Three subsamples of approximately 100 glochidia will be impartially transferred to each of three wells of a 24-well polystyrene tissue-culture plate filled with ~ 2 mL of well water per well. Glochidia in each well will be examined with a dissecting microscope, and the number of closed glochidia will be recorded. After adding one drop of saturated NaCl solution (~ 12 g NaCl in 50 mL of deionized water; Wang et al. 2007) to each well, the numbers of open and closed glochidia will be counted within 1 min. Glochidia that closed in response to NaCl will be classified as alive (or viable), whereas glochidia that are closed before the addition of NaCl or that remained open after the addition of NaCl will be classified as dead (nonviable). The viability of glochidia will be calculated as described in ASTM E2455 (2022) using the following formula:

Viability (%) = $100 \times (\text{number of closed glochidia after adding NaCl solution} - \text{number of closed glochidia before adding NaCl solution}) \div (\text{total number of open and closed glochidia after adding NaCl solution})$.

If glochidia viability from an individual mussel is >80% (preferably >90%; ASTM 2022), the remaining glochidia from that adult mussel will be used for inoculating fish for production of juveniles and for toxicity testing (Fatmucket only). Glochidia isolated from at least three female mussels for each species will be pooled and mixed to obtain organisms to start a toxicity test.

Newly transformed juveniles (ages within 2 days) produced at CERC will be cultured in flow-through auto-feeding system (Kunz et al. 2020) for at least 48 h in test water (i.e., 100 hard water) and test temperature of 23 °C before use in acute toxicity tests.

Toxicity testing (Task 2)

96-hour, acute toxicity tests with juveniles will be conducted in water baths at 23 ± 1 °C under static-renewal conditions (Table 2). Ambient laboratory light (intensity of ~500 lux) will be used with a photoperiod of 16L:8D. Test chambers will be 50-mL glass beakers with 40 mL of test or control (100 hard water) solution. Four replicates will be used per test concentration or control and five juveniles (ideally <10 d old) will be impartially added to each test chamber. Test solutions will be renewed (~75% of water removed and replaced with fresh solutions) at 48-h; mussels will not be fed. At the end of exposures, mussels in each beaker will be examined under a dissecting microscope. The endpoint is mortality (empty shell or gaped shell containing decomposed tissue) plus immobility (no foot or shell movement within a 5-minute observation period).

Table 7. Summary of test conditions for conducting acute toxicity tests with freshwater mussel juveniles in basic accordance with ASTM (2022).

	Test condition	Condition requirements
1	Temperature	23 ± 1 °C
2	Photoperiod (L:D)	16:8
3	Light intensity	~500 lux
4	Test chamber size	50 mL glass beakers
5	Test solution volume	40 mL
6	Nominal test concentrations	TBD
7	Age of organisms at start of test	4-7 days
8	Dilution water/control	100 hard
9	Substrate	none
10	# organisms per chamber	5
11	# chambers per treatment	4
12	Feeding	none

	Test condition	Condition requirements
13	Aeration	none
14	Test type	static, renewal
15	Renewal frequency	on day 2 (~75%)
16	Test duration	96 hours
17	Endpoint	survival
18	Test acceptability criterion	≥ 90% control survival

Water quality and analytical chemistry

Water temperature will be monitored daily in all tests. Other water quality parameters (dissolved oxygen, pH, conductivity, alkalinity, and hardness) will be measured using standard methods in composite samples from all test concentrations at the beginning and end of acute tests.

For verification of test concentrations in preliminary experiments and definitive toxicity tests with mussels, samples will be collected from each test concentration and control at the beginning of the test, before and after the 48-h renewal, and at 96-h. 5 mL samples will be drawn from each replicate tank for a given concentration (four tanks containing water with the same toxicant concentration), combined, and then sub-sampled by transferring 1 mL of the composite to a 4 mL amber glass vial to be stored immediately at –20 °C.

