DEPARTMENT OF
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State of Washington

Quality Assurance Project Plan

Environmental Effects-Based Concentrations for Total Petroleum Hydrocarbons (TPH) in Marine Water and Freshwater

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This Quality Assurance Project Plan is available on Ecology's website at https://fortress.wa.gov/ecy/publications/SummaryPages/1703101.html

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Quality Assurance Project Plan

Environmental Effects-Based Concentrations for Total Petroleum Hydrocarbons (TPH) in Marine Water and Freshwater

March 2017

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TCP: Toxics Cleanup Program
WQP: Water Quality Program
EAP: Environmental Assessment Program

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2.0 Abstract

The Washington State Department of Ecology (Ecology) Toxics Cleanup Program is responsible for identifying and remediating sites impacted by hazardous substances. Under Washington's Model Toxics Control Act, Ecology undertakes cleanup of contaminated sites. At many contaminated sites, Ecology has the need to establish surface water concentrations for petroleum contaminants that are protective of aquatic life in both the marine water and freshwater environment. Currently there are no cleanup standards within state regulations that are based on dose-response relationships or effects-based concentrations. The goal of this study is to determine petroleum concentrations that are protective of marine and freshwater organisms.

This study will use chronic exposure toxicity tests on one fish and one invertebrate from marine and freshwater exposed to concentrations of total petroleum hydrocarbons (referred to as *Northwest TPH* or *NWTPH* after the lab method). The NWTPH diesel (Dx) and gasoline (Gx) fractions will be tested on all organisms. The tests will consist of six dilutions of each NWTPH fraction and will be based on literature effects-based concentrations and range-finding tests.

Chronic exposure toxicity tests on aquatic organisms provide knowledge of the lowest-observed effect concentration (LOEC) and no-observed effect concentration (NOEC). The test endpoints include survival rate, growth, and fertilization. The main goal of this project is to provide a statistically defendable maximum NOEC that could be used in the future as guidance for the Washington State Surface Water Cleanup Standards.

3.0 Background

3.1 Introduction and problem statement

The Washington State Department of Ecology (Ecology) Toxics Cleanup Program (TCP) is responsible for identifying and remediating sites impacted by hazardous substances. Under Washington's Model Toxics Control Act (MTCA; WAC 173-340), Ecology sometimes undertakes cleanup of contaminated sites for the federal Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) program. TCP has identified a number of tasks for fiscal years 2017 and 2018 to improve the program's ability to participate in the CERCLA response program. One of the main tasks is the development of standards for aquatic organisms.

At many contaminated sites, Ecology must establish surface water concentrations for petroleum contaminants that are protective of aquatic life in both the marine (salt) water and freshwater environment. Petroleum hydrocarbon contamination in waters of the state is often broadly classified using the analytical methodology for total petroleum hydrocarbon concentrations (TPH; Ecology, 1997). The approach to evaluate TPH includes two methods: NWTPH – gasoline range organics (Gx) and NWTPH – diesel range organics (Dx)[1]. Currently, there are no environmental effects-based concentrations under state or federal regulations for these TPH fractions.

The goal of this study is to use a laboratory-based toxicity test dilution series for NWTPH-Dx and -Gx to determine the no-observed effect concentration (NOEC) and lowest-observed effect concentration (LOEC) for two marine and two freshwater organisms. These effects levels would be directly applicable to whole effluent testing (WET) that is carried out under MTCA as per WAC 173-205. The TCP's Policy and Technical Support Unit would then write an implementation memorandum, recommending protective values under WAC-173-340-730(3)(b)(ii) (Environmental effects) – Surface Water Cleanup Standards.

Washington's WAC 173-205, section 050, states that effluent samples must be tested using multiple species, including at a minimum one fish and one invertebrate. Therefore the toxicity tests in this study will be carried out using both a marine and freshwater fish and invertebrate that have demonstrated sensitivity to hydrocarbons. The organisms include:

Marine water

- Topsmelt (*Atherinops affinis*) – EPA/600/R-95/136, method 1006.0
- Sea urchin (*Strongylocentrotus purpuratus*) – EPA/600/R-95/136

Freshwater

- Fathead minnow (*Pimephales promelas*) – EPA-821-R-02-013, method 1000.0
- Daphnia (*Ceriodaphnia dubia*) – EPA-821-R-02-013, method 1002.0

[1] NWTPH: Northwest total petroleum hydrocarbons, where NWTPH-Gx is in the carbon range C7-C12 and NWTPH-Dx is in the range C10-C24.

3.2 Study area and surroundings

3.2.1 History of study area

Not applicable.

3.2.2 Summary of previous studies and existing data

Using EPA's EcoTox database[2], some idea of effects-based concentrations for marine and freshwater organisms can be inferred (Appendix A). The only fish species in this database common to our organisms – which is exposed to a "petroleum" mixture (CAS# 8002059) – is the marine fish, topsmelt (*Atherinops affinis*) (Table 1). This species has been tested for acute toxicity using crude oil and oil dispersants (Singer et al., 1998). In the acute test, the lowest concentration for the lethal exposure to 50% of the test population was 16,340 µg/L. There were no chronic endpoints in the EcoTox database.

The inland silverside (*Menidia beryllina*) is somewhat comparable to the topsmelt based on life history and habitat; it was tested for similar endpoints (growth and survival by weight) to those in this study. The concentrations of silverside were 700 µg/L for the NOEC and 1500 µg/L for the LOEC (Little et al., 2000).

Benzene, ethylbenzene, toluene, and xylenes (collectively referred to as BTEX) are found within the NWTPH-Gx fraction. EcoTox data is also available for the BTEX compounds. A marine fish similar to topsmelt, atlantic silverside (*Menidia menidia*), has been tested using ethylbenzene and found to have a NOEC of 3,300 µg/L (Table 1; Masten et al., 1994).

In freshwater, there were no EcoTox data for the chronic toxicity of petroleum mixtures on fathead minnows; however, acute testing has been carried out on slimy sculpin, dolly varden, and threespine stickleback using crude oil. Results for the lethal exposure concentration ranged from 1250 to 6890 µg/L (Table 1). Concentrations for NOEC of BTEX on fathead minnows range from 5,400 to 10,200 µg/L.

Given the previous chronic toxicity data of petroleum and BTEX on marine and freshwater fishes, the range-finding tests for this study should begin with a maximum concentration of 10,000 ug/L, with a systematic reduction of contaminant concentration from that point. This concentration (10,000 ug/L) will be below the lethal concentration but above the chronic effects concentration (Table 1).

[2] https://cfpub.epa.gov/ecotox/index.html

Table 1. Summary of effects-based concentrations for fish from EPA's EcoTox database.

Species	Media	Endpoint	Minimum Result (µg/L)	Maximum Result (µg/L)	Contaminant	CAS #
Chronic toxicity endpoints						
inland silverside	SW	LOEC	NA	1500	petroleum	8002059
inland silverside	SW	NOEC	NA	700	petroleum	8002059
fathead minnow	FW	NOEC	NA	10,200	benzene	71432
fathead minnow	FW	LOEC	NA	17,200	benzene	71432
atlantic silverside	SW	NOEC	NA	3,300	ethylbenzene	100414
fathead minnow	FW	NOEC	NA	5,400	toluene	108883
fathead minnow	FW	LOEC	6,000	8,040	toluene	108883
Acute toxicity endpoints						
topsmelt	SW	LC50	16,430	40,200	petroleum	8002059
slimy sculpin, dolly varden, and threespine stickleback	FW	LC50	1,250	6,890	petroleum	8002059
fathead minnow	FW	LC50	12,500	84,000	benzene	71432
pacific herring	SW	LC50	20,000	25,000	benzene	71432
fathead minnow	FW	LC50	9,900	48,510	ethylbenzene	100414
atlantic silverside	SW	LC50	5,100	7,000	ethylbenzene	100414
fathead minnow	FW	LC50	9,390	77,400	toluene	108883
fathead minnow	FW	LC50	13,400	46,000	xylene	1330207

SW: salt water; FW: freshwater; LOEC: Lowest-observed effect concentration;
NOEC: No-observed effect concentration; LC50: Lethal concentration to 50% of test organisms

The effects of the petroleum mixture (CAS#:8002059) also has been tested on marine invertebrates (Table 2). For an urchin, the LOEC for general damage to the organism was found at 60 µg/L (Taban et al., 2004). In a study with effects endpoints similar to our study, O'Clair and Rice (1985) found an LOEC of 200 µg/L for a seastar and an NOEC of 120 µg/L for growth effects. There were no data available for the impacts of petroleum mixture on freshwater invertebrates.

There are also few data available for the chronic effects of BTEX on invertebrates (Table 2). Snell and Moffat (1992) found that a freshwater invertebrate, the rotifer *Brachionus calyciflorus*, had an LOEC of 40,000 µg/L for xylenes and an NOEC of 20,000 µg/L xylenes. There is a considerable increase in concentrations causing mortality (acute toxicity) in both marine and freshwater rotifers for BTEX (Table 2).

The previous data appear to show that rotifers are less sensitive to petroleum and BTEX than other invertebrates. For seastars and urchins, concentrations of petroleum mixtures < 1000 µg/L elicit effects to the organism. The range-finding toxicity tests should assess one to two concentrations below 1000 µg/L.

Table 2. Summary of effects-based concentrations for invertebrates from EPA's EcoTox database.

Species	Media	Endpoint	Minimum Result (µg/L)	Maximum Result (µg/L)	Contaminant	CAS #
Chronic toxicity endpoints						
green sea urchin	SW	LOEC	NA	60	petroleum	8002059
seastar	SW	LOEC	NA	200	petroleum	8002059
seastar	SW	NOEC	120	720	petroleum	8002059
rotifer	FW	LOEC	NA	20,000	xylenes	1330207
rotifer	FW	NOEC	NA	40,000	xylenes	1330207
Acute toxicity endpoints						
hydra	FW	LC50	NA	34,000	benzene	71432
rotifer	FW	LC50	113,000	113,300	toluene	108883
rotifer	SW	LC50	NA	552,600	toluene	108883
rotifer	FW	LC50	252,700	253,000	xylenes	1330207
rotifer	SW	LC50	NA	496,000	xylenes	1330207
rotifer	SW	EC50	NA	99,000	xylenes	1330207

SW: salt water; FW: freshwater; LOEC: Lowest-observed effect concentration;
NOEC: No-observed effect concentration; LC50: Lethal concentration to 50% of test organisms;
EC50: Effect concentration for 50% of test organisms.

3.2.3 Parameters of interest and potential sources

The fractions of petroleum hydrocarbons that are of interest in this study are broadly defined as a volatile petroleum hydrocarbon (NWTPH-Gx) and a semi-volatile petroleum hydrocarbons (NWTPH-Dx). Analytically these two fractions are operationally defined by the extraction methods (Ecology, 1997) and weight of carbon compounds within the fraction (Table 3). In the environment, the carbon ranges within the diesel and gasoline fraction can include a number of products (Table 3). The methods published by Ecology (1997) detailing the quantification of NWTPH-Dx and -Gx also include the chromatograms for each of the products listed in Table 3.

