Quality Assurance Project Plan:

Evaluating and Prioritizing Contaminants of Emerging Concern in the Lower Columbia River

Prepared for:

U.S. EPA, Region 10

Prepared by:

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A. Project Management

A.3 Distribution List

Table 1 identifies all individuals who should get a copy of the approved Quality Assurance Project Plan (QAPP), either in hard copy or electronic format, as well as any subsequent revisions. Revisions will be clearly identified. Hayley Mathews will be responsible for distributing the QAPP and any revisions upon approval.

Table 1. Distribution list for approved QAPP and revisions.

Name	Title	Organization	Email
C. Andrew James	Principal Investigator	UWT at CUW	jamesca@uw.edu
Dave Wark	Assistant Research Scientist	UWT at CUW	davewark@uw.edu
Nicole Taylor	Project Manager	EPA	taylor.nicole@epa.gov

UWT at CUW: University of Washington Tacoma research laboratory at the Center for Urban Waters

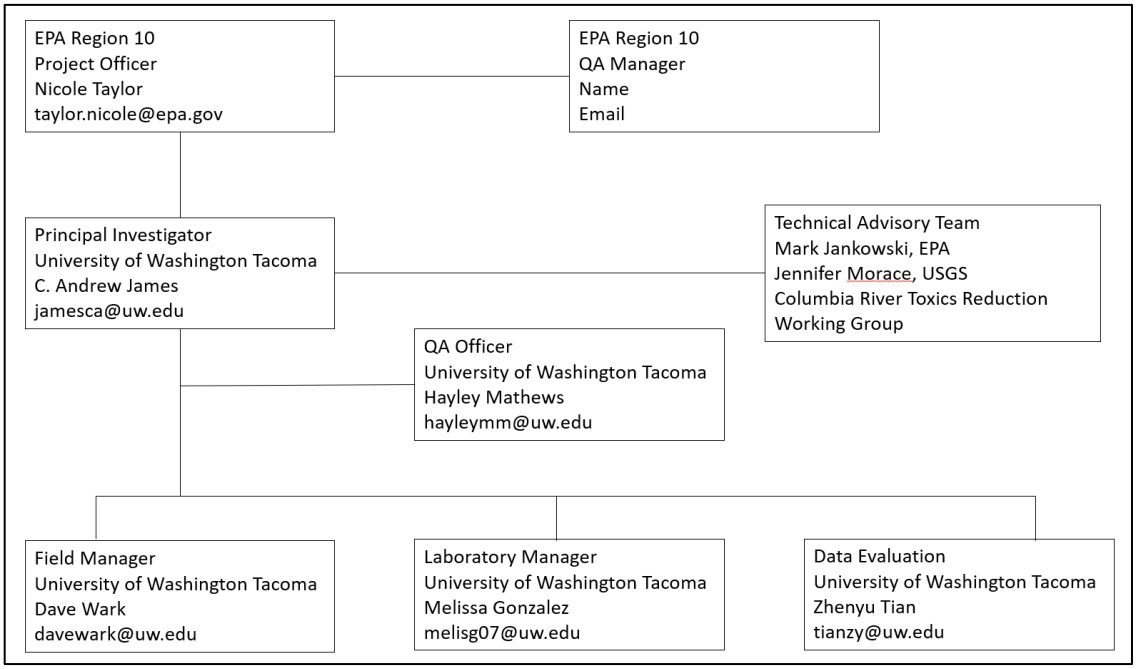
A.4 Project/Task Organization

This work will be performed primarily by personnel at the University of Washington Tacoma Laboratories at the Center for Urban Waters (UWT at CUW) in coordination with, and under the guidance of, the Columbia River Toxics Reduction Working Group. The UWT at CUW team has extensive experience and expertise implementing monitoring programs throughout the Pacific Northwest similar to the one described in this QAPP. This includes project management and the primary sampling, analysis, evaluation, and communication tasks.

Table 2. Organization of project staff and other key individuals and their responsibilities.

Key Individuals	Title	Responsibilities
C. Andrew James, PhD UWT Center for Urban Waters jamesca@uw.edu	Principal Investigator	Manages overall project, co-writes QAPP, prepares budget report, coordinating field sampling, collaborating with NORMAN group and EPA personnel, development of communication products.
Hayley Mathews UWT Center for Urban Waters hayleymm@uw.edu	QA Officer	Co-writes and distributes QAPP, revises QAPP as needed.
Dave Wark UWT Center for Urban Waters davewark@uw.edu	Field Manager	Conducts field sampling and reviews QA/QC. Reports on QA assessment, data handling and analysis.
Nicole Taylor US EPA taylor.nicole@epa.gov	EPA Project Manager	Clarifies scope of the project, provides internal review of the QAPP, and approves the final QAPP.
Donald Brown US EPA brown.donaldm@epa.gov	EPA Quality Assurance Manager	Reviews draft QAPP and recommends approval of final QAPP. May comment on draft project report

Figure 1. Project organizational chart



A.5 Problem Definition and Background

A.5.1 Problem Statement

This project will provide occurrence information on previously unmonitored contaminants such as endocrine disruptors in Columbia River and utilize a range of effects information to evaluate potential for harm to important species. This project specifically addresses the Columbia River Basin Restoration Program (CRBRP) priority, "increased monitoring and access to data from monitoring in the Columbia River Basin with a focus on toxics with an impact on human health and fish and wildlife."

A.5.2 Background Information

Contaminants of Emerging Concern (CECs) are a class containing thousands of compounds which are not well described in terms of distribution, environmental fate and transport, or regulation. CECs include pharmaceuticals and personal care products, hormones or endocrine disrupting compounds, flame retardants, and agrichemicals present in surface water and groundwater. They generally occur at low levels (µg/L to ng/L) and are unregulated when found in the environment. Importantly, there is growing evidence that they may adversely affect biota. Although direct toxicity and mortality is generally not associated with exposure to CECs, endocrine disruption, reproductive alteration, and behavior modification have all been observed in organisms exposed to CECs, including in studies performed in the region (Lubliner et al. 2010, Meador et al. 2016, WDFW TBiOS 2016, Miller-Schulze et al. 2017). Advances in analytical methods over the last decade have facilitated the investigation and quantification of CECs (Wu et al. 2010, Richardson 2012). Impacted water will contain a different suite of CECs based on its source inputs (e.g., domestic sewage vs. livestock runoff), treatments (e.g., septic system vs. wastewater treatment plant), and pathways (surface water vs. groundwater).

Water quality data collected from the Pacific Northwest and elsewhere indicate the widespread occurrence of CECs in surface and ground water. The available information, however, is limited. Traditional analytical techniques have been developed on a compound-by-compound basis with methods being developed and verified for a specific chemical or group of chemicals. Although there are existing methods that measure perhaps a few hundred unique chemicals (e.g., EPA method 1694), thousands of different compounds are produced and used every day. There is a dearth of information on their occurrence. In order to understand potential exposures, and thus risk to aquatic organisms, it is necessary to investigate the occurrence of a wider range of compounds, but it is also important to study the spatial and temporal variations. This is a primary goal of this study.

High resolution mass spectrometry (HRMS) approaches are ideal for characterizing a wide range of contaminants in a given sample. In contrast with traditional methods, where compounds are pre-selected prior to analysis, HRMS has the capacity to provide information to allow the identification of compounds without prior knowledge of their presence in a given samples (Strynar et al. 2015, Barzen-Hanson et al. 2017, Tian et al. 2017a). Non-target screening, the application of HRMS approaches without any antecedent information on compound, can be used to detect and identify otherwise unexpected chemicals, including metabolites and transformation products. Such approaches will be used in this study.