The samples will be analyzed at the USGS, Organic Geochemistry Research Laboratory in Lawrence, KS, using UPLC/MS/MS (Lane et al. 2024). Methods for analysis follow the validated EPA method 1643 with several exceptions. These methods have been compared to EPA 1634 in a published report by Environment and Climate Change Canada (2025). EPA 1634 was developed for larger-volume stormwater and surface water and the USGS direct-inject method provides a similar lower-volume approach. Differences include the use of freezing as a preservation technique and the elimination of solid-phase extraction and the extracted internal standard (EIS), as these are not necessary for the direct injection of small volumes. LC/MS/MS parameters are equivalent to those of EPA 1634, and the non-extracted internal standard (NIS) is used for quantification by isotope dilution. For quantification, calibration curves use ≥7-points with concentrations ranging from 0.001 to 20 µg/L, a 1/x weighting, R > 0.975, and a dynamic linear range of 0.001–20 µg/L ppb. During analytical instrument analysis, 6PPDQ control samples are analyzed with the following acceptance criteria: check and independent check standards with 80–120% accuracy, spike recovery samples with 80–120% recovery, instrument replicate injections within ±20% of each other, laboratory duplicate samples within ±20% of each other, and non-detect in laboratory method blanks. The method reporting limit is

determined by selecting one calibration point above the lowest standard with a signal-to-noise of 15; the lowest standard is 0.001 µg/L and the method reporting level was 0.002 µg/L.

Mussel reproductive cycle investigation (Task 3)

USGS will conduct research to assess effects of anti-ozonants in tires to western mussel reproductive cycles. Methods development with 6PPD and 6PPDQ will occur first using the Fatmucket Mussel (*Lampsilis siliquoidea*) before any testing occurs on western mussels. The western mussel chosen for use will depend on availability from sources. The goal is to determine if exposure of the compounds on host fish will affect glochidia attachment and development to host fish, an important part of the mussel life cycle.

At least two potential alternatives will be tested, in addition to 6PPD and 6PPDQ, contingent on funding. 6PPD and 6PPD- quinone will be used as a reference value, and alternative anti-ozonants will be identified as being either less toxic, similarly toxic, or more toxic to western mussels.

Methods for testing will be as follows: 20 to 24 fish hosts (Largemouth Bass *Micropterus salmoides* or Smallmouth Bass *Micropterus dolomieu*) will be inoculated with 500 counted Fatmucket glochidia for 10 to 20 minutes in a small bucket with aerated water to insure suspension of glochidia. These fish will then be held for two days in clean water in an inoculation rack (Patterson et al. 2018) to allow development of glochidia. Fish will then be moved to a large diluter, randomly assigned to one of four treatments (control, solvent control, low concentration of chemical, high concentration of chemical), where they will be exposed for two days to simulate a storm event. Chemical concentrations will be selected based on the outcome of acute tests with juveniles, and with consideration for concentrations that may be toxic to the fish hosts. After two days, fish will be returned to inoculation racks and held in clean water until day 20 post-exposure to glochidia. All juveniles that drop from fish will be collected, counted, and visually evaluated for presence of obvious abnormalities. This process will be repeated for 6PPD, 6PPDQ, and up to two different alternative chemicals, and then tests will be conducted with a western mussel species (with Rainbow Trout as host).

Table 8. Measurement methods (laboratory).

Analyte	Sample Matrix	Samples (Number/ Arrival Date)	Expected Range of Results	Detection or Reporting Limit	Sample Prep Method	Analytical (Instrumental) Method
6PPD	Water					
6PPDQ	Water					

9.2 Sample preparation method(s)

Chemicals used in the experiments will be purchased commercially. Suppliers still need to be identified for many of the 6PPD alternatives. Stocks of chemicals will be made by dissolving in solvent (either DMSO or ethanol). Stock will be added to exposures waters so that solvent concentrations are less than or equal to 0.1 mL/L. Some chemicals may require a different solvent concentration or composition to facilitate solubility. Suitable controls will be used to provide context for altered solvent choices. Samples of control exposure media will be analyzed to ensure no cross-contamination.