Unlike the methods for extractable petroleum hydrocarbons (EPHs), the preparation of the samples for the NWTPH-Dx method does not include cleaning the media for naturally-occurring organics that can interfere with the quantification, unless it can be shown that naturally occurring organic matter is a significant component of the TPH being detected in the samples (Ecology, 2016). The silica gel cleanup of the sample can lead to a loss of degradation products and polar organics, possibly biasing the measured concentration low. As a secondary objective in this project, we will test a subset of the NWTPH-Dx samples using silica cleanup and no cleanup for comparison.

Table 3. Summary of NWTPH fractions.

NWTPH-Gx (C7-C12)	NWTPH-Dx (C10-C24)
Gasoline	#2 Diesel Oil
Weathered gasoline	#2 Diesel Oil/Motor Oil
Naphtha	#2 Fuel Oil (38% Aromatic)
Mineral spirits #1, #2, and #3	Kerosene (Deodorized)
	Jet Fuel A
	Bunker C #1 and #2
	Motor Oil 30 Wgt.
	Hydraulic Oil (USP)
	Transformer Oil
	Gas Oil

Within the gasoline fraction, monocyclic aromatic hydrocarbons (MAHs) are thought to contribute significantly to aquatic toxicity because they are relatively water-soluble in comparison to other petroleum hydrocarbons (McGrath and Di Toro, 2009). MAHs contain one benzene ring and are comprised mainly of benzene, toluene, ethylbenzene, and three xylene isomers (collectively referred to as BTEX).

The diesel fraction is a more complex mixture of hydrocarbons. Many of the polycyclic aromatic hydrocarbons (PAHs) are present in the diesel fraction. Aquatic toxicity of individual PAHs have been investigated in the past, and there is a strong relationship between the partition coefficient of the compounds (Kow) and the acute toxicity, where lighter compounds are more acutely toxic (McGrath and Di Toro, 2009; Redman and Parkerton, 2015). Following the final toxicity tests, the sentinel PAH compounds, naphthalene and benzo(a)pyrene, will be analyzed in a small number of samples, if sufficient sample remain.

3.2.4 Regulatory criteria or standards

This study is designed to inform the Washington State Surface Water Cleanup Standards (WAC-173-340-730). In particular, section 3(b)(ii) of this regulation pertains to the use of WET testing under the federal National Toxics Rule (40 C.F.R. Part 131) which states:

> ***(ii) Environmental effects.*** *For hazardous substances for which environmental effects-based concentrations have not been established under applicable state or federal laws, concentrations that are estimated to result in no adverse effects on the protection and propagation of wildlife, fish, and other aquatic life. Whole effluent toxicity testing using the protocols described in chapter* ***173-205*** *WAC may be used to make this demonstration for fish and aquatic life.*

There are currently no numeric environmental effects criteria or standards for surface water in Washington State for NWTPH-Dx and -Gx. The Washington State Water Quality Standards (WAC 173- 201A), section 260(2)(b), state that the "Aesthetic values must not be impaired by the presence of materials or their effects, excluding those of natural origin, which offend the senses of sight, smell, touch, or taste...". This would include oily sheens from hydrocarbon contamination.

Ecology's Toxics Cleanup Program (TCP) has guidance on the remediation of petroleum-contaminated sites (Ecology, 2016). Part of remediating a contaminated site is establishing a level or concentration of petroleum hydrocarbons as a "cleanup standard". For waters of the state, WAC 173-340-730 (3)(b)(iii)(C) provides that Method A groundwater TPH cleanup levels may be used as surface water Method B petroleum cleanup levels protective of human health. The cleanup levels for diesel range organics by NWTPH-Dx and gasoline range organics by NWTPH-Gx are shown in Table 4.

Site-specific surface water Method B petroleum protective concentrations can be derived using Equations 730-1 and 730-2 (WAC 173-340-730 pg. 164). However, the concentration calculated under Method B would then default to the practical quantitation limits (PQLs) for the NWTPH-Dx and -Gx (Table 4) if the calculated protective value is lower than the PQL for the specific contaminant.

Table 4. Cleanup concentrations for petroleum hydrocarbons in surface waters (Ecology, 2016).

Petroleum Hydrocarbons	Method A†	Method B (site-specific)*
NWTPH-Dx	500 µg/L	500 µg/L
NWTPH-Gx (benzene present)	800 µg/L	250 µg/L
NWTPH-Gx (no detectable benzene)	1000 µg/L	250 µg/L

† Table 720-1 WAC 173-340-730.

* The lowest concentration of either equation 730-1 or 730-2 (WAC 173-340-730 pg. 164) or the practical quantitation limits listed in Table 4.

4.0 Project Description

This study is a laboratory-based series of toxicity tests using diesel fuel and gasoline to determine the NOEC and LOEC on two freshwater and two marine organisms. A proposed 0.5 dilution series will be carried out by a contract lab with sub-sampling of the bioassay waters to confirm chemical concentrations (this may be modified after range-finding tests). Data from the lab will be entered into the program, Comprehensive Environmental Toxicology Information System (CETIS), which is Ecology's database for WET testing results and data analysis[3]. Water chemistry data also should be entered into Ecology's Environmental Information Management (EIM) database. Statistically valid NOEC and LOEC concentrations will then be generated and reported.

4.1 Project goal

The project goal is to produce an environmental effects-based NOEC and LOEC concentration of diesel and gasoline range hydrocarbons that can be used to propose numerical cleanup levels protective of aquatic life in marine water and freshwater pursuant to WAC 173-340-730(3)(b)(ii).

4.2 Project objectives

The project objectives include:

- Supervise a laboratory-based series of toxicity tests using diesel fuel and gasoline to determine the NOEC and LOEC on two freshwater and two marine organisms. A proposed 0.5 dilution series (to be determined after range-finding tests) will be carried out by a contract lab with sub-sampling of the bioassay waters to confirm chemical concentrations.
- Enter data into CETIS. Water chemistry data should also be entered into the EIM database.
- Analyze the toxicity test data to establish a statistically defendable NOEC and LOEC for NWTPH-Dx and NWTPH-Gx in marine and freshwater.
- Test whether there is a statistically significant difference between concentrations of NWTPH-Dx when using silica gel cleanup in the lab preparation methods.

4.3 Information needed and sources

Previous bioassays and effects-level concentrations for petroleum hydrocarbons will assist the contract lab in determining starting points for the dilution series. Some of these data are already summarized in this QAPP (section *3.2.2 Summary of Previous Studies and Existing Data*).

[3] http://www.ecy.wa.gov/programs/wq/wet/index.html

4.4 Tasks required

The project tasks include:

- Write the Quality Assurance Project Plan (QAPP) and the Request for Qualifications (RFQ) for the contract bioassay lab.
- Design the dilution bioassay series with the contract lab.
- Ecology's Manchester Environmental Laboratory (MEL) validate an internal NWTPH-Dx and -Gx spike for the contract lab and analyze all subsamples of the toxicity tests.
- Ensuring communication and liaison between the contract lab and MEL.
- Receive and analyze the bioassay data in CETIS to establish the NOEC and LOEC concentrations.
- Enter chemical concentrations into EIM.
- Write the final report.

4.5 Systematic planning process used

This QAPP represents the systematic planning process.

5.0 Organization and Schedule

5.1 Key individuals and their responsibilities

Table 5. Organization of project staff and responsibilities.

Staff	Title	Responsibilities
Arthur Buchan TCP Phone: 360-407-7146	EAP Client	Clarifies scope of the project. Provides internal review of the QAPP, approves the budget and approves the final QAPP.
William Hobbs TSU-SCS-EAP Phone: 360-407-7512	Project Manager	Writes the QAPP and RFQ. Oversees bioassay design by contract laboratory. Conducts QA review of data, analyzes and interprets data, and enters data into CETIS. Writes the draft report and final report.
Randall Marshall WQP Phone: 360-407-6445	Project Scientist	Conducts QA review of data, analyzes and interprets data, and enters data into CETIS.
Siana Wong TSU-SCS-EAP Phone: 360-407-6432	Project Scientist	Assists with data management and enters chemical concentrations in EIM.
TCP Aquatics Unit (Russ McMillan, Pete Adolphson, Fu-Shin Lee)	Project Toxicologists	Conducts QA review of data (both biologic and chemistry). Provides internal review of the QAPP and project scope
Debby Sargeant TSU-SCS-EAP Phone: 360-407-6775	Unit Supervisor for the Project Manager	Provides internal review of the QAPP, approves the budget, and approves the final QAPP.
Jessica Archer SCS-EAP Phone: 360-407-6698	Section Manager for the Project Manager	Reviews the project scope and budget, tracks progress, reviews the draft QAPP, and approves the final QAPP.
Joel Bird MEL-EAP Phone: 360-871-8801	Director	Reviews and approves the final QAPP.
Contract Laboratory	Project Manager	Reviews draft QAPP, coordinates with MEL QA Coordinator; oversees toxicity tests and reporting.
William R. Kammin Phone: 360-407-6964	Ecology Quality Assurance Officer	Reviews and approves the draft QAPP and the final QAPP.

TCP: Toxics Cleanup Program; TSU: Toxics Studies Unit; SCS: Statewide Coordination Section;
EAP: Environmental Assessment Program; WQP: Water Quality Program;
EIM: Environmental Information Management database; QAPP: Quality Assurance Project Plan;
RFQ: Request for Qualifications; MEL: Manchester Environmental Laboratory.

5.2 Special training and certifications

This project requires knowledge of the CETIS program and database. CETIS will be used to analyze the data and calculate a statistically defendable LOEC and NOEC. Randall Marshall, project scientist, is Ecology's WET testing program coordinator and is responsible for the oversight of WET testing in National Pollutant Discharge Elimination System (NPDES) permits. He is a trained toxicologist and has the experience to provide the necessary quality assurance oversight for the bioassay data. In addition, the aquatic toxicologists listed in Table 5 will provide review of, and recommendations for, the biologic and chemistry data.

5.3 Organization chart

Not Applicable - See Table 6.

5.4 Proposed project schedule

Table 6. Proposed schedule for completing field and laboratory work, data entry into CETIS, and reports.

Field and laboratory work	Due date	Lead staff
Lab analyses completed (MEL)	May 2017	
Lab analyses completed (contract)	May 2017	
CETIS database		
Study ID	WHOB005	
Product	Due date	Lead staff
CETIS data loaded	July 2017	Randall Marshall
Environmental Information System (EIM) database		
EIM Study ID	WHOB005	
Product	Due date	Lead staff
EIM data loaded	June 2017	Melissa McCall
EIM data entry review	June 2017	Siana Wong
EIM complete	July 2017	Melissa McCall
Final report		
Author lead / Support staff	William Hobbs and Randall Marshall	
Schedule		
Draft due to supervisor	July 2017	
Draft due to client/peer reviewer	August 2017	
Final (all reviews done) due to publications coordinator	September 2017	
Final report due on web	October 2017	

5.5 Budget and funding

The detailed laboratory budget for the project is shown in Table 7. The total project budget allocated by TCP is $210,000.