Summary of previous studies and existing data

Previous investigations have evaluated the presence of CECs in regional waters. Miller-Schulze et al. (2017) performed a single sampling event in the Puget Sound and reported detecting seven wastewater-associated CECs (sucralose, caffeine, acetaminophen, paraxanthine, sulfamethoxazole, carbamazepine, and mecoprop) in most samples collected at concentrations ranging from 0.01 – 100 ng/L. Meador et al. (2016) collected water from three locations in Puget Sound meant to represent waters with various degrees of impact from wastewater treatment plant (WWTP) effluent. They reported identifying 25 analytes in estuary water and an additional set of 29 analytes in fish tissues, but not in estuary waters indicating that both tissue and water samples should be collected for a complete characterization profile of CECs in the marine environment. Investigations on CECs the Columbia River and San Francisco Bay have reported similar findings (Morace 2012, Klosterhaus et al. 2013a, Klosterhaus et al. 2013b). These studies, along with regional data from additional studies, show that CECs are ubiquitous in the environment. Since general sources of CECs are the same, we expect similar CECs are present in the Columbia River.

There is limited information available on CEC occurrence in marine species. Meador et al. (2016) reported on 27 analytes present in fish tissues, generally at concentrations ranging from <1-10 ng/g. Some of the compounds identified may affect growth and/or reproductive pathways in important aquatic species and could feasibly lead to population level impacts. Washington Department of Fish and Wildlife (WDFW) has performed pilot studies evaluating a suite of CECs in mussel tissue (n=18) and juvenile Chinook tissue that were collected in 2013 (n=12) and 2016 (n=15) samples. The mussel tissue data indicated that several pharmaceuticals such as virginiamycin M (antibiotic), sertraline (antidepressant), and melphalan (chemotherapy) were detected in nearly all samples at concentrations ranging from 0.1 – 100 ng/g tissue (ww) James et al. 2020). The juvenile salmonid tissue data indicated

the presence of a similar suite of compounds, though there were differences in the occurrence patterns. For example, virginiamycin was not detected at all and sertraline (4 of 15) and melphalan (2 of 15) were only sporadically present, while penicillin (antibiotic) and iopomidol (contrasting agent) were detected in all samples. It is not clear at this point whether these differences reflect differences in exposure, uptake, and/or metabolic processes all of which might result in differences in tissue concentrations.

Regional studies have investigated WWTPs, septic systems, and stormwater runoff (Peter et al. 2018) as potential pathways for CEC contamination to the environment (Lubliner et al. 2010, Morace 2012, James et al. 2016). These data indicate that CECs are widely present in human wastewater and stormwater runoff; treatment systems are somewhat effective at reducing the concentration of some of the contaminants, but many remain.

The results of these studies and others will be used to select the sampling locations that are likely to be impacted by wastewater and stormwater inputs. Studies include the following: the Lower Columbia River Estuary Partnership, USGS, and NOAA characterized the water column, suspended sediment, and bed sediments and juvenile salmon at five sites in the Columbia and Willamette rivers in 2004 and 2005 (Lower Columbia River Partnership 2007; Morace 2006); Morace (2012) sampled WWTP effluent and stormwater runoff that directly enters the Columbia River in nine cities across Washington and Oregon; Nilson et al. (2014) sampled three sites along the Columbia River in 2009 and 2010. These studies provide an indication of the spatial and temporal trends of contaminant exposure and provide additional useful exposure data.

Parameters of interest and potential sources

The parameters of interest are defined by a chemical class (e.g., CECs) and analytical approach (e.g., non-targeted screening).

The chemical class are the trace organic contaminants that are amenable to the analytical approaches described herein. The methods focus on small, non-volatile organic molecules that generally be described as CECs. These includes pharmaceuticals and personal care products, illicit drugs, food additives, antimicrobials, and automotive-related compounds. These compound classes can be identified through the non-targeted screening of water samples, performed utilizing a Liquid Chromatography Quadrupole Time of Flight, Tandem Mass Spectrometry (LC-QTOF-MS/MS) system. This analytical approach differs somewhat from traditional approaches, which typically characterize the occurrence of pre-selected contaminants (e.g., lead, copper, PCBs, etc.) through defined methods. The LC-QTOF-MS/MS High Resolution Mass Spectrometry (HRMS) system allows the collection of accurate mass information on thousands of compounds in a given sample. Subsequent data reduction and data processing techniques, generally known as non-target screening approaches, are applied for compound identification (Schymanski et al. 2014a, Schymanski et al. 2014b, Gago-Ferrero et al. 2015, Hernández et al. 2015).

A.6 Project/Task Description

A.6.1 Project Summary

The primary objectives of this work are to: 1) characterize the occurrence of priority chemicals in the Columbia River through a focused monitoring program, 2) evaluate the occurrence based on their potential to cause harm to important species, and 3) communicate the results to stakeholders.

Task 1: Monitoring

Task 1 will consist of a focused monitoring program to characterize the occurrence of priority chemicals in the Columbia River. The monitoring will consist of approximately four samplings event, with approximately 12-14 locations sampled for each event. The timing of the sampling events will be selected in order to characterize the influence of two primary pathways of chemicals into the river system focusing on stormwater runoff from the built environment (e.g., roads, commercial and industrial landscapes, residential neighborhoods, etc.) and WWTP effluent. To capture the influence of stormwater and/or wastewater we propose to collect samples during two wet weather events, when stormwater is likely to enter into the Columbia River, and during two dry weather events, when stormwater influence is likely to be low and wastewater influence would be more important. By approaching the sampling in this way, we can associate priority chemicals with a given pathway, which will be valuable in informing a management or regulatory response. For example, if a priority compound were associated with WWTP effluent, then treatment system process alterations or upgrades could be explored.

Key considerations in selecting sampling locations are land use and point source inputs to the river. It is expected that the most problematic areas in terms of contaminant loading (and thus exposures to biota) would be those closest to outfalls which direct stormwater or wastewater into the river. The sampling locations, then, will be selected to bracket these potentially important sources/pathways of contaminant loading, to help isolate impacts and contributions. See Table 5 for complete list of planned sampling locations.

Water samples will be analyzed utilizing HRMS methods at the University of Washington Tacoma laboratories at the Center for Urban Waters. HRMS analytical methods allow a more holistic evaluation of compounds in any given sample compared to the traditional methods.

Samples are analyzed utilizing an Agilent 1290 Liquid Chromatograph paired with an Agilent 6530 Quadrupole Time of Flight mass spectrometry unit, with data processed utilizing proprietary and open source analytical software. This monitoring approach is consistent with previous work evaluating wastewater and stormwater-associated contaminants in stream and estuarine waters (see James et al. 2016, Du et al. 2017, Peter et al. 2018, James et al. 2019 and Tian et al. 2020).

Task 2: Evaluation

Monitoring during Task 1 will provide chemical occurrence information. Task 2 will focus on evaluating the resulting information to better understand spatial and temporal occurrence patterns, and to determine if the detected chemicals might pose some risk to biota.

Occurrence Evaluation

One of the main points of this work is to understand where and when potentially harmful chemicals are occurring in the Columbia River so that we understand the exposure environment, and potential sources. As described above, we will collect samples at different locations, and under varying conditions (rain vs dry) to help us better understand where the chemicals are coming from. Spatial and temporal patterns will be used to evaluate sources (e.g., compounds that are only present in dry weather sampling and downstream of a WWTP outfall might be specific to a wastewater pathway). Further, specific chemicals have been identified which can help identify contributions from specific sources/pathways such as artificial sweeteners in human wastewater (Van Stempvoort et al. 2013, James et al. 2016, James et al. 2017).