9.3 Special method requirements

Not applicable. No special methods are needed for this project.

9.4 Laboratories accredited for methods

Laboratory accreditation is not required for these methods. The laboratories will be following standard validated methods using prescribed quality controls to ensure reliability of results.

For verification of test concentrations in preliminary experiments and definitive toxicity tests with fish, samples will be collected from each test concentration and control daily. 5 mL samples will be drawn from each replicate tank for a given concentration, combined, and then sub-sampled by transferring 1 mL of the composite to a 4 mL amber glass vial to be stored immediately at -20 °C. The samples will be analyzed at the USGS, Organic Geochemistry Research Laboratory using UPLC/MS/MS following methods outlined in Lane et al. (2024).

10.0 Quality Control Procedures

All research will be conducted in accordance with the [USGS Quality Management System](https://www.usgs.gov/office-of-science-quality-and-integrity/quality-management-system-usgs-laboratories)⁴ (QMS). The USGS QMS establishes foundational requirements for all USGS laboratories to ensure laboratory activities meet defined quality standards, supporting the USGS mission to provide reliable scientific information to the Nation by ensuring quality in laboratory processes, products, and services. Additional policies and center-specific guidance are outlined in the various center and laboratory specific documentation.

For this study, laboratory data recording will follow standard documentation procedures. Data will be recorded in ink, signed, and dated by the person making the entry. Any changes will be corrected by crossing out with a single line, initialed, and dated by the person responsible for the change. All project records and supporting documents will be archived at USGS upon project completion.

Only trained staff will be used for data collection. All instrumentation will be properly calibrated and maintained, and SOPs for use of equipment will be followed.

For UPLC-MS/MS analysis at OGRL, the standard protocol for a positive identification of the analytes is based on the retention time and the ratio of the quantitation and confirmation ion transitions being within $\pm 25\%$. For quantification, calibration curves use a ≥ 6 -points with a $1/x$ weighting, and $R > 0.975$. Quality controls during analysis include check and independent check standards of 80-120% accuracy, spike recovery samples of 80-120% recovery (including both matrix and blank spikes), instrument replicate injections within $\pm 20\%$ of each other, laboratory duplicate samples within $\pm 20\%$ of each other, and non-detects in laboratory method blanks.

10.1 Field and laboratory quality control

Laboratory controls using 100 hard water will be included in this project during toxicity testing to serve as the basis for comparison with all tested chemicals. This will ensure organisms are healthy and responses seen during testing are only due to chemical exposure.

10.2 Corrective action processes

If analysis results do not meet measurement quality objectives (MQOs), we will convene project personnel to decide on the next steps that need to be taken to improve experimental outcomes. This may include repeating the exposures and/or reanalyzing water samples that do not meet quality control criteria. Ecology's project manager will be informed of anomalies and corrective action taken during the execution of the project.

⁴ <https://www.usgs.gov/office-of-science-quality-and-integrity/quality-management-system-usgs-laboratories>

11.0 Data Management Procedures

11.2 Laboratory data package requirements

Data recording practices in the laboratory will follow standard procedures of documentation. Data will be recorded in ink, signed, and dated by the person making the entry. Changes to data will be corrected by crossing out with a single line, initialed, and dated by the person responsible for the change. All project supporting records and documents will be archived at USGS after project completion.

Research conducted, and samples collected/analyzed will adhere to plans in place for data management and quality assurance. The USGS follows a rigorous QA/QC program including sample replicates, blanks, spikes, certified standards, instrument verification checks, and data review to the extent possible with every study. Procedures are documented in SOP for routine activities/analyses or are recorded in field/laboratory notebooks for new, modified, and non-routine activities/analyses.