Table 7. Project budget and funding.

Parameter	Number of Samples	Number of QA Samples	Total Number of Samples	Cost Per Sample	Lab Subtotal
NWTPH-Gx (incl. BTEX)	153	50	203	$100	$20,300
NWTPH-Dx (no silica clean-up)	153	50	203	$100	$20,300
NWTPH-Dx (silica clean-up)	50	10	60	$100	$6,000
PAHs (naphthalene and benzo(a)pyrene)	10	2	12	$200	$2,400
				MEL Subtotal	$49,000
				Contract Lab Subtotal	$81,000
				Lab Grand Total	$130,000

Budget Items	Estimated Cost
Salary, benefits, and indirect/overhead	$61,000
Contracts (Bioassay contract lab)	$81,000
Laboratory (MEL)	$49,000
Laboratory contingency	$19,000
Project Total	**$210,000**

NWTPH-Dx: Northwest Total Petroleum Hydrocarbon Diesel fraction;
NWTPH-Gx: Northwest Total Petroleum Hydrocarbon Gasoline fraction;
BTEX: benzene, toluene, ethylbenzene, and xylenes.

6.0 Quality Objectives

6.1 Data quality objectives (DQO)

Before beginning the toxicity tests, MEL will certify an internal standard for NWTPH-Dx and -Gx or work with the contract lab to purchase a certified standard. The contract lab will use the standard to make the stock dilution series. The standard will be mixed with lab-grade solvents in order to remain dissolved and act as a carrier and dispersant when diluted with water. The MEL standard will be mixed well ahead of time and tested for stability over time (at least one week) as part of the certification. The variability during the certification period should be no more than 40% relative standard deviation (RSD) which is compatible with the NWTPH method (Table 8).

Before the contract lab begins the toxicity tests, they will be asked to mix a trial stock dilution series consisting of three concentrations (e.g., 10000 ppb, 1000 ppb, and 100 ppb for NWTPH-Gx). Each concentration will be subsampled three times for confirmation of the nominal concentrations. The contract lab must meet an RSD of 40% to continue. If not, the protocols for mixing reliable stock solutions will need to be re-evaluated.

During the range-finding toxicity tests, the nominal and measured concentrations of the fresh stock solutions will continue to be evaluated, as per an agreed-upon sampling schedule with MEL and the contract lab. If the samples are consistently within 40% relative percent difference (RPD), the number of confirmatory samples will be reduced.

6.2 Measurement quality objectives (MQO)

6.2.1 Targets for precision, bias, and sensitivity

The MQOs for project results, expressed in terms of acceptable precision, bias, and sensitivity, are described in this section and summarized in Table 8. There are no defined MQOs for toxicity testing; however, the factors affecting precision, bias, and sensitivity in establishing effects-based concentrations are discussed below.

Table 8. Measurement quality objectives for water chemistry.

Parameter	Precision		Bias			Sensitivity
	Duplicate Samples	Matrix Spike-Duplicates	Verification Standards (LCS,CCV)	Matrix Spikes	Surrogate Standards*	MDL or Lowest Conc. of Interest
	Relative Percent Difference (% RPD)		Recovery Limits (%)			Concentration Units
NWTPH-Dx†	< 40%	< 40%	70-130%	70-130%	50-150%	0.15 mg/L
NWTPH-Gx‡	< 50%	<40%	70-130%	70-130%	70-130%	0.07 mg/L
Benzene	< 50%	< 50%	70-130%	70-130%	70-130%	0.26 µg/L
Ethylbenzene	< 50%	< 50%	70-130%	70-130%	70-130%	0.15 µg/L
Toluene	< 50%	< 50%	70-130%	70-130%	70-130%	0.11 µg/L
Xylenes	< 50%	< 50%	70-130%	70-130%	70-130%	0.24 µg/L
Naphthalene	< 40%	< 40%	80-120% (41-105% LCS)	40-100%	10-140%	0.05 µg/L
Benzo(a)pyrene	< 40%	< 40%	70-130% (14-129% LCS)	40-110%	30-120%	0.05 µg/L

*Surrogate recoveries are compound specific
† Based on the analysis of #2 Diesel (CAS#: 68476-34-6)
‡ Based on the analysis of gasoline (CAS#: 86290-81-5)
LCS: Lab Control Sample
CCV: Continuing Calibration Verification Standard
MDL: Method Detection Limit

6.2.1.1 Precision

Precision is a measure of variability between results of replicate measurements that is due to random error. Laboratory duplicate precision for the water chemistry is detailed in Table 8. With each sampling event of the bioassay test chambers, a replicate sample will be taken. The RPD for the field replicates (samples from the test chambers) will be <40% for the NWTPH-Dx and PAHs and <50% for the NWTPH-Gx and BTEX.

Precision for the toxicity tests is measured and controlled through the use of reference toxicants. The contract lab will provide an assessment of precision for toxicity tests on the organisms to be tested in this project. Based on an inter-laboratory study by the United States Environmental Protection Agency (USEPA, 2000), expected coefficient of variations (CV) for precision around the toxicity tests is expected to meet the median results in Table 9.

A number of factors in the lab influence the variability of the toxicity tests and therefore the precision, including (USEPA, 2002a): test organism, dilution water quality, temperature control, and the quality and quantity of food provided. The optimal ranges of these factors are described in each toxicity test method and also described in section *9.0 Laboratory Methods*.

Table 9. Percentiles of the coefficient of variation for the NOEC (USEPA, 2000).

Test Organism	Method	Percentiles		
		25th	50th (median)	75th
Fathead minnow larval survival	1000.0	0.26	0.39	0.48
Fathead minnow larval growth	1000.0	0.22	0.37	0.53
Ceriodaphnia survival	1002.0	0.21	0.30	0.43
Ceriodaphnia reproduction	1002.0	0.25	0.33	0.49
Topsmelt larval survival*	1010.0	0.42	0.42	0.42
Topsmelt larval growth*	1010.0	0.31	0.31	0.31
Sea urchin fertilization	EPA/600/R-95/136	0.40	0.50	0.69

* One lab participated using this method

6.2.1.2 Bias

Bias is the difference between the population mean and the true value. Bias is usually addressed by calibrating field and lab instruments, and by analyzing lab control samples, matrix spikes, and/or standard reference materials. The necessary targets for the water chemistry samples are listed in Table 8.

Bias in the toxicity tests is controlled through replication of the control samples using reference toxicants. The USEPA (2002b,c) guidance documents on chronic toxicity testing recommend that labs summarize the last 20 control toxicity tests when reporting effluent toxicity data.

6.2.1.3 Sensitivity

Sensitivity is a measure of the capability of a method to detect a substance. It is commonly described as a detection limit. The method detection limits for the water chemistry analysis are listed in Table 8.

The sensitivity of the toxicity tests is dependent on the number of replicates per concentration. The sensitivity is assessed by comparing against the control tests that are run concurrently. There is a recommended minimum significant difference (MSD) for each method (USEPA, 2000). The MSD is the smallest difference between the control and another test treatment that can be determined as statistically significant. The MSD is often expressed as the %MSD of the mean control value. In Washington State, WAC 173-205 defines a "Chronic statistical power standard" that represents the maximum %MSD of the test:control. The chronic statistical power standard is 39%, meaning the percent difference in a statistically significant response (i.e., %MSD) must be less than or equal to 39% to be acceptable. The power standards prevent large effects from being classified as non-toxic (i.e., false negatives).

6.2.2 Targets for comparability, representativeness, and completeness

6.2.2.1 Comparability

Sampling techniques for the petroleum hydrocarbons will follow the Ecology Spills Program SOP SPL003 (Davis, 2011). The bioassays will follow EPA methods to ensure comparability with future bioassays and WET testing. The EPA methods include:

- Topsmelt (*Atherinops affinis*) – EPA/600/R-95/136, method 1006.0
- Sea urchin (*Strongylocentrotus purpuratus*) – EPA/600/R-95/136
- Fathead minnow (*Pimephales promelas*) – EPA-821-R-02-013, method 1000.0
- Daphnia (*Ceriodaphnia dubia*) – EPA-821-R-02-013, method 1002.0

6.2.2.2 Representativeness

The water samples from the bioassays will be representative of both the fresh stock solution used in the bioassays and the stale old solution that the organisms were exposed to. The fresh stock solution will be sampled as it is being added to the chambers to ensure that it represents the starting concentration of the assay. The concentration of the stale solutions will be used as the exposure concentration to establish the NOEC and LOEC. These samples will be taken directly from the test chambers.

The test species selected in this project are representative of west coast marine water and freshwater organisms. In addition, the test species represent organisms that are sensitive to hydrocarbons, particularly polycyclic aromatic hydrocarbons (PAHs) (Marshall, 2016).

6.2.2.3 Completeness

The project will be considered complete if 95% of the bioassays are carried out and the samples are successfully taken.

6.3 Acceptance criteria for quality of existing data

Not applicable.

6.4 Model quality objectives

Not applicable.

7.0 Study Design

7.1 Study boundaries

Not applicable

7.2 Field data collection

7.2.1 Sampling location and frequency

Subsamples of the fresh stock water will be taken during the filling of the test chambers. Subsamples of the old test chamber solutions will be collected as a composite of the chamber replicates (see section *9.1 Lab Procedures Table*) prior to renewal or the end of the test. For quality control (QC) of the water chemistry, a replicate sample of the stock solution will be collected from one concentration for each species and each contaminant during each sampling event.

Each of the toxicity tests will require the renewal of water during the period of exposure as described in EPA guidance documents on the short-term methods for chronic exposure (static renewal tests) (USEPA, 2002b,c), except the sea urchin bioassay. In addition to the static renewal testing, full spectrum UV exposure will be used (Ecology, 2015). The contract lab will mix fresh stock solution and dilutions for the renewal. The proposed strategy for subsampling the stock solutions, fresh and stale, is detailed in Table 10. The total number of samples collected is estimated to be between 203 and 257, depending on whether stock solutions can be shared between dilution series. However, these estimates may change if the contract lab has an alternate strategy or the initial range-finding tests suggest that a more conservative sampling schedule for the fresh solutions is necessary.

The lab will begin with an exercise to test the precision of the mixing protocols over three concentrations. Triplicate samples will be taken of each concentration. The concentration of the fresh solution is what is used as the effects-based concentration; therefore, we will assure the precision of this concentration is adequate before proceeding with the toxicity tests.

Following confirmation that the nominal concentrations are accurate, range-finding toxicity tests will be completed for all organisms using three concentrations. It is likely that the range-finding tests will be conducted using a 0.3 dilution series based on the previous data presented in section *3.2.2 Summary of Previous Results and Existing Data*. The fresh solutions will be tested each day to continue to confirm that the nominal concentration is accurate. If the nominal and measured concentrations of the stock solutions are < 20% relative percent difference (RPD) in the range-finding tests, then the number of subsamples for the fresh and stale solutions will be reduced in the follow-up chronic toxicity tests.

