Quality Assurance Project Plan

Stormwater Contaminants of Emerging Concern in Western Washington

Prepared for
City of Tacoma

Prepared by
Herrera Environmental Consultants, Inc.

HERRERA
Science + Planning + Design

Quality Assurance Project Plan

Stormwater Contaminants of Emerging Concern in Western Washington

Prepared for
Brandi Lubliner
Stormwater & Watershed Planning, Principal Engineer
Department Environmental Services
Center for Urban Waters
326 East D Street
Tacoma, Washington 98421

Prepared by
Herrera Environmental Consultants, Inc.
2200 Sixth Avenue, Suite 1100
Seattle, Washington 98121
Telephone: 206-441-9080

May 14, 2025

Approval Signatures of Key Individuals

Quality Assurance Project Plan

Stormwater Contaminants of Emerging Concern in Western Washington

Prepared for: City of Tacoma
Prepared by: Herrera Environmental Consultants, Inc.
May 14, 2025

Approval signatures indicate that each key member of the project team has reviewed this Quality Assurance Project Plan (QAPP) and agree to follow the methods and quality assurance (QA) procedures contained herein.

Signature: _____ Date: _____

Brandi Lubliner, Project Lead, City of Tacoma

Signature: _____ Date: _____

Dylan Ahearn, Project Manager, Herrera Environmental Consultants

Signature: _____ Date: _____

James Packman, Assistant Project Manager, Herrera Environmental Consultants

Signature: _____ Date: _____

Ed Kolodziej, Project Partner/Toxics, University of Washington Tacoma

Signature: _____ Date: _____

Bryan Berkompas, Project Partner/Monitoring, Geosyntec Consultants

Signature: _____ Date: _____

Meredith Seeley, Lab/Microplastics, Virginia Institute of Marine Science

Signature: _____ Date: _____

Win Cowger, Lab/Microplastics, Moore Institute for Microplastics Pollution Research

Signature: _____ Date: _____

Tiffany Ryan, Primary Lab Director, Tacoma Environmental Service Laboratory

Signature: _____ Date: _____

Chris Dudenhoeffer, Ecology QA Coordinator, Washington State Department of Ecology

Signature: _____ Date: _____

Chelsea Morris, Ecology Project Manager, Washington State Department of Ecology

Contents

Appendices

Tables

Figures

1. Abstract

Under Special Condition 8 Section D (S8.D) of the 2007 National Pollutant Discharge Elimination System (NPDES) Phase I Municipal Stormwater Permit for western Washington, permittees were required to collect data on water quality and settled stormwater suspended particulate matter (SSPM) quality. This stormwater characterization data set has proven extremely valuable for permittees in the region. The project described in this Quality Assurance Project Plan (QAPP) will revisit S8.D monitoring locations and conduct a synoptic monitoring study throughout western Washington. The study will correlate land use (high-density residential, low-density residential, industrial, and commercial) to concentrations of contaminants of emerging concern (CEC) in stormwater, which were either unknown or not measured during the issuance of the 2007 NPDES Permit. Specifically, the study will focus on 6PPD-quinone (6PPDQ), microplastics/tire wear particles (TWP), and per- and polyfluoroalkyl substances (PFAS). Because Herrera is reusing the same monitoring locations from the previous S8.D study, additional parameters will be measured to compare concentrations over time.

In this QAPP, Herrera describes the methods to collect and analyze water and SSPM samples at 16 monitoring locations. Flow-weighted composite and grab sampling will be conducted at 8 of the 16 locations. Only grab samples will be collected at the remaining 8 sites. The QAPP defines the target water quality parameters, the approach to hydrologic monitoring at 8 of the 16 locations, method quality objectives, and analysis methods. The study is designed to determine if pollutant export from the monitored basins has increased or decreased over time. In addition to also measuring the CECs mentioned above, water sample spectra from non-targeted high resolution mass spectrometry (HRMS) analysis will be archived for future investigations as new CECs are discovered.

The project will entail the targeting of 9 storm events for 2 years, for a total of 18 events at each monitoring location. SSPM will be sampled annually for a total of 2 samples per monitoring location. Monitoring will commence in March of 2025.