The HRMS approaches to be used here are particularly effective in comparing samples sets which can support source identification, source apportionment, and identifying differential inputs. An HRMS sample produces a lot of data; over 15,000 unique compounds can be found in each WWTP effluent sample. This amount of data allows us to 1) develop signature profiles for many different sources, and 2) use statistical approaches to quantify the similarity of different samples. Both techniques will be applied to samples collected in the Columbia River.

Source signatures are developed by first, repeatedly sampling a given source type such as WWTP effluent, to determine the suite of compounds that are consistently present in all such samples. Second, we compare those compounds to samples from many other sources (e.g., road runoff, agricultural runoff, etc.) to identify those that are unique to the original source type. In that way we have a signature consisting of a suite of compounds that is consistently found in, and unique to, a given source. We have developed signatures for WWTP effluent, road runoff, boat waste, and a series of automotive fluids and can use that information to evaluate putative source contributions at a given sampling site.

Effects/Health Evaluation

The purpose of the second evaluation is to determine if a detected chemical will present a risk to fish or other aquatic species. The general approach will be to compare a concentration to an effects level. If the concentration is higher than the effects level then there is some reason to believe that the presence of the chemical might be worrisome, and so it should be prioritized. The general approach can be described by the following formula:

$$Effect\ Ratio\ (ER) = \frac{Concentration}{Effects\ Level}$$

If cases where ER > 1, the concentration is greater than effects levels, and there is the potential for risk.

To do this, we will compare measured environmental concentrations of a given compound (as determined in this project) with a Predicted No Effects Concentration (PNEC) as described in von der Ohe et al. (2011) and Hollender et al. (2019). Briefly, the PNEC is the concentration below which there is expected to be to no harm or biological response. PNECs have been developed for over 70,000 compounds by the NORMAN network, a group of European laboratories and regulatory

representatives who have collectively been working on prioritizing CECs for the last 10 years. The Puget Sound Ecosystem Monitoring Program (PSEMP) Toxics Workgroup and members of this project team, have collaborated with NORMAN in the past and have full access to, and experience with, the NORMAN ecotoxicological databases (https://www.norman-network.com/nds/ecotox/lowestPnecIndex.php).

Importantly, the PSEMP Toxics Workgroup is working on a project to prioritize CECs in the Puget Sound (in coordination with members of the Columbia River Toxics Reduction Working Group) and currently has a funded project to evaluate occurrence and effects information. Part of that project is to evaluate the PNECs to make sure that they are applicable and valid in the Pacific Northwest.

In addition, PSEMP is evaluating a second approach to risk screening is described in Pinto et al.(2019) and Corsi et al.(2019). The approach is similar to that described above but incorporates bioaccumulation potential into the concentration measure and accesses toxicological screening data from the US EPA ToxCast (https://comptox.epa.gov/dashboard/chemical_lists/toxcast) to support the comparison. The benefit of the second approach is that it allows the use of a large dataset produced by the ToxCast program, which is not completely integrated with the NORMAN PNEC approach.

We feel there is a benefit to utilizing both approaches as it allows us to access a much wider set of effects data compared to using one method alone. This is particularly important when dealing with poorly characterized chemicals and so want to maximize available data sources to support informed decision making.

The results of this PSEMP work form an integral part of this evaluation task, it is both necessary and directly applicable. As such, the value of that work is included as an in-kind contribution to meet the cost share obligations of this proposal.

Task 3: Communication

Task 3 under this project is communications. Results of the work will be communicated at different venues, and through different means, to maximize potential impacts. Project personnel will provide updates and a final presentation to the Columbia River Toxics Reduction Working Group, the Lower Columbia Estuary Partnership, and PSEMP. There is an existing connection with the PSEMP as they have been implementing a CEC prioritization program for several years, which includes participation with members of the Columbia River Toxics Reduction Working Group. We will work with the groups to arrange presentations of findings and its relevance to the health of the Columbia River. We will also prepare one or two communication briefs, intended to promote messaging to decision makers and other interested parties (for example, see https://pspwa.box.com/s/kl726eieqaikx6meeggg4gqg5slv8a7c). These products will be developed based on lessons learned during the development of a focused communication plan for the Puget Sound region which will identify key messages and stakeholder groups and support the development of products. This communication plan will be consulted as it is likely that messages, and some stakeholders, will be in common between the two programs. Detailed findings will be summarized in a project report, which we expect to develop into a manuscript for publication in the scientific, peer-reviewed literature.

A.6.2 Project Schedule

Table 3. Anticipated project schedule, including activities and deliverables.

Tasks/Milestones	2020				2021												2022
	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan
Project Begins	x																
QAPP Development and Finalization		x	x	x													
Field Sampling 1 (wet)					x	x											
Field Sampling 2 (wet)							x	x									
Field Sampling 3 (dry)											x	x					
Field Sampling 4 (dry)												x	x				
Data Evaluation								x	x	x	x	x	x	x	x		
Effects Evaluation								x	x	x	x	x	x	x	x		
Communication Products											x	draft	x	x	x	final	
Guidance Team Meetings		x			x			x			x			x			
Progress Report					x						x						
Final Reporting															x	x	x

A.6.3 Project Locations

The project will take place in the lower Columbia River ranging from upstream of the Portland metropolitan area to Wauna, with several sites on the Willamette River. Based on potential stormwater and wastewater discharges, it is expected that these sites will effectively characterize potential contaminant exposures. See Table 5 for site names and coordinates, in section B.1.1.

A.6.4 Resource and Time Constraints

The primary practical constraint is the physical distance between sampling sites and careful planning will be required to collect samples from all sites during any given day. Extra laboratory personnel may assist during collection and processing for sampling events.

The current COVID-19 pandemic is a possible source of constraints. The UWT at CUW laboratory is following strict guidelines set by the University of Washington, which includes avoiding working on-site as much as possible, limiting the number of staff working in the laboratory, and requiring all staff to leave sufficient distance when working with others. The pandemic is also impact supply chains for scientific supplies, such as gloves. At the time of QAPP development, current restrictions will have little impact on this project.

A.7 Quality Objectives and Criteria

A.7.1 Data Quality Objectives (DQOs)

The main DQOs for this project are to collect and analyze samples from the lower Columbia River in approximately four sampling events, with 12-24 locations sampled during each event. Samples will be collected in both the wet and dry seasons. These samples will be analyzed using HRMS methods to obtain pollution profiles that meet the Measurement Quality Objectives (MQOs) described below.

A.7.2 Measurement Quality Objectives

The MQOs describe the performance metrics and criteria for acceptance that provide the basis for evaluating data quality and usability. They indicate the minimum threshold levels for measures of bias, repeatability, precision, accuracy, and sensitivity that must be associated with the data.

The primary types of quality control samples used to evaluate the control the quality of the laboratory analyses are check standards, replicates, blanks, and spikes. While consensus protocols for assessment of non-target HRMS QTOF data have not yet been established, the UWT at CUW laboratories have established several internal QA/QC protocols and workflows for non-target and suspect screening of contaminants in environmental samples. These protocols and workflows are described in Du et al. (2017) and UWT at CUW Laboratory Standard Operating Procedures (SOPs) for sample collection, sample processing, sample analysis, and data handling, data analysis, and data management. Links to

these documents are provided in the references and Appendix A; copies are available upon request from the authors.

Data collected will be analyzed relative to the following indicators, many of which are defined per the US EPA Quality Assurance Glossary (US Environmental Protection Agency 1997). Relevant numerical criteria are provided in Table 4.