Table 10. Proposed subsampling schedule for water chemistry.

	t=0	t=24	t=48	t=72	t=96	t=120	t=144	Total samples
Stock dilution series confirmation (triplicate samples; 3 concentrations)								**9**
Range-Finding Test								
Topsmelt								
Fresh Stock	3 + 1 QC	3 + 1 QC	3 + 1 QC	3 + 1 QC	3 + 1 QC	3 + 1 QC		**24**
Test chamber		3 + 1 QC			3 + 1 QC		3 + 1 QC	**12**
Urchin								
Fresh Stock	6 + 1 QC							**7**
Test chamber								**0**
Fathead minnow								
Fresh Stock	3 + 1 QC	3 + 1 QC	3 + 1 QC	3 + 1 QC	3 + 1 QC	3 + 1 QC		**24**
Test chamber		3 + 1 QC			3 + 1 QC		3 + 1 QC	**12**
Daphnia								
Fresh Stock‡								**0**
Test chamber		3 + 1 QC			3 + 1 QC		3 + 1 QC	**12**
Chronic Bioassay Test								
Topsmelt								
Fresh Stock	4 + 1 QC	4 + 1 QC	4 + 1 QC	4 + 1 QC	4 + 1 QC	4 + 1 QC		**30**
Test chamber		3 + 1 QC			3 + 1 QC		3 + 1 QC	**12**
Urchin								
Fresh Stock	6 + 1 QC							**7**
Test chamber								**0**
Fathead minnow								
Fresh Stock	4 + 1 QC	4 + 1 QC	4 + 1 QC	4 + 1 QC	4 + 1 QC	4 + 1 QC		**30**
Test chamber		3 + 1 QC			3 + 1 QC		3 + 1 QC	**12**
Daphnia								
Fresh Stock‡								**0**
Test chamber		3 + 1 QC			3 + 1 QC		3 + 1 QC	**12**
								203

t = time in hours.
‡ = assumes the same concentration of fresh stock can be used for both freshwater organisms.
QC = quality control

7.2.2 Field parameters and laboratory analytes to be measured

The bioassay test chambers will be monitored for temperature, dissolved oxygen, salinity (if applicable), conductivity, pH, and light. All limits and range of these parameters are discussed in section *9.1 Lab Procedures*.

Water samples collected of the stock solutions and stale test chamber solutions will be analyzed for:

- NWTPH-Gx (C7-C12).
- Benzene, ethylbenzene, toluene, and xylenes (BTEX).
- NWTPH-Dx (C10-C24).
- Naphthalene and benzo(a)pyrene if sufficient sample exist following analysis of the above.

7.3 Modeling and analysis design

Not applicable. No model is being developed in this project.

7.4 Assumptions in relation to objectives and study area

The major assumption affecting study design is that the spiked petroleum hydrocarbons will remain in solution throughout the period of exposure and will not form droplets or bind to organics (i.e., feces) in the test chambers. In other words, we are assuming that the measured concentrations of the test chambers represent the true exposure concentrations.

7.5 Possible challenges and contingencies

In an article by Redman and Parkerton (2015), a number of considerations were highlighted that can impact the efficacy and interpretability of aquatic toxicity tests using petroleum. Considerations that are relevant to the bioassays we will run are summarized in the sections below.

7.5.1 Logistical problems

One of the main issues with conducting aquatic toxicity tests using petroleum hydrocarbons can be ensuring the concentrations of the dilutions or mixtures are accurate. Working with low solubility materials will be a prerequisite of the contract lab. MEL will be certifying an internal standard for the contract labs to dilute and use in the toxicity tests. MEL will also assess the stability of the standards over a one- to two-week period prior to the toxicity tests. In order for the gasoline and diesel standards to be diluted and remain dissolved in the standards, a solvent will need to be used as a carrier and dispersant. However, the concentration of the solvent must be below the adverse-effects level for the test organism. Consensus between MEL and the contract lab on the most appropriate concentrations to mix, and how, will take place before the tests begin.

Properly quantifying the composition of the hydrocarbon mixtures and concentrations in the exposure media is an important step in establishing reliable effects concentrations. We will use known mixtures (standards) and dilute to our own internal standards. These standards will be

certified by MEL. Before the contract lab starts the tests, the accuracy of the stock solutions mixed from the internal standard will be assessed (see section *6.1 Data Quality Objectives*).

Maintaining the proper conditions for the test chambers (i.e., dissolved oxygen, pH, temperature, and salinity) is prescribed under the EPA methods. Contract labs accredited by Ecology have SOPs in place for monitoring and responding to instances when the conditions are outside the optimal range. The lab may have to repeat a test if the test conditions are violated.

In addition to the test conditions, toxicity tests with hydrocarbons must maintain a consistent headspace among replicates and concentrations. Because of the potential for losing more volatile compounds during the exposure, it is also advisable to monitor the loss between fresh and stale solutions. We plan to constrain this potential loss of hydrocarbons at numerous times throughout the toxicity tests. Furthermore, the headspace needs to be a balance between minimizing volatile loss and allowing for sufficient oxygen for the test organisms. The contract lab will advise Ecology on the optimal headspace in the test chambers and also document the conditions in lab bench sheets.

7.5.2 Practical constraints

The toxicity tests being carried out in this project are commonly used, and we do not foresee any practical constraints conducting the tests. MEL routinely mixes internal standards, and we do not foresee any practical constraints with the analysis of the water chemistry.

The only practical constraint is the time period funding is available: laboratory analysis must be completed by May 31, 2017.

7.5.3 Schedule limitations

MEL will certify an internal standard for the contract lab to use in mixing the dilution series. MEL will also analyze all the water chemistry samples. Possible schedule limitations with sample receipt and flow will be planned around existing sample load at MEL.

8.0 Field Procedures

8.1 Invasive species evaluation

Not applicable.

8.2 Measurement and sampling procedures

Sample collection for the analysis of hydrocarbons will follow the Ecology general SOP for the Spills Program; *Standard Operating Procedure for Collecting Oil Spill Water Samples, SPL003* (Davis, 2011).

8.3 Containers, preservation methods, holding times

Table 11. Sample containers, preservation, and holding times.

Parameter	Matrix	Minimum Quantity Required	Container	Preservative	Holding Time
NWTPH-Dx	water	500 ml	1 L Amber glass bottle	1:1 HCl, Cool to ≤6 °C	14 days preserved
NWTPH-Gx w/ BTEX	water	40 mL no headspace	(3) 40 mL vials w/septum	HCl, Cool to ≤6 °C	14 days
PAHs	water	500 ml	1 L Amber glass bottle	1:1 HCl, Cool to ≤6 °C	14 days preserved

8.4 Equipment decontamination

Not applicable

8.5 Sample ID

Sample names for the water chemistry will follow the convention: Species-dilution-hour-stock, where the species abbreviations will be Ppro (*Pimephales promelas*), Cdub (*Ceriodaphnia dubia*), Aaff (*Atherinops affinis*), and Spur (*Strongylocentrotus purpuratus*). An example would be the 50% dilution of the daphnia bioassay at the 96-hour mark and a new stock renewal: Cdub-50-96-new.

8.6 Chain-of-custody

Water chemistry samples from the bioassays will be assigned a MEL work order number for either range-freshwater, range-marine, chronic-freshwater, or chronic-marine. Sample chain-of-custody and submission will be overseen by Ecology with the contract lab.

8.7 Field log requirements

Bench sheets documenting each toxicity test will be required from the contract lab. Observations necessary include:

- A readable copy of all bench sheets and chain-of-custody, including toxicological and nominal and actual water chemistry data. Water chemistry will be confirmed and supplied by MEL.
- Test chamber parameters: temperature, dissolved oxygen, salinity (if applicable), conductivity, pH, and light.
- Bench sheets must record counts of number alive (not percentages or number dead) in order to be acceptable.
- Start counts must be clearly recorded on the bench sheet.
- Test report must include computer printouts of test data and summary results of statistical analyses. The full details of the statistical analyses do not need to be printed and included in reports
- Test organism source, age, and unusual conditions (e.g., lethargy, hyperactivity, spots or filaments, discoloration, excessive ventilation) should be reported.
- Special circumstances such as treatment system upsets known to exist at the time of sample collection must be reported.
- Time that water was renewed and sampled.

8.8 Other activities

Not applicable

9.0 Laboratory Procedures

9.1 Lab procedures table

The project consists of four chronic toxicity tests (Table 12) based on a dilution series using a stock solution mixed from hydrocarbon standards of gasoline (NWTPH-Gx) and diesel (NWTPH-Dx). A series of range-finding tests will be carried out before the chronic toxicity tests. The toxicity tests will consist of six concentrations of petroleum hydrocarbons on both a vertebrate and invertebrate organism in marine and freshwater. The dilution series will be run from the upper limit of range-finding tests. For example, if the range finding tests show some effect between 100 – 1000 µg/L, then the dilution series will start at 1000 µg/L. Dilutions may be set at 0.5 with an additional 75% concentration added, or the dilution series may be set at a 0.75.

Table 12. Description of chronic toxicity test methods.

test organism and EPA method	test type	chamber size (minimum)	solution volume	# organisms per chamber	# replicates (minimum)	age	temperature	aeration	feeding	endpoints
Ceriodaphnia dubia EPA-821-R-02-013, method 1002.0	7-day static renewal (80% renewal daily)	30 mL	15 mL	1 from a female with ≥ 8 neonates in the 3rd or subsequent broods	10	< 24 hrs and within an 8 hr age range	25° ± 1°C	if DO < 2.0 mg/L	0.1 mL YCT and 0.1 mL algal suspension daily	Number of survivors at 7 days and number of neonates per female at 3 broods
Pimephales promelas EPA-821-R-02-013, method 1000.0	7-day static renewal (80% renewal daily)	500 mL	250 mL	minimum 10	4	< 24 hrs (< 48 hrs if shipped)	25° ± 1°C	if DO < 4.0 mg/L	0.1 g wet weight per container 3 times daily at 4-hour intervals or 0.15 g wet weight per container twice daily at 6-hour intervals: no food in final 12 hours	Survival rate; Total weight of survivors divided by the initial count (biomass); Total weight of survivors divided by the final count (weight)
Atherinops affinis EPA/600/R-95/136, method 1006.0	7-day static renewal (80% renewal daily)	600 mL	200 mL	minimum 5	5	9 - 15 days post-hatch	20° ± 1°C	if DO < 4.0 mg/L	Twice daily (40 Artemia nauplii/fish at each feeding) morning and afternoon; no food on day 7	Survival rate; Total weight of survivors divided by the initial count (biomass); Total weight of survivors divided by the final count (weight)
Strongylocentrotus purpuratus EPA/600/R-95/136	24-hr static	20 mL	5 mL	about 5 X $l0^7$ sperm/mL and about 2000 eggs/mL	4	< 4 hrs after collection of gametes	20° ± 1°C	if DO < 4.0 mg/L	NA	Fertilization of eggs

DO: dissolved oxygen

YCT: yeast-cerophyl-trout mixture

There are established EPA protocols describing the appropriate test methods for the bioassays, and these are also summarized relative to WET testing in Washington State by Marshall (2016). Summaries of those being used in this project are found in Appendix B.