2. Background

2.1. Introduction and Problem Statement

In order to obtain local water quality data, the Washington State Department of Ecology (Ecology) required stormwater discharge monitoring in the 2007 NPDES Phase I Municipal Stormwater Permit (Phase I Permit) Section S8.D. This resulted in a large, robust dataset for characterizing stormwater in Municipal Separate Storm Sewer Systems (MS4) in western Washington (Ecology 2015). These data have proven valuable for Ecology and Permittees in the region. These data are used for multiple purposes, including modelling, planning, and adaptive stormwater management. For example, the data have been included in The Nature Conservancy's Hot Spot Model, King County's Water Quality Benefits Evaluation (WQBE) model, and Seattle's Integrated Plan (SPU 2015). However, these models and planning efforts do not account for CECs because little, if anything, was known about them when the S8.D data were collected. The 2020 discovery of the linkage between 6PPDQ and coho salmon mortality has shown that emerging pollutants can drive biological degradation in waterways. This discovery demands focus on current CECs in order to characterize their prevalence in runoff and distribution across the landscape.

This project will revisit the locations monitored under S8.D and conduct a synoptic monitoring study throughout western Washington to correlate land use to current CECs. These include 6PPDQ, microplastics/TWP, and PFAS (Tian, et al. 2021, Werbowski, et al. 2021, Saifur and Gardner 2021, Shafi, et al. 2024).

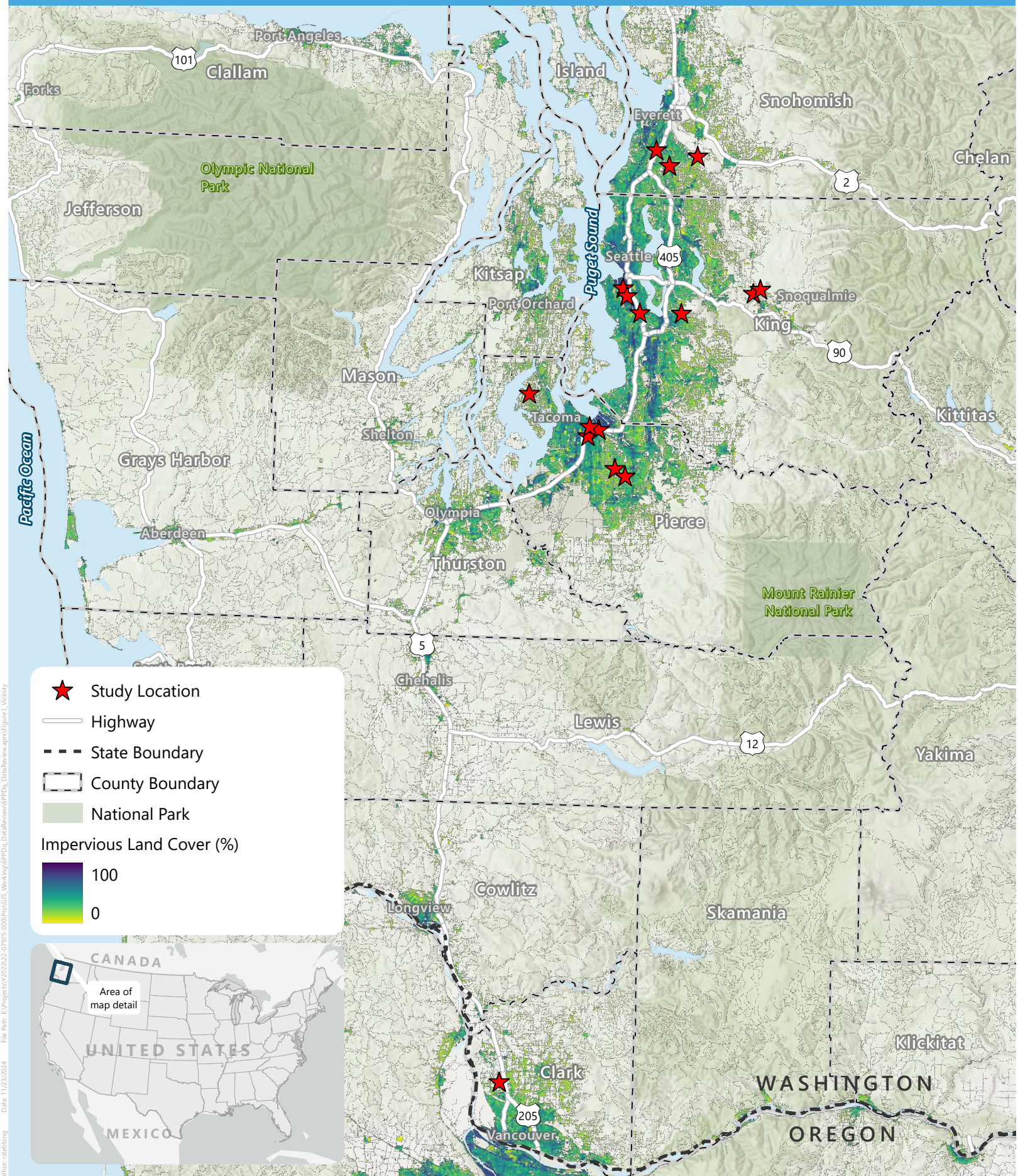
The purpose of this study is to generate a stormwater characterization dataset to augment and update the S8.D data and produce long-term benefits by helping inform future stormwater management decisions. Ecology reissued the Municipal Stormwater Permits in 2024, with more requirements and guidance aimed at source control of pollutants from roadways, stormwater management planning, and retrofitting existing development. With an increased focus on managing and retrofitting existing MS4s, there is a growing need for research on where these efforts will be most effective and what pollutants they should be targeting. The data from this study will provide this foundational information, so that Permittees can make informed decisions on where their best management practices (BMPs) should be located to target these CECs. To help assure information durability, the data generated from this project will be hosted online and easily accessible.