Data and results are recorded either electronically or in laboratory notebooks and field datasheets (notebooks and datasheets are scanned to electronic format). Electronic files are backed up to the CERC server and redundant external hard drives. The data management plan template which addresses data sharing, documentation, and archiving is found at [Data Management Plans | U.S. Geological Survey](#).⁵ All data will be released in USGS ScienceBase in accordance with the Office of Science and Technology Policy (OSTP) public access and the Office of Management and Budget (OMB) open data directives. In addition, data will be collected following the Branch specific quality control and quality assurance practices which are in accordance with the requirements of the USGS's Quality Management System (USGS OSQI 2022). A Quality Assurance Project Plan (last updated 21 Feb 2013) is available on the CERC intranet. All data will be collected following the Guidelines for Developing Research Study Plans/Protocols, following relevant SOP files, entry into lab data books and data sheets (Appendix 5), and archived following guidance.

Study outlines are developed for each experiment and archived in a shared toxicology folder on the CERC server. For this study statistical analyses of data for each toxicity endpoints will be performed in accordance with guidance outlined in USEPA (2000) and in ASTM (2023) (e.g., calculation of LC₅₀S, EC₅₀S).

11.3 Electronic transfer requirements

Data will be archived in accordance with USGS policies. Metadata including a dataset with individual replicate responses of test organisms and replicate chemical measurements will be created and submitted for USGS review during the manuscript review and released to the

⁵ <https://www.usgs.gov/data-management/data-management-plans>

public when the paper is published. Project plans and data files will be shared through USGS Sharepoint™ collaborative software. Project files are stored on CERC's 120 Terabyte NetApp FAS2620 Network Attached Storage System and backed up incrementally every night and a full backup occurs once a week. Full backups are stored off site using CommVault CommCell backup software and the NetApp AltaVault AVA 400 Cloud-Integrated Storage system. The latter is a Disk to Disk to Cloud backup system in which the off-site backups are stored in a dedicated storage area in the cloud on Amazon Web Services.

11.4 EIM/STORET data upload procedures

Not applicable.

11.5 Model information management

Not applicable. The only modeling for this project is dose-response curve modelling and output is median lethal concentration.

12.0 Audits and Reports

12.1 Field, laboratory, and other audits

Audits will be performed once per year in the form of telephone audits or presentations if data has been obtained.

12.2 Responsible personnel

The Contract Manager (Stephanie Gill) will conduct the audit and will ensure that the project is being performed in a timely manner and done according to the contract and QAPP.

12.3 Frequency and distribution of reports

Quarterly updates will be conducted via email and virtual meetings with presentations to review progress with all project participants. The final report will be a journal article.

12.4 Responsibility for reports

Stephanie Gill, David Soucek, James Kunz.

13.0 Data Verification

13.1 Field data verification, requirements, and responsibilities

Not applicable. There is no fieldwork component to this project.

13.2 Laboratory data verification

At USGS, a series of reviews by technical personnel are implemented to ensure that the data generated for this project meets the data quality objectives and indicators. These reviews include the following:

- Data will be reviewed by laboratory personnel at the end of each working day and at the completion of each task (experiment) to ensure that toxicity testing and analytical activities are completely and adequately documented.
- A 100% verification of data entry into spreadsheets will be performed. The staff member performing the verification will ensure accuracy by comparing the spreadsheet entries with the original hard copy data. Any errors identified will be corrected, and an updated data listing will be generated.

13.3 Validation requirements, if necessary

Chemistry data validation will consist of a review of initial calibrations, calibration checks, and all results for QC samples, such as duplicates, and procedures. Ten percent of the data will be randomly selected for full validation to verify the accuracy of calculations and transcriptions. Validation will follow EPA stage 2B requirements and will be independent of the data generator. Additional full validation will be performed if errors are found, to the extent required to ensure an accurate database.