All bioassays will require monitoring for temperature, pH, dissolved oxygen, salinity (if applicable), and conductivity. The EPA methods describe the optimal conditions for these parameters. The contract lab will be required to (1) document these parameters throughout the tests and (2) provide any description of mitigation protocols and actions if the chambers required adjusting.

The general conditions of the bioassays must reflect the following (Marshall, 2016):

- The approved chronic test manual is EPA-821-R-02-012 (USEPA, 2002a).
- Dual endpoint tests must meet conditions in the chronic manual to have a valid chronic result.
- Illumination must be for 16 hours at 10 - 20 μE/m2/s (50 - 100 ft-c) followed by 8 hours of darkness.
- Effluent holding time is 36 hours maximum prior to test initiation. The original sample (up to 84 hours old) may be used for renewals at 48 hours if held at 0-6° C in the dark.
- Controls must have at least 90% survival, or the test should be repeated as soon as possible on a fresh sample.

The laboratory methods for the water chemistry are described in Table 13. An additional objective of this study is to test whether there is a significant difference in the results of NWTPH-Dx when using a silica gel cleanup in the sample preparation methods. A subset of NWTPH-Dx samples will be split; one will go through cleanup while the other will not.

Table 13. Laboratory measurement methods.

Analyte	Sample Matrix	Samples		Expected Range of Results (µg/L)	Detection or Reporting Limit (µg/L)	Sample Prep Method	Analytical (Instrumental) Method
		Number	Arrival Date (2017)				
NWTPH-Dx	water	203	Feb–Apr	500 – 10,000	500	NWTPH-DXP	NWTPH-DX
NWTPH-Dx	water	60	Feb–Apr	500 – 10,000	500	SCP; NWTPH-DXP	NWTPH-DX
NWTPH-Gx	water	203	Feb–Apr	250-10,000	250	NWTPH-GXP	NWTPH-GX
benzene	water	203	Feb–Apr	1-1,000	0.26	SW5030B	SW8021B
toluene	water	203	Feb–Apr	1-1,000	0.15	SW5030B	SW8021B
ethylbenzene	water	203	Feb–Apr	1-1,000	0.11	SW5030B	SW8021B
xylenes	water	203	Feb–Apr	1-1,000	0.24	SW5030B	SW8021B
naphthalene	water	12	May	0.5-500	0.05	SW3535A	SW8270DSIM
benzo(a)pyrene	water	12	May	0.5-500	0.05	SW3535A	SW8270DSIM

SCP: silica gel cleanup

9.2 Sample preparation methods

Sample preparation will follow standard protocols for the water chemistry and toxicity tests. It is of interest to Ecology's TCP to determine whether there is a significant difference between NWTPH-Dx concentrations that have gone through a silica gel cleanup and those that have not. A silica gel cleanup is typically used on samples where natural organic matter may interfere with the analysis of hydrocarbons (Ecology, 2016). We will split 50 samples for NWTPH-Dx and analyze them with and without silica cleanup for comparison.

9.3 Special method requirements

Not applicable.

9.4 Laboratories accredited for methods

One accredited contract lab will be selected to carry out all four bioassays. A *Request for Qualifications* will be posted as per Ecology's procurement guidance. All chemical analyses will be run by MEL.

10.0 Quality Control Procedures

Communication among the project manager, contract lab, and MEL during the initial stages of the project will ensure the water chemistry results are meeting the project DQOs for precision and accuracy. As part of this, the project team will discuss quality control.

10.1 Table of field and laboratory quality control

Table 14. Quality control samples, types, and frequency.

Parameter	Field		Laboratory			
	Blanks	Replicates	Check Standards	Method Blanks	Analytical Duplicates	Matrix Spikes
NWTPH-Dx	NA	50	1/batch	1/batch	1/batch	1/sample
NWTPH-Dx w/ silica cleanup	NA	10	1/batch	1/batch	1/batch	1/sample
NWTPH-Gx w/ BTEX	NA	50	1/batch	1/batch	1/batch	1/sample
PAHs	NA	2	1/batch	1/batch	1/batch	1/sample

Quality control for the toxicity tests is detailed in Table 12. In addition, each chronic toxicity test has a reference toxicant control test conducted concurrently. The petroleum hydrocarbon toxicity test endpoints must be significantly different from the control tests and meet the general test conditions outlined in section *9.1 Lab Procedures Table*.

10.2 Corrective action processes

A number of DQOs are built into the initial stages of this project. Continued evaluation and communication among project personnel will ensure that corrective actions are taken if necessary, including:

- Re-analysis of samples
- Re-running toxicity tests
- Re-evaluation of nominal hydrocarbon concentrations in the dilution series

A laboratory contingency of 20% is built into the project budget to accommodate corrective actions.

11.0 Management Procedures

11.1 Data recording and reporting requirements

Lab bench sheets from the contract lab will be used to document all toxicity tests and transcribed to the lab final reports and CETIS output. Copies of the original bench sheets will be included in the final report for review by Ecology project staff.

11.2 Laboratory data package requirements

The contract lab will submit a data package that meets the following requirements:

1. Report data to Ecology as a printed copy test report or PDF file, including the following:
    i. A readable copy of all WET test bench sheets and chain-of-custody, including toxicological and nominal and actual water chemistry data. Water chemistry will be confirmed and supplied by MEL.
    ii. Bench sheets must record counts of number alive (not percentages or number dead) in order to be acceptable. Bench sheets must also include observations of oily sheens in the test chambers.
    iii. Start counts must be clearly recorded on the bench sheet.
    iv. Test report must include computer printouts of test data and summary results of statistical analyses. The full details of the statistical analyses do not need to be printed and included in reports.
    v. Test organism source, age, and unusual conditions (e.g., lethargy, hyperactivity, spots or filaments, discoloration, excessive ventilation) should be reported.
    vi. Special circumstances such as treatment system upsets known to exist at the time of sample collection must be reported.
    vii. The report must contain a description and justification of any dechlorination procedure used, including the stoichiometric calculations for determining the proper amount of dechlorinating agent.
    viii. The report must contain a description and justification for any filtration, aeration, hardness adjustment, UV disinfection, or pH control procedure used.
    ix. Each test report must contain a section where deviations from test protocols are listed or their absence noted.
2. If the lab uses CETIS, then an export of the CETIS file should be submitted. CETIS is not required; however, if the lab does not use CETIS then a MS Excel spreadsheet of the data in a format compatible with CETIS must be submitted. CETIS is produced by Tidepool Scientific Software (https://tidepool-scientific.com/). The lab may work with Ecology to confirm the format of the final data submission.

The water chemistry lab data package will be generated by MEL. MEL will provide a project data package that will include: a narrative discussing any problems encountered in the analyses, corrective actions taken, changes to the referenced method, and an explanation of data qualifiers. Quality control results will be evaluated by MEL (discussed below in *Section 13.0 Data Verification*).

The following data qualifiers will be used:

- "J" – The analyte was positively identified. The associated numerical result is an estimate.
- "UJ" – The analyte was not detected at or above the estimated reporting limit.
- "U" – The analyte was not detected above the reporting limit.
- "NJ" – The analysis indicates the presence of an analyte that has been "tentatively identified" and the associated numerical value represents its approximate concentration.

The qualifiers will be used in accordance with the method reporting limits such that:

- For non-detect values, the estimated detection limit (EDL) is recorded in the "Result Reported Value" column, and a "UJ" is recorded in the "Result Data Qualifier" column.
- Detected values that are below the quantitation limits (QL) are reported and qualified as estimates ("J").

11.3 Electronic transfer requirements

All water chemistry lab data will be accessed and downloaded from MEL's Laboratory Information Management System (LIMS) into Excel spreadsheets. MEL will provide an electronic data deliverable (EDD).

MEL will provide the contract lab an EDD of the water chemistry data. The contract lab will provide output data files from the CETIS program or an Excel data file that is compatible with CETIS for importing by Ecology.

11.5 Model information management

Not Applicable.

12.0 Audits and Reports

12.1 Field, laboratory, and other audits

There is no defined audit for the field work in this project.

Ecology's Environmental Laboratory Accreditation Program evaluates a laboratory's quality system, staff, facilities and equipment, test methods, records, and reports. It also establishes that the laboratory is capable of providing accurate, defensible data. All assessments are available from Ecology upon request, including MEL's internal performance and audits.

Based on the proximity of the contract lab, a site visit by the project manager will be conducted during the toxicity tests.

12.2 Responsible personnel

No audits will be conducted during this project.

12.3 Frequency and distribution of report

At the end of the project, one final report will summarize the results for Ecology's TCP Policy and Technical Support Unit. The report will be accessible on Ecology's *Reports and Publications* webpage.

12.4 Responsibility for reports

The final report will be co-authored by William Hobbs and Randall Marshall.

13.0 Data Verification

13.1 Field data verification, requirements, and responsibilities

Not applicable.

13.2 Laboratory data verification

As previously described, MEL will oversee the review and verification of all lab data packages. All data generated by the contract lab must be included in the final data package. Randall Marshall will conduct the data verification of the contract lab to ensure the data meet the guidelines for WET testing (Marshall, 2016).

13.3 Validation requirements, if necessary

Verification of the contract lab data will be completed by Randall Marshall. In order to validate the data, all requirements of the data package outlined in section *11.2 Laboratory Data Package Requirements* must be met. This includes all the monitoring data of the pH, temperature, salinity, and dissolved oxygen in the test chambers.

13.4 Model quality assessment

Not applicable.

14.0 Data Quality (Usability) Assessment

14.1 Process for determining project objectives were met

This project has a series of DQOs that must be met in the initial stages. Each DQO has a specific RPD or RSD as outlined in section *6.0 Quality Objectives*. Furthermore, the contract lab must show that the control test chambers for each organism are within the median RSDs listed in Table 9, based on the last 20 tests.

The project manager and MEL will determine if the water chemistry data are useable by assessing whether the data have met the MQOs outlined in Table 8. Based on this assessment, the data will either be accepted, accepted with appropriate qualifications, or rejected and re-analysis considered.

14.2 Treatment of non-detects

There is no specific approach necessary for the treatment of non-detects. MEL will report whether the analyte was not detected at or above the estimated reporting limit. It is not anticipated that non-detects will be an issue for the parameters being measured.

14.3 Data analysis and presentation methods

The goal of this project is to produce a statistically sound LOEC and NOEC for four organisms, two in marine water and two in freshwater. Using the quality control objectives described throughout this QAPP, the necessary data set will be compiled for analysis in CETIS. CETIS is the industry standard for data analysis of toxicity data.