2.2. Study Area and Surroundings

The study area encompasses four counties and two cities in western Washington, consisting of Snohomish, King, Pierce, and Clark Counties, and the cities of Tacoma and Seattle (Figure 1).

HERRERA
Science + Planning + Design

Figure 1.
Vicinity Map.



2.2.1. History of Study Area

The study area consists of select drainage areas within four counties (Clark, King, Pierce, and Snohomish) and two cities (Seattle and Tacoma) in western Washington. For thousands of years, these areas have been inhabited by numerous indigenous tribes including but not limited to the Snohomish, Duwamish, Muckleshoot, Puyallup, Steilacoom, Nisqually, and Cowlitz peoples (Native Land 2024). The various counties and cities as they are known today were established in the mid- to late 1800s. Since then, the area has been highly developed, with a large percentage of impervious area (Figure 1).

The monitoring locations for this study represent stormwater and stormwater SSPM (also commonly referred to as suspended sediment or suspended solids) from typical residential, commercial, and industrial land uses. A geographical information system (GIS) analysis will be performed to determine if land uses contributing to the monitoring locations have significantly changed since the previous study. The results of the analysis will be presented in the final report.

2.2.2. Summary of Previous Studies and Existing Data

Between 2007 and 2014, the Phase I permittees collected stormwater and SSPM data under S8.D in their jurisdictions, all located in western Washington. This stormwater characterization data represented runoff from multiple land uses, across different storm characteristics, and across seasons and years. The Phase I permittees consist of four counties (Clark, King, Pierce, and Snohomish), two cities (Seattle and Tacoma), and two ports (Seattle and Tacoma).

The 2007 to 2014 study provides a western Washington regional baseline of MS4 water and sediment quality (Ecology 2015). The study found that metals, hydrocarbons, phthalates, total Kjeldahl nitrogen and phosphorus, pentachlorophenol, and polychlorinated biphenyls (PCBs) were detected more frequently and at higher concentrations from commercial and industrial areas than from residential areas. Residential areas exported stormwater with the highest dissolved nutrient concentrations. Data from this study are available from Washington state's open data portal (Ecology 2024a; https://data.wa.gov/).