Toxicity test data will be validated based on test acceptability criteria (e.g., temperature, dissolved oxygen, pH, negative controls), precision (measurement duplicates), and completeness. Procedures will be compared to toxicity test methods and chemical analytical methods, and all manual data manipulations and transcriptions will be reviewed for accuracy.

13.4 Model quality assessment

Not applicable. The only modeling for this project is dose-response curve modelling and output is median lethal concentration.

14.0 Data Quality (Usability) Assessment

14.1 Process for determining project objectives were met

Any data that do not meet the data quality objectives and indicators will be flagged and discussed in the final report. Any limitations on the use of the data will also be reported.

14.2 Treatment of non-detects

Exposure concentrations for 6PPD and 6PPDQ will be known to be above measurable concentration limits and therefore, non-detects are not expected. Furthermore, testing with alternatives will involve use of nominal concentrations only.

14.3 Data analysis and presentation methods

Median lethal concentrations (LC_{50} s) and median effect concentrations (EC_{50} s) will be calculated using Toxicity Relationship Analysis Program (TRAP; Erickson 2015). If TRAP is unable to calculate a satisfactory model fit, the Trimmed Spearman-Kärber method will be used (Hamilton et al. 1977). For task 3, comparison of treatments will involve analysis of variance and post-hoc pairwise testing after testing for normality and variance heterogeneity assumptions using Sigma Plot vers. 15.0 (RRID:SCR_003210).

14.4 Sampling design evaluation

The sampling design will have the necessary statistical power because it follows established guidelines designed to address the issue of statistical power.

14.5 Documentation of assessment

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

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16.0 Appendices

Appendix A. Glossaries, Acronyms, and Abbreviations

Glossary of General Terms

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

Conductivity: A measure of water's ability to conduct an electrical current. Conductivity is related to the concentration and charge of dissolved ions in water.

Diel: Of, or pertaining to, a 24-hour period.

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

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

Glochidia: Parasitic life stage of a freshwater mussel before they fully develop into juveniles where they require nutrients from a host fish to survive.

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.

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

Sediment: Soil and organic matter that is covered with water (for example, river or lake bottom).

Stormwater: The portion of precipitation that does not naturally percolate into the ground or evaporate but instead runs off roads, pavement, and roofs during rainfall or snow melt. Stormwater can also come from hard or saturated grass surfaces such as lawns, pastures, playfields, and from gravel roads and parking lots.

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.

90th percentile: An estimated portion of a sample population based on a statistical determination of distribution characteristics. The 90th 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

DO	(see Glossary above)
e.g.	For example
Ecology	Washington State Department of Ecology
EPA	United States Environmental Protection Agency
et al.	And others
i.e.	In other words
QA	Quality assurance
QC	Quality control
RPD	Relative percent difference
SOP	Standard operating procedures
SRM	Standard reference materials
TOC	Total organic carbon
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey

Units of Measurement

°C	degrees centigrade
G	gram, a unit of mass
mm	millimeter
mg	milligram
mg/L	milligrams per liter (parts per million)
mL	milliliter
mL/L	milliliter per liter
ng/g	nanograms per gram (parts per billion)

Quality Assurance Glossary

Accreditation: A certification process for laboratories, designed to evaluate and document a laboratory'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, 2016).

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, 2016).

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, Laboratory 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, 2016).

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 2016).

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 data quality indicator 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 method quality objectives 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, 2016).

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, 2016).

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, 2016).

Duplicate samples: Two samples taken from and representative of the same population, and carried through all 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, 2016).

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, 2016).

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, 2016).

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, 2016; 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, 2016).

Population: The hypothetical set of all possible observations of the type being investigated (Ecology, 2016).

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, 2016).

Quality control (QC): The routine application of measurement and statistical procedures to assess the accuracy of measurement data (Ecology, 2016).

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, 2016).

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, 2016).

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 data quality objective process is a specialized type of systematic planning (USEPA, 2006).

References for QA Glossary

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