Table 4. MQOs for HRMS analysis

MQO	Precision Target	Bias Target	Sensitivity Target
Instrument Tune Agilent 6530 QTOF	< 2 ppm mass accuracy	--	Resolving power for tune solution ions 118 m/z >5,900 322 m/z > 9,000 622 m/z > 10,000 922 m/z > 12,000 1221 m/z > 12,500 1520 m/z >13,000 Tune solution ion response height 100k-400k (118 m/z > 40k)
Continuous Reference Mass (Continuous Injection)	Continuous detection of purine (m/z 121.0509) and HP-921 (m/z 922.0098)	Detection throughout analytical run	>5,000
Background ions	Retention time precision <0.1 min: triethyl citrate (RT 6.13 min) oleamide (RT 15.76 min) stearamide (RT 16.36 min) unidentified background ion (300.2019 Da @ 3.68 min)	--	--
CEC Standard Mix	Retention time variation <0.1 min; mass accuracy variation <5 ppm	Reference ions detected	Area response within ~20% of initial response
Internal Standard Mix	Retention time variation <0.1 min; mass accuracy variation <5 ppm	--	Area response within ~20% of initial response
Replicates	--	Features present in ≥ 3 field/lab replicates	--
Blanks	--	Blank features abundance < 5x sample	Sample features present at abundance ≥ 5 times blank area abundance

A.7.3 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 are summarized in Table 4. If the instrument fails these criteria,

performance will be corrected by conducting instrument tuning or detector maintenance, as outlined in the QTOF SOP titled, "LC-QTOF – MS/MS Setup, Operation, and Data Analysis" (see Appendix).

Precision

Precision is a measure of the repeatability of a set of replicated results and is considered to represent random error in the measurement process. Poor precision is due to difficulties in obtaining samples under identical conditions (e.g., contamination, variability of field conditions during the time replicate samples are collected) or poor sensitivity of laboratory and/or field procedures.

Method precision is demonstrated through the reproducibility of analytical results. Five key aspects are considered to evaluate precision of non-target data: instrument tuning, reference mass, background signals, repeated injections of reference standards, and field replicates (Table 4).

- Instrument tuning: Instrument tuning ensures consistent mass accuracy during a given analytical run and throughout the duration of the experimentation. Instrument tuning procedures are described extensively in the QTOF SOP titled, "LC-QTOF – MS/MS Setup, Operation, and Data Analysis". A check tune is performed prior to each analytical run, and the detector is re-tuned or re-calibrated if mass error exceeds 2 ppm.
- Reference Mass: a commercially available mixture of calibration compounds containing purine and HP-921 are concurrently analyzed during sample runs within the QTOF which provide reference mass (m/z 121.0509 and m/z 922.0098)for ongoing mass calibration. Reference ions must be observed during the analytical run.
- Background signals: Background signals, including triethyl citrate (RT 6.13 min), oleamide (RT 15.76 min), stearamide (RT 16.36 min), and an unidentified background ion (300.2019 Da @ 3.68 min) are used to monitor chromatographic stability.
- CEC Mix and Internal Standard injections: A mixture of CEC standards, each at 25-100 ng/mL (CEC-CAL-8; preparation described in CEC Calibration Set Preparation SOP) and a mixture of internal standards, each at 25-100 ng/mL (Internal Standard; preparation described in Stocks and Standards Preparation) are analyzed every 12 samples to check chromatography and sensitivity during data acquisition. Mass accuracy was limited to <5 ppm and retention time variability was limited to <0.1 minutes.
- Field replicates: Field replicates are samples taken from, and are representative of, the same locations during the same sampling event, and carried through all steps of the sampling and analytical procedures in an identical manner. Field replicates are used to assess variance of sampling and analysis and prevent false positives. A minimum of three replicates are collected for each location during each sampling event. Chemical feature identification is based on feature occurrence in multiple replicates.

Bias

Bias is the systematic or persistent distortion of a measurement process which makes the result non-representative (i.e., the measured parameter is different than its true value in a given sample). Potential sources of bias include sampling and analytical procedures that introduce contamination, instability of samples during transportation and storage, interference from other constituents in the sample matrix, inability of the analytical method to measure all forms of the constituent of interest, and faulty calibration of the measurement process. Errors of bias are minimized through use of standardized procedures by properly trained staff.

Bias will be assessed by use of a continual injection of reference solution during instrument analysis. The reference solution contains two ions (122 m/z and 922 m/z) that are continuously injected during the analytical run. If these two ions are not detected, the mass calibration cannot be ensured. Bias will also be assessed by the analysis of blanks, including field blanks, method blanks, and instrument blanks. Field blanks are prepared coincident with sample collection and may provide an indication of contamination due to bottle cleanliness, transport conditions, exposure to surroundings during sampling, and transfer from equipment. Method (or laboratory) blanks are prepared in the laboratory and processed in the same manner as the field samples and can, thus, provide information on the preparation process. Instrument blanks are solvent-only (e.g., methanol) samples that are analyzed along with the sample set. They can provide information on contamination or bias associated with the instrumentation.

Identification and alignment of peaks in non-target data (referred to as features, or unique exact mass-retention time pairs) is performed concurrently for all samples and blanks in Agilent MassHunter Profinder software (B.06.00). Peaks are defined as having mass height counts above 300 (noise level) for positive adducts ($[M + H]^+$, $[M + Na]^+$, and $[M + NH_4]^+$) or negative adducts ($[M - H]^-$). Alignment of features across sample groups in MassHunter Profinder is based upon matching retention time and mass within spans of 0.3 min and 30 ppm, respectively. To screen ions, only features with mass height above 5000 (S/N ~17) and appearing in 50% of replicates from at least one condition (2 of 4 replicates) are extracted. The recursive feature extraction rescanned samples and extracted ions with heights above 3000 (S/N ~10) and match score (based on mass accuracy, isotopic abundance, and isotopic spacing) >50 to capture any features missed during the first feature extraction.

Field, method, and instrument blanks are utilized to identify features that are associated with the sample process. Some features may be present in both the blanks and the field samples. For those features identified in both samples and blanks, the MQO for feature reporting is that the peak area (abundance) in the field sample must be 5 times greater compared to the peak area in the blank.

Representativeness

The sampling design incorporates several factors to ensure resulting data is representative of environmental conditions. First, samples will be collected at a suite of locations along the Lower Columbia River in the Willamette River (near to the confluence) to characterize along a spatial gradient. Sample sites are located to characterize conditions entering the Lower Columbia River as well

as upstream and downstream potential source inputs. The results will be a range of sample thought to be representative of conditions in the study area.

Second, sufficient volume will be collected at each sample location for triplicate processing and analysis. The use of triplicate samples, along with field, method, and laboratory blanks, will ensure that identified compounds are actually representative of environmental conditions and not a relic of sample collection, processing, or handling procedures. Additionally, established data reduction procedures have been developed and evaluated to ensure sample representativeness.

Third, there will be multiple field sampling events to: 1) determine the consistency of occurrence patterns during similar conditions, and 2) determine the changes in occurrence patterns during different environmental conditions. Multiple sampling events will ensure that the range of occurrence patterns are captured and represented in the final data.

Comparability

To ensure comparability across all samples collected and analyzed across UWT at CUW projects, relevant procedures are detailed in laboratory SOPs and followed closely. Samples are collected, processed, and handled in a consistent manner. Relevant SOPs are included in Appendix A.