The analysis of toxicity data is based on the concept of a dose-response relationship of the test organism. The data analysis is used to describe thresholds or endpoints (LOEC and NOEC) in this dose-response relationship. Guidance from EPA outlines the various tests appropriate for determining a statistically defendable endpoint (Figure 1; USEPA, 2002a). Significance tests between the test chambers and controls can include: Dunnett's procedure, t-test with Bonferroni adjustment, steel's many-one rank test, and Wilcoxon rank-sum test with Bonferroni adjustment. The selection of the appropriate test depends on the heterogeneity of the test replication among concentrations (USEPA, 2002a). As described in the flow chart in Figure 1, data transformation also may be necessary depending on the distribution of the data. The program CETIS has the capacity to carry out the above statistical tests and also can suggest appropriate tests.

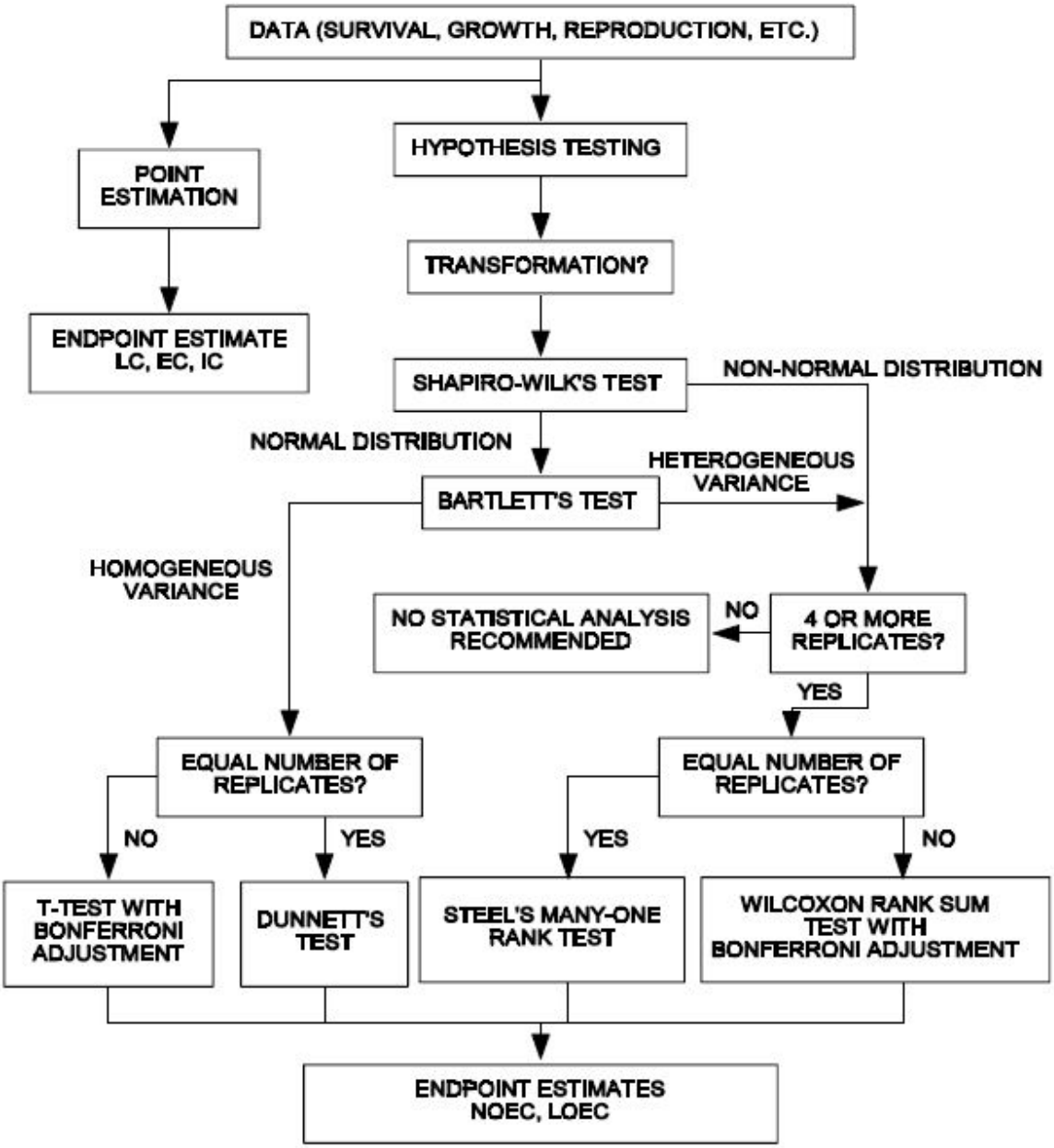

Figure 1. Flow chart of statistical analysis for toxicity endpoints (USEPA, 2002a).

14.4 Sampling design evaluation

The standard methods for the toxicity tests are designed to provide sufficient replication for statistically relevant findings. The goal is to determine the NOEC for each test organism. The lowest estimate of a NOEC for any of the responses would be used as the NOEC for each test (USEPA, 2002a). There is sufficient laboratory budget contingency to replicate any tests if necessary.

14.5 Documentation of assessment

Data usability will be described in the *Quality Control* section of the *Results* section in the final report.

15.0 References

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16.0 Appendices

Appendix A. Summary of EcoTox Data

Table A-1: USEPA EcoTox data for petroleum (CAS# 8002059) chronic and acute endpoints in marine and freshwater fish.

Species		Media	Endpoint	Effect	Effect Measurement	Result (µg/L)	Reference
Chronic toxicity endpoints							
Clupea pallasii	Pacific Herring	SW	LOEL	GRO	WGHT	10	Kocan et al., 1996
Menidia beryllina	Inland Silverside	SW	LOEC	MOR	MORT	1500	Little et al , 2000
Menidia beryllina	Inland Silverside	SW	LOEC	MOR	MORT	1500	Little et al , 2000
Menidia beryllina	Inland Silverside	SW	LOEC	MOR	MORT	1500	Little et al , 2000
Menidia beryllina	Inland Silverside	SW	NOEC	MOR	MORT	700	Little et al , 2000
Menidia beryllina	Inland Silverside	SW	NOEC	MOR	MORT	700	Little et al , 2000
Menidia beryllina	Inland Silverside	SW	NOEC	MOR	MORT	700	Little et al , 2000
Acute toxicity endpoints							
Clupea pallasii	Pacific Herring	SW	LC50	MOR	MORT	370	Carls, 1987
Atherinops affinis	Topsmelt	SW	LC50	MOR	MORT	35730	Singer et al., 1998
Atherinops affinis	Topsmelt	SW	LC50	MOR	MORT	16340	Singer et al., 1998
Atherinops affinis	Topsmelt	SW	LC50	MOR	MORT	40200	Singer et al., 1998
Atherinops affinis	Topsmelt	SW	EC50	ITX	IMBL	31760	Singer et al., 1998
Atherinops affinis	Topsmelt	SW	EC50	ITX	IMBL	48220	Singer et al., 1998
Atherinops affinis	Topsmelt	SW	EC50	ITX	IMBL	26630	Singer et al., 1998
Menidia beryllina	Inland Silverside	SW	LC50	MOR	MORT	930	Little et al , 2000
Menidia beryllina	Inland Silverside	SW	LC50	MOR	MORT	510	Little et al , 2000
Menidia beryllina	Inland Silverside	SW	LC50	MOR	MORT	1270	Little et al , 2000
Cottus cognatus	Slimy Sculpin	FW	LC50	MOR	MORT	3000	Moles et al., 1979
Salvelinus malma	Dolly Varden	FW	LC50	MOR	MORT	1250	Moles et al., 1979
Salvelinus malma	Dolly Varden	FW	LC50	MOR	MORT	2680	Moles et al., 1979
Gasterosteus aculeatus	Threespine Stickleback	FW	LC50	MOR	MORT	6890	Moles et al., 1979

SW: salt water; FW: freshwater; LOEL: Lowest-observed effect level;
LOEC: Lowest-observed effect concentration; NOEC: No-observed effect concentration;
LC50: Lethal concentration to 50% of test organisms; EC50: Effective concentration to 50% of test organisms;
GRO: growth; MOR: mortality; ITX: intoxication; WGHT: weight; MORT: mortality; IMBL: immobile.

Table A-2: USEPA EcoTox data for BTEX chronic and acute endpoints in marine and freshwater fish.

Contaminant	Species		Media	Endpoint	Effect	Effect Metric	Result (µg/L)	Reference
Chronic toxicity endpoints								
Benzene	*Pimephales promelas*	Fathead Minnow	FW	NOEC	GRO	GGRO	10200	Marchini et al., 1992
Benzene	*Pimephales promelas*	Fathead Minnow	FW	NOEC	MOR	SURV	10200	Marchini et al., 1992
Ethylbenzene	*Menidia menidia*	Atlantic Silverside	SW	NOEC	MOR	MORT	3300	Masten et al., 1994
Ethylbenzene	*Cyprinodon variegatus*	Sheepshead Minnow	SW	NOEC	MOR	MORT	88000	Heitmuller et al., 1981
Toluene	*Pimephales promelas*	Fathead Minnow	FW	NOEC	GRO	GGRO	5440	Marchini et al., 1992
Toluene	*Pimephales promelas*	Fathead Minnow	FW	NOEC	MOR	SURV	5440	Marchini et al., 1992
Toluene	*Cyprinodon variegatus*	Sheepshead Minnow	SW	NOEC	MOR	MORT	3200	Ward et al., 1981
Benzene	*Pimephales promelas*	Fathead Minnow	FW	LOEC	MOR	SURV	17200	Marchini et al., 1992
Benzene	*Pimephales promelas*	Fathead Minnow	FW	LOEC	GRO	GGRO	17200	Marchini et al., 1992
Ethylbenzene	*Menidia menidia*	Atlantic Silverside	SW	LOEC	MOR	MORT	5900	Masten et al., 1994
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LOEC	GRO	GGRO	8040	Marchini et al., 1992
Toluene	*Cyprinodon variegatus*	Sheepshead Minnow	SW	LOEC	MOR	MORT	7700	Ward et al., 1981
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LOEC	GRO	GGRO	6000	Devlin, 1982
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LOEC	MOR	SURV	8040	Marchini et al., 1992
Acute toxicity endpoints								
Benzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	SURV	24600	Marchini et al., 1992
Benzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	SURV	14010	Marchini et al., 1992
Benzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	12500	Brooke, 1987
Benzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	84000	Slooff, 1982
Benzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	34420	Pickering and Henderson, 1966
Benzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	35080	Pickering and Henderson, 1966
Benzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	32000	Pickering and Henderson, 1966
Benzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	12600	Geiger et al., 1990
Benzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	24600	Geiger et al., 1990
Benzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	35560	Pickering and Henderson, 1966
Benzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	32000	Pickering and Henderson, 1966
Benzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	35700	Brooke, 1987
Benzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	SURV	15590	Marchini et al., 1992
Benzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	84000	Slooff, 1982
Benzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	33470	Pickering and Henderson, 1966
Ethylbenzene	*Menidia menidia*	Atlantic Silverside	SW	LC50	MOR	MORT	5800	Masten et al., 1994