The City of Tacoma has monitored the sites they used for the 2007 S8.D monitoring requirement, outfall (OF) 235, OF237B, and OF245, since 2001. The most recent published report is the *Thea Foss and Wheeler-Osgood Waterways 2022 Source Control and Water Year 2022 Stormwater Monitoring Report* (Tacoma 2023).

2.2.3. Parameters of Interest and Potential Sources

Parameters of interest for this study were selected to align with previously sampled parameters, while adding new CECs and removing contaminants that were rarely detected in the previous study. New CEC additions (e.g., PFAS, 6PPDQ, microplastics/TWP) reflect contaminants where recent research has indicated growing concern and risk to humans or ecosystems, or reflect possible parameters subject to future regulation. Table 1 summarizes parameters of interest and specifies which were previously monitored.

Table 1. Parameters of Interest.				
Sample Type	**Previously Sampled Parameters (2007 S8.D)**		**Parameters in This Study**	
Water	• Total Suspended Solids • Turbidity • Conductivity • Chloride • BOD_5 • Particle Size Distribution • pH • Hardness • Methylene Blue Activated Substances • PAHs • Phthalates • Pesticides (Prometon, Diazinon) • Herbicides (2,4-D, MCPP, Triclopyr, Dichlobenil, Pentachlorophenol)	• Total phosphorus • Orthophosphate • Total Kjeldahl nitrogen • Nitrate+Nitrite as N • Fecal Coliform • Total and dissolved metals (Cadmium, Copper, Lead, Mercury, Zinc) • Total Petroleum Hydrocarbons (diesel and gas range)	• Total Suspended Solids • Total phosphorus • Orthophosphate • Total Kjeldahl nitrogen[a] • Nitrate+Nitrite as N • Total and dissolved metals (Copper and Zinc) • Total Petroleum Hydrocarbons (diesel) • PFAS • 6PPDQ • Microplastics/TWP	
SSPM	• Total Solids • Total organic carbon • Grain size • Total Phosphorus • Total Volatile Solids • Total recoverable metals (Cadmium, Copper, Lead, Mercury, Zinc)	• PAHs • Phthalates • Phenolics • PCB Aroclors • Pesticides (Diazinon, Chlorpyrifos, Malathion) • Herbicides (Pentachlorophenol) • Total Petroleum Hydrocarbons (diesel range)	• Total Solids • Total organic carbon • Grain size • Total Phosphorus • Total Volatile Solids • Total recoverable metals (Arsenic, Cadmium, Copper, Lead, Mercury, Zinc)	• PAHs • Phthalates • Phenolics • PCB Aroclors • Pesticides (Bifenthrin) • Herbicides (Dichlobenil) • Total Petroleum Hydrocarbons (diesel range) • PFAS • 6PPDQ • Microplastics/TWP

[a] Total Kjeldahl nitrogen will be calculated as Total Nitrogen – Nitrate+Nitrite.

SSPM = stormwater suspended particulate matter
PFAS = Per- and poly- fluoroalkyl substances
PCB = Polychlorinated biphenyl
BOD_5 = Biological oxygen demand
MCPP = methylchlorophenoxypropionic acid
PAHs = Polycyclic aromatic hydrocarbon

Due to project constraints, not all water quality parameters analyzed in the 2007 S8.D effort are included in the current study (Table 1). The list was narrowed based on prevalence of non-detects in the previous study, environmental relevance, feasibility, and cost.

In addition to the parameters listed in Table 1, the chemical composition of collected samples will be screened using non-targeted analytical methods (described in Appendix A) to provide electronic data amenable to retrospective analyses. Non-targeted high resolution mass spectrometry (HRMS) analysis (see Appendix A for analytical details) will be used to collect qualitative chemical composition data for samples. This approach can identify both suspected and yet-unknown contaminants in samples and semi-quantify detected chemicals, enabling immediate compositional analysis while also electronically

archiving data for future retrospective analyses as new CECs are discovered. As a non-target approach, HRMS spectra contains all the ionized chemical features present in each sample that are ionizable and amenable to liquid chromatographic separation, including those where little to no identify or abundance information yet exists. All these data will be stored on Ecology servers and be available for retrospective analysis. When new CECs, chemical classes, or transformation products are identified, these spectra may be used to determine their presence and abundance in samples from this study. Refer to Appendix A for guidance on the High-Resolution Mass Spectrometry (HRMS) Analysis.