Sensitivity

Sensitivity is a measure of the capability of a method to detect a substance and discriminate between measurement responses representing variable levels of interest. Sensitivity is measured through reporting limit performance, and in a regulatory setting, the method detection limit (MDL) is often used to describe sensitivity. In the case of HRMS approaches, the sensitivity of the method can be assessed based on the detector resolving power, the results of repeated injections of reference standards, and by setting standards for minimum response of non-target features. The following measures will be used to measure sensitivity.

- Instrument resolving power: The resolving power of the QTOF detector is typically 6,000-13,000 within the acquisition range (100-1700 m/z, MS only; 50-1700 m/z, MS/MS). A standard tune solution is used to tune the QTOF prior to each analytical run; specific targets for resolving power are set for individual masses in the tune solution (see Table 4). Additionally, peak height of the tuning ions is expected to be 100,000-400,000 (with the exception of 118 m/z, which is expected to be > 40,000).
- Reference standard injections: A mixture of reference standards, each at a concentration of approximately 100 ng/mL (CEC-CAL-8; preparation described in CEC Calibration Set Preparation SOP) is analyzed every 8-10 samples to check chromatography and sensitivity during data acquisition. Area counts are monitored and expected to be within 20% of initial sensitivity.
- Instrument response: After alignment of features in samples and blanks, only compounds with mass height above 5000 (S/N ~17) in the sample are considered for further analysis.

A.8 Special Training/ Certifications

Project staff have a combined >15 years of experience analyzing environmental samples for various pollutants, including CECs. UWT at CUW staff have the necessary skills in environmental analytical chemistry, especially non-target screening for pollutants and HRMS applications (Tian et al. 2017a, Tian et al. 2017b, Tian et al. 2018). All laboratory personnel are required to take laboratory training courses as administered by the University of Washington Environmental Health and Safety (http://www.ehs.washington.edu/psotrain/). These training records are managed by the UWT at CUW laboratory manager and documented in department employee files. There are no additional training or certification required for project personnel above and beyond what is required per the project staff job classifications. All project staff are trained to demonstrate competency in the water quality monitoring and data analysis program components.

A.9 Documentation and Records

Reporting documentation provided by UWT at CUW labs to the US EPA will be submitted in PDF or .docx format, whichever is preferred by EPA. All UWT at CUW files are stored digitally on local computers, as well as in online cloud storage.

A.9.1 Data recording and reporting requirements

All electronic data, including documents, analytical output, statistical analysis, reports, etc. will be stored on project computers at the UWT at CUW labs that are backed up by a commercial cloud-based system that maintains continuously updated copies of all materials. A Microsoft Excel-based electronic record of all sampling events, stored samples, and associated data will be maintained for the project, and will be stored on UWT at CUW project computers. Data entry errors will be detected by comparison of field records and electronic data records.

Three types of documentation will be managed: (1) field operation records, (2) laboratory records, and (3) QAPP revision documentation.

Field Operation Records

Sample ID

All sample containers and extracts will be labeled with a unique identifier. Sample labels will include the date of collection, unique site name/identifier, replicate number, and initials of field personnel.

Chain-of-custody

A laboratory chain-of-custody form will be completed by project field staff. The information on the chain-of-custody form will include project area name, staff name and contact information, billing information, sample identification, time collected, method of analysis and any comments pertinent to the sample. The form will be signed and dated by the project field staff, and also by laboratory staff who verify receipt of samples.

Field log requirements

The field log will be a bound, waterproof notebook with pre-numbered pages. Permanent, waterproof ink will be used for all entries. Corrections will be made with single line strikethroughs, initialed, and dated. The following details will be included in field entries:

- Clearly record the event name, date, staff names, weather conditions, collection time, sample location identification, and field notes.
- For every site, the latitude and longitude of the sample location should be verified during the initial sampling event using a GPS unit. The site conditions should be recorded with digital photographs. Photographs are helpful for locating sampling stations during subsequent surveys. It is not necessary to record GPS coordinates or take site photographs for every sampling event.
- Record detailed sample location descriptions so that sites can be resampled by different staff, if necessary.
- Descriptions, maps, and aerial photos marked with sampling locations should also be included in the project files.
- Note the identity of QC samples collected, and any unusual circumstances that might affect interpretation of results.
- Note any changes or deviations from the QAPP

Laboratory records

Internal and contract laboratories will provide a data package to the Principal Investigator, or designee, and will be available to EPA. The data package will report the test results clearly and accurately. The test report will include the information necessary for interpretation and validation of data and will include the following:

- Report title
- Name and address of laboratory
- Cover narrative
- Study name
- Sample identifiers
- Data and time of sample collection and sample analysis
- Analytical methods and results
- Results of QC samples
- An explanation of any failed QC or non-standard conditions that may have affected quality, including corrective actions and plan to prevent loss of quality
- Chain of custody forms

QAPP

On approval of the QAPP and any subsequent revisions, it will be distributed by Hayley Mathews via email to project staff and those listed on the distribution list found in Table 1.

If the scope of the study changes in a substantial way, then a revised version or addendum will be prepared and submitted to EPA for review and approval. The approved version of the QAPP will remain in effect until the revised version or addendum has been approved. Justifications, summaries and details of all QAPP changes will be documented and distributed by Hayley Mathews to all persons in the QAPP distribution list. Minor changes to the QAPP shall be discussed in the final report.

B. Data Generation and Acquisition

B.1 Sampling Process Design (Experimental Design)

Task 1 will consist of a focused monitoring program to characterize the occurrence of priority chemicals in the Columbia River. The monitoring will consist of approximately four sampling events, with grab samples of Columbia River water taken from approximately 12-14 locations for each event. The timing of the sampling events will be selected in order to characterize the influence of two primary pathways of chemicals into the river system, focusing on stormwater runoff from the built environment (e.g., roads, commercial and industrial landscapes, residential neighborhoods, etc.) and WWTP effluent.

To capture the influence of stormwater and/or wastewater we plan to collect samples during two wet weather events, when stormwater is likely to enter into the Columbia River, and during two dry weather events, when stormwater influence is likely to be low and wastewater influence would be more important. By approaching the sampling in this way, we can associate priority chemicals with a given pathway, which will be valuable in informing a management or regulatory response. For example, if a priority compound were associated with wastewater effluent, then treatment system process alterations or upgrades could be explored.