Contaminant	Species		Media	Endpoint	Effect	Effect Metric	Result (µg/L)	Reference
Ethylbenzene	*Cyprinodon variegatus*	Sheepshead Minnow	SW	LC50	MOR	MORT	360000	Heitmuller et al., 1981
Ethylbenzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	48510	Pickering and Henderson, 1966
Ethylbenzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	48510	Pickering and Henderson, 1966
Ethylbenzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	12100	Geiger et al., 1990
Ethylbenzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	42330	Pickering and Henderson, 1966
Ethylbenzene	*Cyprinodon variegatus*	Sheepshead Minnow	SW	LC50	MOR	MORT	300000	Heitmuller et al., 1981
Ethylbenzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	9100	Brooke, 1987
Ethylbenzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	11900	Brooke, 1987
Ethylbenzene	*Cyprinodon variegatus*	Sheepshead Minnow	SW	LC50	MOR	MORT	280000	Heitmuller et al., 1981
Ethylbenzene	*Menidia menidia*	Atlantic Silverside	SW	LC50	MOR	MORT	7000	Masten et al., 1994
Ethylbenzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	48510	Pickering and Henderson, 1966
Ethylbenzene	*Menidia menidia*	Atlantic Silverside	SW	LC50	MOR	MORT	5100	Masten et al., 1994
Ethylbenzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	42330	Pickering and Henderson, 1966
Ethylbenzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	42330	Pickering and Henderson, 1966
Ethylbenzene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	9090	Geiger et al., 1990
Ethylbenzene	*Menidia menidia*	Atlantic Silverside	SW	LC50	MOR	MORT	6400	Masten et al., 1994
Ethylbenzene	*Cyprinodon variegatus*	Sheepshead Minnow	SW	LC50	MOR	MORT	320000	Heitmuller et al., 1981
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	SURV	36200	Marchini et al., 1992
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	56000	Pickering and Henderson, 1966
Toluene	*Cyprinodon variegatus*	Sheepshead Minnow	SW	LC50	MOR	MORT	13000	Ward et al., 1981
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	28000	Devlin et al., 1982
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	SURV	9390	Marchini et al., 1992
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	36200	Geiger et al., 1990
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	72000	Devlin et al., 1982
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	36000	Devlin et al., 1982
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	77400	Mayes et al., 1983
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	66000	Devlin et al., 1982
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	SURV	17030	Marchini et al., 1992
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	12600	Pearson et al., 1979
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	56000	Pickering and Henderson, 1966
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	46310	Pickering and Henderson, 1966

Contaminant	Species		Media	Endpoint	Effect	Effect Metric	Result (µg/L)	Reference
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	46310	Pickering and Henderson, 1966
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	56400	Mayes et al., 1983
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	25000	Devlin et al., 1982
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	42330	Pickering and Henderson, 1966
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	31700	Geiger et al., 1990
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	22100	Brooke, 1987
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	34270	Pickering and Henderson, 1966
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	31000	Devlin et al., 1982
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	26000	Devlin et al., 1982
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	59000	Devlin et al., 1982
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	55000	Devlin et al., 1982
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	30000	Devlin et al., 1982
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	54000	Mayes et al., 1983
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	18000	Devlin et al., 1982
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	27000	Devlin et al., 1982
Toluene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	16000	Lawry, 1985
Xylene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	28770	Pickering and Henderson, 1966
Xylene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	46000	Mattson et al., 1976
Xylene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	42000	Mattson et al., 1976
Xylene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	28770	Pickering and Henderson, 1966
Xylene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	28770	Pickering and Henderson, 1966
Xylene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	26700	Pickering and Henderson, 1966
Xylene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	42000	Masten et al., 1994
Xylene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	27710	Pickering and Henderson, 1966
Xylene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	42000	Mattson et al., 1976
Xylene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	28770	Pickering and Henderson, 1966
Xylene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	42000	Mattson et al., 1976
Xylene	*Pimephales promelas*	Fathead Minnow	FW	LC50	MOR	MORT	13400	Geiger et al., 1990

SW: salt water; FW: freshwater; LOEL: Lowest-observed effect level;
LOEC: Lowest-observed effect concentration; NOEC: No-observed effect concentration;
LC50: Lethal concentration to 50% of test organisms; EC50: Effective concentration to 50% of test organisms;
GRO: growth; MOR: mortality; ITX: intoxication; GGRO: general growth; WGHT: weight; MORT: mortality;
IMBL: immobile.

Table A-3: USEPA EcoTox data for petroleum chronic and acute endpoints in marine invertebrates.

Species		Media	Endpoint	Effect	Effect metric	Result (µg/L)	Reference
Chronic toxicity endpoints							
Evasterias troschelii	Seastar	SW	LOEL	GRO	WGHT	200	O'Clair and Rice, 1985
Strongylocentrotus droebachiensis	Green Sea Urchin	SW	LOEL	GEN	DAMG	60	Taban et al., 2004
Lytechinus anamesus	White Sea Urchin	SW	LOEL	DVP	ABNM	0.1	Hamdoun et al., 2002
Evasterias troschelii	Seastar	SW	NOEL	MPH	SMIX	720	O'Clair and Rice, 1985
Evasterias troschelii	Seastar	SW	NOEL	GRO	WGHT	120	O'Clair and Rice, 1985
Acute toxicity endpoints							
Evasterias troschelii	Seastar	SW	EC50	INJ	GINJ	710	O'Clair and Rice, 1985
Evasterias troschelii	Seastar	SW	LC50	MOR	MORT	820	O'Clair and Rice, 1985

SW: salt water; FW: freshwater; LOEL: Lowest-observed effect level; NOEL: no-observable-effect-level;
LC50: Lethal concentration to 50% of test organisms; EC50: Effective concentration to 50% of test organisms;
GRO: growth; GEN: general; DAMG: damage; MPH: morphology; SMIX: organ weight in relation to body weight;
WGHT: weight; DVP: development; ABNM: abnormal; INJ: injury; GINJ: general injury; MORT: mortality.

Table A-4: USEPA EcoTox data for BTEX chronic and acute endpoints in marine and freshwater invertebrates.

Contaminant	Species		Media	Endpoint	Effect	Effect metric	Result (µg/L)	Reference
Chronic toxicity endpoints								
Xylenes	*Brachionus calyciflorus*	Rotifer	FW	NOEC	REP	GREP	20000	Snell and Moffat, 1992
Xylenes	*Brachionus calyciflorus*	Rotifer	FW	LOEC	REP	GREP	40000	Snell and Moffat, 1992
Acute toxicity endpoints								
Benzene	*Hydra oligactis*	Hydra	FW	LC50	MOR	MORT	34000	Slooff, 1983
Benzene	*Hydra oligactis*	Hydra	FW	LC50	MOR	MORT	34000	Slooff et al., 1983
Toluene	*Brachionus calyciflorus*	Rotifer	FW	LC50	MOR	MORT	113300	Ferrando and Andreu-Moliner, 1992
Toluene	*Brachionus calyciflorus*	Rotifer	FW	LC50	MOR	MORT	113000	Snell, 1991
Toluene	*Brachionus plicatilis*	Rotifer	FW	LC50	MOR	MORT	552600	Ferrando and Andreu-Moliner, 1992
Toluene	*Brachionus calyciflorus*	Rotifer	FW	LC50	MOR	MORT	113000	Snell et al., 1991
Xylenes	*Brachionus calyciflorus*	Rotifer	FW	LC50	MOR	MORT	253000	Snell, 1991
Xylenes	*Brachionus calyciflorus*	Rotifer	FW	LC50	MOR	MORT	252700	Ferrando and Andreu-Moliner, 1992

Contaminant	Species		Media	Endpoint	Effect	Effect metric	Result (µg/L)	Reference
Xylenes	*Brachionus calyciflorus*	Rotifer	FW	LC50	MOR	MORT	253000	Snell, 1991
Xylenes	*Brachionus plicatilis*	Rotifer	SW	LC50	MOR	MORT	496000	Snell et al., 1991
Xylenes	*Brachionus plicatilis*	Rotifer	SW	LC50	MOR	MORT	495900	Ferrando and Andreu-Moliner, 1992
Xylenes	*Brachionus calyciflorus*	Rotifer	FW	LC50	MOR	MORT	253000	Snell et al., 1991
Xylenes	*Brachionus calyciflorus*	Rotifer	FW	LC50	MOR	MORT	253000	Snell and Moffat, 1992
Benzene	*Pseudokirchneriella subcapitata*	Green Algae	FW	EC50	GRO	GGRO	29000	Galassi et al., 1998
Benzene	*Pseudokirchneriella subcapitata*	Green Algae	FW	EC50	GRO	GGRO	41000	Herman et al.., 1990
Ethylbenzene	*Pseudokirchneriella subcapitata*	Green Algae	FW	EC50	GRO	GGRO	4800	Herman et al.., 1990
Ethylbenzene	*Pseudokirchneriella subcapitata*	Green Algae	FW	EC50	GRO	GGRO	4600	Galassi et al., 1998
Toluene	*Pseudokirchneriella subcapitata*	Green Algae	FW	EC50	GRO	GGRO	12500	Galassi et al., 1998
Toluene	*Pseudokirchneriella subcapitata*	Green Algae	FW	EC50	GRO	GGRO	9400	Herman et al.., 1990
Xylenes	*Brachionus calyciflorus*	Rotifer	FW	EC50	REP	GREP	99000	Snell and Moffat, 1992

SW: salt water; FW: freshwater; LOEL: Lowest-observed effect level;
LOEC: Lowest-observed effect concentration; NOEC: No-observed effect concentration;
LC50: Lethal concentration to 50% of test organisms; EC50: Effective concentration to 50% of test organisms;
GRO: growth; MOR: mortality; ITX: intoxication; GREP: general reproduction; GGRO: general growth;
WGHT: weight; MORT: mortality.

Appendix B. Summary of Toxicity Test Methods (Marshall, 2016)

Topsmelt survival and growth

Test species: *Atherinops affinis*

Approved test method: EPA/600/R-95/136

Test type: 7-day static-renewal (75% renewal of test solution in each test chamber daily)

Temperature: 20° ± 1°C

Illumination: Illumination must be for 16 hours at 10 - 20 µE/m2/s (50 - 100 ft-c) followed by 8 hours of darkness.