B.1.1 Project Sampling Locations

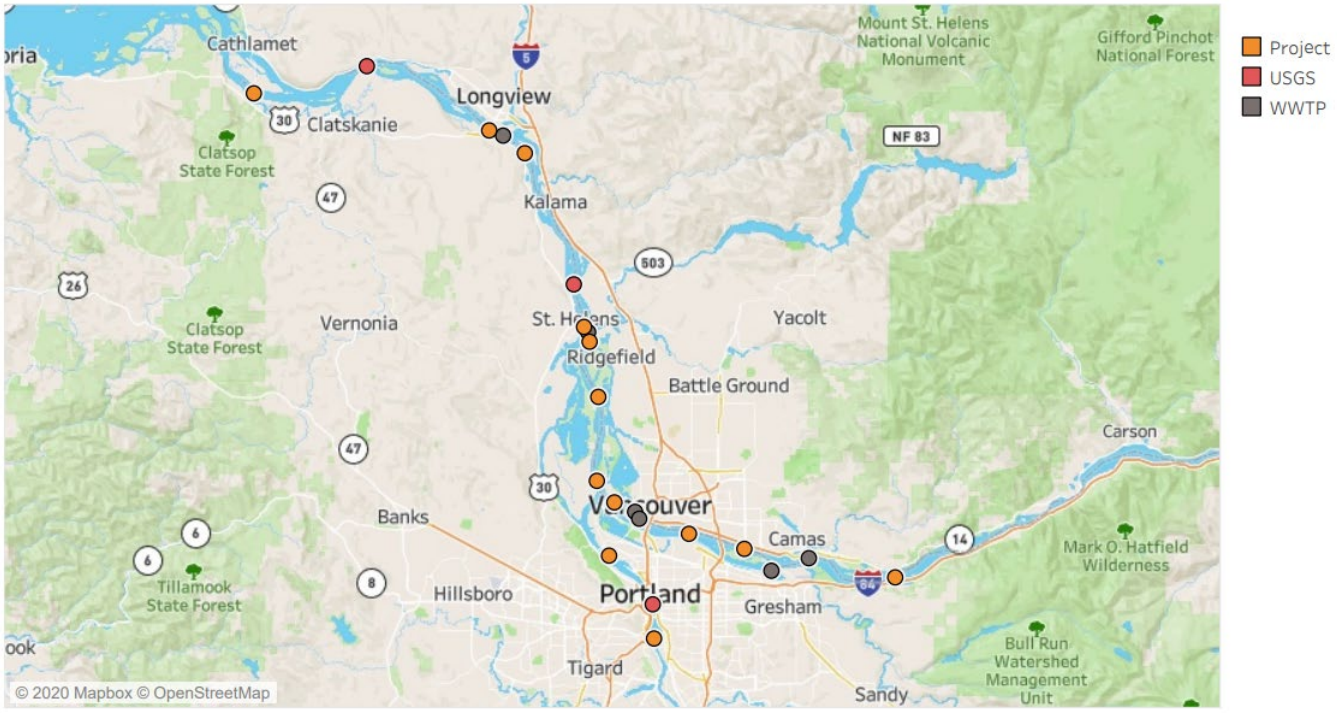
Key considerations in selecting sampling locations are land use and point source inputs to the river. It is expected that the most problematic areas in terms of contaminant loading (and thus exposures to biota) would be those closest to outfalls which direct stormwater or wastewater into the river. The sampling locations were selected to capture these potentially important sources of contaminant loading, (Figure 1). Figure 1 shows the sites unique to this project (“project”), sites that overlap with USGS sampling locations (“USGS”), and nearby WWTPs (“WWTP”); the WWTP outfall locations are included for reference. These sample sites were selected based on land use and other information provided in Morace (2012).

Table 5. Project sampling locations with locations of major WWTP outfalls for reference.

Location Type	Location Name	Latitude	Longitude
Sample	Rooster Rock State Park	45.55087	-122.23786
Sample	Peach Beach (Portland)	45.58636	-122.50683
Sample	Broughten Beach (Portland)	45.60512	-122.60562
Sample	West End of Hayden Island	45.644231	-122.738537
Sample	Blurock Landing (Willamette Confluence)	45.67101	-122.76981
Sample	Rainier/Cowlitz/Three Rivers WWTP	46.10507	-122.96171
Sample	Wahkiakum County Ferry	46.15082	-123.38132
Sample	Stevens Point (Willamette)	45.4746	-122.66788
Sample	Willamette River at Portland, OR**	45.517297	-122.670155
Sample	Columbia River near Columbia City, OR**	45.914810	-122.810876
Sample	Columbia River at Beaver Army Terminal, OR**	46.184315	-123.179853
Sample	Confluence of Multnomah Channel and Columbia River	45.8621	-122.792625
Sample	Confluence of Columbia River and Lake River (Vancouver)	45.843795	-122.782735
Sample	Willamette River at Burlington Northern RR Bridge	45.57796	-122.748029
Sample	Upstream Longview	46.076974	-122.898199
Sample	Ridgefield Refuge	45.775324	-122.767249
Reference	Vancouver Westside WWTP effluent at Vancouver, WA	45.63245	-122.70163
Reference	Columbia Blvd WWTP effluent at Hayden Island, OR	45.62395	-122.69427
Reference	City of St. Helens WWTP effluent at St. Helens, OR	45.85559	-122.7853
Reference	Three Rivers Regional WWTP effluent at Longview, WA	46.0987	-122.9369
Reference	Gresham WWTP effluent	45.559097	-122.458628
Reference	Camas WWTP effluent	45.57479	-122.391897

**Existing USGS sites

Figure 2. Map of sampling locations and nearby WWTPs.



Samples will be collected at both project and USGS sites. WWTP outfall locations are shown for reference.

All sampling sites are mid-river and it is anticipated will be accessible via boat during each sampling event. In the event that a site is not accessible, for whatever reason, we will sample at the nearest location that would be representative of similar conditions. Alternative sampling location may be selected with the description and selection criteria document in sampling notes.

B.1.2 Project Schedule

We intend to conduct two sampling events in the wet season (October through May) and two events in the dry season (June through September). During each event, project staff will collect samples and transport them back to the UWT at CUW laboratory in the same day. Due to the distance to the sampling sites, it is unlikely that all sites will be sampled in the same day. Sample processing will begin within 24 hours of collection, and HRMS analysis will take place within 7 calendar days.

See earlier discussion in section A.6.2 for full project schedule.

B.1.3 Project Assumptions

This study intends to use non-target HRMS data to develop contaminant profiles of regional waters to support the evaluation of risk. The assumptions and hypotheses supporting the ability to achieve these outcomes are:

1. The sampling sites selected represent close to the full range of concentrations for CECs in Columbia River waters.
2. Non-target QTOF-LC-MS/MS analysis of samples influenced by regional stormwater and WWTPs can provide a representative description of contaminant profiles in regional waters.
3. Non-target contaminant profiles can be used to identify compounds associated with exposure risks in marine and freshwaters.
4. New, high concentration, and potentially high-risk pollutants can be identified in stormwater and surface waters within non-target toxicant signatures by QTOF-LC-MS/MS analysis.

B.2 Sampling Methods

Sample collection materials and methods are detailed in Du et al. (2017) and in section 2 of "Solid Phase Extraction of Trace Organic Contaminants from Water Samples for LC-MS/MS (Targeted) or LC-HRMS (Non-Target) Analysis" dated October 2020 (see Appendix). If any deviations from these procedures occur, they will be noted in the lab notebook and reviewed by the project PI.

B.2.1 Containers, preservation methods, holding times

Water samples will be collected in 4 L amber glass jars (decontaminated by procedures outlined in "Solid Phase Extraction of Trace Organic Contaminants from Water Samples for LC-MS/MS (Targeted) or LC-HRMS (Non-Target) Analysis" dated October 2020). A minimum of 1 L is required for each sample replicate. Each sample will be analyzed in triplicate. Water will be transported on ice from the field site to the Center for Urban Waters laboratory, held at 4°C until extraction, and extracted within 24 hours of sample collection. Sample containers, preservation, and holding times are outlined in Table 6.

Table 6. Sample containers, preservation, and holding times.

Parameter	Matrix	Minimum Quantity Required	Container	Preservative	Holding Time
Non-target analysis	Stormwater or surface water	4 L (minimum 1 L per replicate x 3 replicates)	Amber glass jar	n/a	24h at 4°C

B.3 Sample Handling and Custody

Sample handling and custody procedures are detailed in section 2 of "Solid Phase Extraction of Trace Organic Contaminants from Water Samples for LC-MS/MS (Targeted) or LC-HRMS (Non-Target) Analysis" dated October 2020 (see Appendix).

If any deviations from the SOP occur, they will be noted in the lab notebook and reviewed by the project PI.

B.4 Analytical Methods

Water samples will be analyzed utilizing HRMS methods at the UWT at CUW laboratory, one of the few labs in the United States that can support such evaluations. Samples are analyzed utilizing an Agilent 1290 Liquid Chromatograph paired with an Agilent 6530 Quadrupole Time of Flight mass spectrometry unit, with data processed utilizing proprietary and open source analytical software. The development of this monitoring approach is based on our extensive work in the Puget Sound watershed, evaluating wastewater and stormwater-associated contaminants in stream and estuarine waters (see James et al. 2016, Du et al. 2017, Peter et al. 2018, James et al. 2019 and Tian et al. 2020).

The QToF mass spectrometer performs MS/MS using a quadrupole, a hexapole—collision cell—and a time-of-flight chamber to acquire spectra. The quadrupole selects precursor ions that are fragmented in the collision cell into product ions, which are then impelled to the detector, at an angle perpendicular to the original path. Accurate mass measurements are determined based on the time it takes for ions to traverse the flight chamber. Ionization occurs via electro spray ionization in ether positive or negative modes.

Additional system information, including diagrams, can be found in the overview section of "Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS System Concepts Guide" found in the Appendix.

B.4.1 Standard Operating Procedures

Samples will be analyzed by HRMS using a method developed at UWT at CUW. Details of the method are provided in the SOP "LC-HRMS Operation and Data Analysis" dated November 2019 (see Appendix).

B.4.2 Laboratories accredited for methods

The acquisition and analysis of high-resolution mass spectrometry data described in this QAPP is not subject to the laboratory accreditation system. Analyses will be performed at the UWT at CUW laboratory, which has laboratory accreditation waivers approved by the Washington State Department of Ecology for the water sampling and analysis methods described herein.

B.4.3 Corrective action processes

Project personnel will review field and sample documentation to ensure that processes were performed according the QAPP procedures, and to check for deficiencies and nonconformances. Deficiencies are unauthorized deviations from procedures documented in the QAPP. Nonconformances are deficiencies that affect quality and render the data unacceptable or indeterminate. Examples include:

- Deficiencies

  - Chain of custody deviation such as incorrect sample time, resulting in holding time exceedances.
  - Conducting field Quality Control sampling at a rate less than described in the QAPP.
- Nonconformance
  - Failure to analyze samples within holding times

Deficiencies or nonconformances are reported to the Project PI, and corrective actions are applied (when possible) in a timely manner. Laboratory sample results found outside of limits will be flagged for further evaluation and potentially re-analyzed. The Project PI is responsible for implementing and tracking corrective action procedures based on review findings. Records of corrective actions are maintained by the laboratory data evaluator (chemistry), or the Project PI (field). Field deficiencies and nonconformances are documented in sample logbooks.

B.5 Quality Control

The quality control procedures that will help identify problems or issues associated with data collection and data analysis while the project is underway will include reviewing field notes prior to leaving each site, following the field and laboratory procedures outlined in section B, comparing preliminary results to the quality indicators outlined in section A.7, and regular project meetings to review data outputs and analyses.

When analytical control limits are exceeded (see Table 4), sample analysis will stop, and the cause of the exceedance will be identified and corrected prior to restarting the analytical run. All data acquired up until that point will be reviewed prior to acceptance. Following corrective action, the instrument may be tuned, as described in the SOP titled "Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Setup, Operation, and Data Analysis for Non-Target Analysis of Trace Organic Contaminants" dated November 2019, prior to resuming the analysis.

B.5.1 Table of field and laboratory quality control

Table 7. Quality control samples, types, and frequency for non-target analysis.

Field Blanks	Minimum 1 per event
Field Replicates	Minimum 3 per event
Check standards	Every 8-10 samples through an analytical run
Instrument blanks	Every 8-10 samples through an analytical run
Method blanks	1 per sample event (water)

For additional information, see section A.7.

B.6 Instrument/Equipment Testing, Inspection, and Maintenance

The Agilent 6530 Accurate-Mass quadrupole time-of-flight (QTOF) is a HRMS system that provides accurate mass measurements for profiling, identifying, characterizing, and quantifying low molecular-weight compounds and biomolecules with confidence. Coupled with an Agilent 1290 liquid chromatograph (LC) system, this LC-HRMS workflow supports both non-targeted approaches and suspect screening and analysis of environmental samples. The modules in use on the UWT at CUW system are:

- Agilent LC
    - 1290 Binary pump – G4220A, serial number DEBAA03418
    - 1290 TCC – G1316A, serial number DEBAC06325
    - 1290 Sampler – G4226A, serial number DEBAP04055
- Agilent HRMS
    - QTOF 6530 – G6530B, serial number SG13122004

Information on testing, inspection and maintenance can be found in section 2 of the SOP titled "Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Setup, Operation, and Data Analysis for Non-Target Analysis of Trace Organic Contaminants" dated November 2019. Ongoing maintenance is performed by UWT at CUW staff or by Agilent technicians. Maintenance logs are kept by the UWT at CUW laboratory manager and are available on request.

B.7 Instrument/Equipment Calibration and Frequency

This information can be found in section 2 of the SOP titled "Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Setup, Operation, and Data Analysis for Non-Target Analysis of Trace Organic Contaminants" dated November 2019.

B.8 Inspection/Acceptance of Supplies and Consumables

All laboratory supplies and consumables are received and inspected by the UWT at CUW lab manager, Melissa Gonzalez, or by project staff member Dave Wark. Supplies are not used if they are visibly broken or damaged. All solvents and reagents will be HPLC grade (or equivalent) or higher. When necessary, supplies and consumables are solvent washed or baked in an industrial oven before use to avoid contaminating samples.

Table 8. Supplies and consumables expected to be used in this project.

Item Name	Part Number/ID	Supplier	Acceptance Criteria
Oasis HLB SPE Cartridges (200 mg)	WAT106202	Waters	N/A
Empore glass beads (filtration aid)	EW-35211-50	Cole Parmer	Cleaned in lab before use
Methanol	A456-4	Fisher Scientific	Optima grade
Ethyl Acetate	E196SK4	Fisher Scientific	Optima grade
Acetonitrile	AX0156-6	Sigma Aldrich Inc	LC-MS grade
Ammonium acetate	A11450	Fisher Scientific	Optima grade
Glacial acetic acid	AX0073-9	Sigma Aldrich Inc	Optima grade
Reference standard for QTOF	G1969-85001	Agilent Scientific	N/A
Deionized water	N/A	Produced in-lab using Thermo Scientific Barnstead nanopore system	N/A

For explanation of Fisher Scientific's reagent purity grades, visit https://fscimage.fishersci.com/cmsassets/downloads/segment/Scientific/pdf/Chemicals/fisherchem_grades.pdf

B.9 Data Management

Data management is described in the following SOPs:

- Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Setup, Operation, and Data Analysis for Non-Target Analysis of Trace Organic Contaminants
- Solid Phase Extraction of Trace Organic Contaminants from Water Samples for LC-MS/MS (Targeted) or LC-HRMS (Non-Target) Analysis

Management of project data is the responsibility of Dave Wark, under the oversight of Andrew James. See section A.9 for data backup and storage procedures.

B.9.1 Electronic transfer requirements

All final results from non-target analyses and chemical identification efforts will be entered into Microsoft Excel spreadsheets. The data may subsequently be reformatted for transfer into EPA's STORET or Ecology's EIM databases.

B.9.2 EIM/STORET data upload procedures

If required, the project manager will work with EPA and/or Ecology Information Technology staff to ensure that final results are formatted correctly for upload. The final data set would then be uploaded to STORET or EIM for agency review and acceptance.