Salinity: 30 or 34 ± 2‰

Test chamber size: 600 mL (minimum)

Test solution volume: 200 mL (minimum)

Age of test organisms: 9 - 15 days post-hatch

Number of organisms/chamber: 5

Number of replicates/concentration: 5 (minimum)

Feeding: Twice daily (40 *Artemia* nauplii/fish at each feeding) morning and afternoon; no food on day 7

Aeration: None unless DO < 4.0 mg/L; aerate all chambers with < 100 bubbles/minute

Test duration: 7 days

Endpoints: Survival rate

Total weight of survivors divided by the initial count (biomass)

Total weight of survivors divided by the final count (weight)

Control performance criteria: ≥ 80% survival and average dry weight ≥ 0.85 mg/surviving fish

Reference toxicant acceptability criteria:

- Copper chloride is the only acceptable reference toxicant. The survival LC50 must be < 205 µg/L Cu. The median scaled difference probabilities (PMSD) must be < 25% for survival and < 50% for biomass. The results should also be used for QC as discussed in the "Reference Toxicant Tests".

Data entry: Because biomass can be zero, total weight equals tare weight for each replicate with zero survival. Because division by zero is undefined, the pan count should be blank for each replicate with zero survival. See Appendix C for more explanation.

Echinoderm fertilization

Test species: *Strongylocentrotus purpuratus* or *Dendraster excentricus*

Approved test method: EPA/600/R-95/136

Test type: static (nonrenewal)

Temperature: 12° ± 1°C

Salinity: 30 ± 2‰

Test chamber size: 16 × 100 mm or 16 × 125 mm disposable culture tubes

Test solution volume: 5 mL

Age of test organisms: < 4 hours after collection of gametes

Number of spawners: Gametes pooled from ≤ 4 males and ≤ 4 females (≤ 6 female sand dollars).

Number of organisms/chamber: Approximately 1,120 eggs and ≤ 3,360,000 sperm

Number of replicates/concentration: 4

Aeration: None in test chambers; the sample may be aerated if DO < 4.0 mg/L

Test duration: 40 minutes (20 minutes exposure of sperm; 20 minutes with eggs)

Endpoints: Fertilization of eggs (elevation of the fertilization membrane)

Test acceptability criteria:

- A test is acceptable if ≥ 70% of eggs in the control are fertilized. Control fertilization percentages close to 100% are to be avoided if possible.
- A test is acceptable if the minimum significant difference is < 25%.
- Fertilization at the NOEC must be within 80% of control fertilization.
- A concurrent reference toxicant test must be conducted with each batch of tests.
- Both dilution water and effluent egg blanks should have essentially no eggs with elevated fertilization membranes.

The density of the final sperm stock must be ≤ 33,600,000/mL and one of these options met:

1. *Option 1*, trial fertilization used - The sperm count for the final sperm stock must not exceed double the target density determined from the fertilization trial test used to determine the sperm density that will provide about 80% to 100% fertilization without oversperming. 90% to 95% fertilization is the ideal range.
2. *Option 2*, sperm/egg ratio kept ≤ 500:1 - confirmation of a sperm stock density of ≤ 5,600,000/mL
3. *Option 3*, use any reasonable sperm stock density and run two extra sets of controls (a high and a low density control) - the high density control (0.2 mL sperm stock) must have at least 5% higher fertilization than the low density control (0.05 mL sperm stock).

Fathead minnow survival and growth

Test species: *Pimephales promelas*

Approved test method: EPA-821-R-02-013, method 1000.0

Test type: 7-day static-renewal (80% renewal of test solution in each test chamber daily)

Temperature: 25° ± 1°C

Illumination: Illumination must be for 16 hours at 10 - 20 μE/m_2/s (50 - 100 ft-c) followed by 8 hours of darkness.

Test chamber size: 500 mL (minimum)

Test solution volume: 250 mL (minimum)

Age of test organisms: < 24 hours (< 48 hours if shipped)

Number of organisms/chamber: 10

Number of replicates/concentration: 4 (minimum)

Feeding: 0.1 g wet weight (approximately 1,000 Artemia nauplii) per container 3 times daily at 4-hour intervals (4 times/day at 2.5- to 3.0-hour intervals is acceptable) or 0.15 g wet weight (approximately 1,500 Artemia nauplii) per container twice daily at 6 hour intervals: no food in final 12 hours

Aeration: none unless DO < 4.0 mg/L; aerate all chambers and use < 100 bubbles/minute

Test duration: 7 days

Endpoints: Survival rate

Total weight of survivors divided by the initial count (biomass)

Total weight of survivors divided by the final count (weight)

Control performance criteria: ≥ 80% survival in the control

Average dry weight ≥ 0.25 mg per surviving fish in the control

Data entry: Because biomass can be zero, total weight equals tare weight for each replicate with zero survival. Because division by zero is undefined, the pan count should be blank for each replicate with zero survival. See Appendix C. for more explanation.

Ceriodaphnia survival and reproduction

Test species: *Ceriodaphnia dubia*

Approved test method: EPA-821-R-02-013, method 1002.0

Test type: 7-day static-renewal (> 90% renewal of test solution in each test chamber daily by transfer of test organism to another container with fresh test solution)

Temperature: 25° ± 1°C

Illumination: Illumination must be for 16 hours at 10 - 20 µE/m2/s (50 - 100 ft-c) followed by 8 hours of darkness.

Test chamber size: 30 mL (minimum)

Test solution volume: 15 mL (minimum)

Age of test organisms: < 24 hours and within an 8 hour age range

Number of organisms/chamber: 1 from a female with ≥ 8 neonates in the 3rd or subsequent broods

Number of replicates/concentration: 10 (minimum)

Feeding: 0.1 mL YCT and 0.1 mL algal suspension daily

Aeration: None unless DO < 2.0 mg/L and then optional at lab discretion using a very low bubbling rate

Test duration: The duration of exposure is expressed in terms of time (7 days) for the survival endpoint and in terms of life cycle (3 broods) for the reproduction endpoint. Final survival counts must be taken at the end of 7 days. Final counts of neonate production should be taken immediately upon production of the third brood by 60% of the surviving control organisms. The third brood will usually occur on the 6th, 7th, or 8th day. The maximum test duration allowed is 8 days as long as test solutions are renewed on each full day. Tests may not be continued beyond the third brood in order to get 15 neonates/surviving adult in the control.

Endpoints: Number of survivors at 7 days and number of neonates per female at 3 broods (# neonates per concentration divided by the # females at test initiation)

Control performance criteria: ≥ 80% survival in the control

An average of 15 neonates per surviving adult in the control

≥ 60% of the surviving control organisms producing 3 broods

Other test acceptability criteria: ≤ 10% males in the surviving test organisms over all test concentrations

≤ 20% males in the surviving test organisms in the ACEC, CCEC, or LOEC

Specific concerns

All surviving *C. dubia* producing no neonates in the test must be examined to determine gender and the results of the determination reported unless reproduction has been nearly eliminated in a test concentration and this fits an expected concentration-response relationship. It is understood that very young *C. dubia* can be difficult to sex and any *C. dubia* that dies in the first two days of

the test may be excluded from calculations for reproduction if gender is difficult to determine and it is one of no more than two mortalities in a concentration. Otherwise, difficult to sex young *C. dubia* must be considered to be female and included in all calculations.

Each successive brood from 1 to 4 tends to increase in neonate count from 50% to 75% over the previous brood. Differences in the number of broods or in the neonate totals due to differences in age or the timing of counting are a big source of variability. The test method requires that all of the *C. dubia* used in a test be less than 24 hours old and be within 8 hours of the same age. Because of the very short lifecycle of *C. dubia*, this restriction cannot completely eliminate these age-related differences in reproduction. The test method also says that all observations at test termination should be completed within 2 hours or the last containers counted might have produced significant numbers of neonates after the first containers received final counting. Labs must therefore strive to keep differences in age and the timing of counting to as small as possible and never exceed the limits in the test method.

Neonate counts are made at 24-hour intervals and will not occur for many females at a time between broods. A daily count may include neonates from only a partial brood or from two separate broods. A skilled technician is needed to tell the difference between broods in order to properly judge when 60% of the surviving control organisms have produced 3 broods. Judging brood occurrence requires experience, a good stereomicroscope, and sufficient time.

The tendency toward reduced neonate production in some females but not in others when the culture condition is borderline goes beyond the normal variation in individual test organism response. It is analogous to testing with organisms from two different life stages, each with its own baseline for the response being quantified. Labs must monitor culture health and reproduction daily, renew cultures both on a regular schedule and when needed, and immediately replace poorly performing batches of food and water.

Temperature inequalities that exceed the ± 1° C in the test method can influence the rate of neonate production for different containers.

Sources of error are unavoidable in any test and the proper solution is to distribute them randomly to avoid bias and invalid conclusions. For the *C. dubia* reproduction endpoint to be valid, all of the listed sources of error plus any others must be randomly distributed throughout the test.

Neonates from a single brood female must be placed in all test chambers assigned the same replicate number so that they appear only once in each test concentration (blocking by known parentage). *The process for achieving blocking by known parentage must be described in the report for each test*. The technique recommended by EPA seems to be to place cups into a test board and assume that each column is a test concentration and that each row contains replicates that have the same replicate number but are each from a different concentration. Test solutions are added accordingly. The cups in each row are then placed into a random order. One neonate from the same brood female is added to each cup in that row but not to cups in any other. All of the cups are then randomized together and the test conducted. Differences due to parentage are then evenly distributed among concentrations. Replicates can be compared at the end to see if there are differences due to parentage.

Appendix C. Glossaries, Acronyms, and Abbreviations

Glossary of General Terms

Clean Water Act: A federal act passed in 1972 that contains provisions to restore and maintain the quality of the nation's waters. Section 303(d) of the Clean Water Act establishes the TMDL program.

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.

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

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

Lowest-observable-effect-concentration (LOEC): the lowest concentration used in a toxicity test that has a statistically significant adverse effect on the exposed population of test organisms relative to the control 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-observable-effect-concentration (NOEC): the greatest concentration used in a toxicity test that has no statistically significant adverse effects on the exposed population of test organisms relative to the control 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.

Toxicity: the degree to which a substance can damage an organism. Can refer to damage on the whole organism or components (e.g. cells or organs).

Acronyms and Abbreviations

DO	(see Glossary above)
DQO	Data quality objective
e.g.	For example
EC50	Effective concentration to 50% of test organisms
Ecology	Washington State Department of Ecology
EIM	Environmental Information Management database
EPA	U.S. Environmental Protection Agency

et al.	And others
LC50	Lethal concentration to 50% of test organisms
LOEC	Lowest-observed effect concentration
LOEL	Lowest-observed effect level
MEL	Manchester Environmental Laboratory
MQO	Measurement quality objective
NOEC	No-observed effect concentration
NPDES	(See Glossary above)
PMSD	Median scaled difference probabilities
QC	Quality control
RPD	Relative percent difference
RSD	Relative standard deviation
SOP	Standard operating procedure
WAC	Washington Administrative Code
YCT	Yeast-cerophyl-trout mixture

Units of Measurement

°C	degrees centigrade
mg/L	milligrams per liter (parts per million)
mL	milliliter
µg/L	micrograms per liter (parts per billion)

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 population 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 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 is usable for intended purposes.
- J (or a J variant), data is estimated, may be usable, may be biased high or low.
- REJ, data is 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. (USEPA, 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:

$$[Abs(a-b)/((a + b)/2)] * 100$$

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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