C. Assessment and Oversight

C.1 Assessments and Response Actions

Assessment	Description	Frequency/Timing	Assessor	Reporting	Corrective Actions
Data Acquisition	Review of compliance with data MQOs during data acquisition to ensure data quality	Coincident with each sampling event	UW Field Manager	Notes corrective actions taken. Report to PI.	As needed during data acquisition
Sample event	Review of sample collection, processing, data acquisition, and data reduction procedures	Following completion of sampling event	UW Data Evaluation Manager	Report to PI. Include with regular semi-annual reporting	As needed
Project	Review of overall project compliance with QAPP procedures	50% project completion	UW QA Manager	Report to PI. Include with regular semi-annual reporting	As needed

C.2 Reports to Management

C.2.1 Frequency and distribution of reports

Reports will include, per the project agreement, , semi-annual reports on progress, semi-annual FEATS reporting, and STORET data collection and reporting and a final project report. Reports will be submitted by email to the EPA project officer.

The semi-annual progress reports will summarize experimental design and data analysis, present and discuss findings, and describe all activities performed. The final report will summarize the background and goals of the project, the experimental design, data collection and analysis methods, data quality, and final results. It will also provide conclusions and recommendations for follow-up activities, as appropriate. A draft report will be distributed for review to the EPA project officer before being finalized.

C.2.2 Responsibility for reports

Reports will be prepared by Andrew James or his designee.

D. Data Validation and Usability

D.1 Data Review, Verification, and Validation

Data verification is a systematic process for evaluating performance and compliance of a set of data to ascertain its completeness, correctness, and consistency using the methods and criteria defined in the QAPP. All data obtained from field and laboratory measurements will be reviewed and verified. Verifying the data quality will help detect inaccuracies, characterize uncertainties, and identify other potential deficiencies. Only data that meet appropriate quality objectives and quality control procedures will be considered acceptable and used in the study. The verification process will involve assembling and comparing the raw data and QC sample results to determine if project MQOs have been met.

Field data verification, requirements, and responsibilities

The Project Manager will review all records associated with quality objectives, and QC procedures described in section B.5, for evidence of proper sample handling, record keeping, and collection of blanks. Sample compliance (e.g., sampling method and SOP were followed, no substantial difficulties were encountered collecting samples, correct sample handling and identification) will be verified.

Data validation

The lab data validation procedures will involve assessing:

- Proper calibration and tuning of analytical instrumentation prior to use
- Laboratory QA/QC (i.e., did the lab meet the MQOs?)

The project manager will conduct a compliance screening by evaluating sample chain-of-custody records, sample holding times, evidence of blank contamination, precision (replicate analyses, background ions, reference standard analyses, tune reports), bias (feature alignment, fold change analysis), and sensitivity (instrument resolving power, reference standard analyses). Instrument screening (initial calibration, continuing calibration, tuning, sensitivity and degradation) will be performed as part of regular laboratory operations.

D.2 Verification and Validation Methods

D.2.1 Treatment of non-detects

In the context of non-target data, non-detects are defined as the lack of a peak (with height 5,000) in the chromatogram for an exact mass-retention time pair (within a span of 0.3 min and 30 ppm) that is observed in other samples with which the sample of interest is aligned. Non-detects will be reported as ND in processed data outputs.

D.2.2 Documentation of assessment

The data usability assessment will be documented in the data report by reporting relevant parameters for the MQOs, as listed in Table 4.

D.2.3 Data analysis using Agilent software

Data Acquisition:
The data acquisition is performed in Agilent MassHunter Data Acquisition Version B.05.01, Build 5.01.5125.3. Data analysis is performed in Profinder B.08.00 Build 8.0.898.0, Qualitative Analysis B.06.00 Build 6.0.633.10 Service Pack 1, and in Mass Profiler Professional (MPP) B.13.00. Recursive extraction of non-target features is performed in Profinder.

Data Processing:
Data files that are acquired from the instrumental analysis using MassHunter Data Acquisition are processed with Profinder. Profinder identifies, extracts, and aligns features across samples/data files which allows the comparison of samples in a single event or across multiple events.

Data Analysis:
The resulting, aligned data is evaluated with MPP, a statistical package used to perform data QAQC, filter (i.e., blank subtractions), analyze, and compare occurrences and occurrence patterns across samples and sample sets.

MPP is also used to perform a series of statistical evaluations such as hierarchical cluster analyses and principal component analysis to determine and evaluate relationships between samples. Feature identification is performed by via the MPP module Agilent ID Browser B.07.00 Build 7.0.799.1 to screen against suspect screening databases, using accurate mass, isotope abundance, and isotope spacing information.

The databases that are used in conjunction with Agilent software packages are managed with Agilent PCDL Manager B.07.00 Build 7024.0 and generally contain formula, structure, exact mass, and MS/MS spectra information.

The analysis of individual sample data files is performed with Agilent MassHunter Qualitative Analysis. This supports a focused analysis of individual features including the associated MS and MS/MS spectra information. The primary use is confirming the identification of suspect and non-target features that are of interest.

D.3 Reconciliation with User Requirements

The data collection and validation is being performed to: 1) provide a screening-level characterization of chemical occurrence in the Lower Columbia River, 2) provide a basis for source evaluation, and 3) provide information sufficient for comparison of ecotoxicological evaluations. Properly validated data will be sufficient for those purposes.

As this project is primarily focused on the acquisition of occurrence information, few untested assumptions were made during its development. One key assumption is that the sampling locations will be sufficient to characterize the range of contaminant profiles that occur in this system. This was based on a review of existing water quality monitoring results and through direct consultations with personnel who have years of experience working in this area. It is possible that there are exposure "hot spots" that exist that are outside the sampling locations. Evaluation and comparison across the spatial and temporal sampling gradient will likely provide evidence of such locations, and could be used to inform future monitoring.

Finally, the use of this data for ecotoxicological screening is based on findings and approaches in the peer-reviewed literature. It is still an area of active research both regionally and nationally. The project technical advisory team includes subject experts; data review meetings will help reconcile resulting information with end-use, ecotoxicological evaluations.

D.3.1 Process for determining project objectives were met

To evaluate whether the project outcomes have met the original objectives, the project manager will assess if the data were collected consistent with the study design (with no reason to question the study design assumptions), study methods, and study procedures described in the final approved QAPP, and if enough of the data (>95%) are deemed usable after verification.

Non-target data will be rejected and will not be used in further analyses if MQOs are not met, including if:

- Reference masses are not observed during the analytical run;
- < 100 non-target features are present at a peak area with a fold-change of 5 relative to field, method, and instrument blanks;
- Reference mix analytes are not observed with mass accuracy <5 ppm, retention time variability <0.1 minutes, and area counts within 20% of initial sensitivity.
- Background ions (oleamide, stearamide, triethyl citrate, and 300.2019 Da @ 3.68 min) are not observed at expected retention times (within <0.1 minutes)

The name of the data files for which non-target data are rejected will marked with "_Rejected" at the end of the filename, and the rejection reason will be noted in the project laboratory notebook.

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Appendices

- Stocks and Standards Preparation SOP
- Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Setup, Operation, and Data Analysis for Non-Target Analysis of Trace Organic Contaminants
- Solid Phase Extraction of Trace Organic Contaminants from Water Samples for LC-MS/MS (Targeted) or LC-HRMS (Non-Target) Analysis
- Du, B., J. M. Lofton, K. T. Peter, A. D. Gipe, C. A. James, J. K. McIntyre, N. L. Scholz, J. E. Baker, and E. P. Kolodziej. 2017. Development of suspect and non-target screening methods for detection of organic contaminants in highway runoff and fish tissue with high-resolution time-of-flight mass spectrometry. Environmental Science: Processes & Impacts **19**:1185-1196.
- Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS System Concepts Guide
- Laboratory Accreditation Waiver from Department of Ecology