Continuous water temperature will also be logged at all of the monitoring locations.

2.2.4. Regulatory Criteria or Standards

Regulatory compliance will not be assessed in this study.

2.3. Water Quality Impairment Studies

This QAPP does not describe a water quality impairment study.

2.4. Effectiveness Monitoring Studies

This QAPP does not describe an effectiveness monitoring study.

3. Project Description

The project team will revisit the locations monitored under S8.D of the 2007 Phase I Permit and will conduct a monitoring study throughout western Washington to correlate land use to stormwater concentrations of current CECs (6PPDQ, microplastics/TWP, and PFAS). Through stormwater and SSPM sample collection across western Washington, this project will build a dataset of average CEC concentrations associated with different land uses.

3.1. Project Goals

The primary goal of the project is to undertake a region-wide stormwater monitoring effort to build a dataset of average CEC concentrations associated with the following land uses: Industrial (IND), Commercial (COM), High-Density Residential (HDR), and Low-Density Residential (LDR). A secondary goal is to make this information widely available for Permittees to use in their modeling and pollutant management efforts. Finally, a third goal is to help determine if water and SSPM quality has improved in the decade since the last study.

3.2. Project Objectives

The project objectives are as follows:

- Develop a dataset correlating CECs with land use, by collecting water grab samples and SSPM samples at 16 locations (composite samples will be collected at half the locations, see below).
- Compare the current SSPM chemistry with the chemistry of SSPM samples previously collected, under the 2007 Phase I Permit, at the same locations.
- Use flow-weighted composite sampling (at 8 of the 16 locations) to compare a subset of project water quality parameters with the sample results previously analyzed under the 2007 Phase I Permit.
- Provide data that can be easily accessed via web-based dashboards and used for future planning and modeling efforts.
- Produce and archive the non-targeted HRMS for future CEC analysis of the samples collected in this study.
- Characterize temperature variability in MS4s across western Washington.

3.3. Information Needed and Sources

Existing information and resources needed for the project include the following:

- Data from previous stormwater and SSPM monitoring efforts at the monitoring sites—to be downloaded from https://data.wa.gov/.

- Geospatial information, including stormwater infrastructure features, traffic usage, land cover, and delineated drainage basins—to be obtained from the relevant jurisdictions
- Prior research and data on sampling and analysis methods for CECs—to be found through a literature review

New data collected during the course of the study will be the chemical analytical results of water and SSPM samples and the hydrologic data from 8 of the 16 locations.

3.4. Tasks Required

Tasks required to meet the project objectives include the following:

- Perform GIS land use analysis.
- Select two new industrial land use monitoring locations.

  Note: The 2007 Study had more COM, HDR, and LDR stations than IND. To balance the n-value among the land uses, two more IND sites will be added.
- Review available literature on CECs and compile available data.
- Install in-line SSPM traps at 16 monitoring locations.
- Install automated samplers, flow monitoring equipment, and rain gauges at five locations (the three locations in Tacoma already have this equipment installed).
- Install water level/temperature sensors at 16 locations.
- Collect grab samples at 16 locations and composite samples at eight of the 16 locations during 18 events.
- Retrieve SSPM samples annually from each site by removing the sample material from the traps and homogenizing.
- Submit samples to analytical laboratories for microparticle (microplastics/TWP) and chemical analysis.
- Review and manage hydrologic and water quality data.
- Summarize findings in a final report and three public presentations.

3.5. Systematic Planning Process

Preparation of this QAPP is adequate systemic planning for this project.

4. Organization and Schedule

4.1. Key Individuals and Responsibilities

Table 2 includes a summary of the key individuals involved in project implementation and their roles.

Table 2. Key Personnel and Roles.

Organization	Personnel	Role	Responsibilities
City of Tacoma	Brandi Lubliner	Project Lead	Coordinates grant with Ecology. Directs the consultant team.
	Monica Herbert	Laboratory Coordinator	Coordinates lab analyses and sub labs
	Steve Shortencarrier	City of Tacoma Field Lead	Leads collection of samples in Tacoma and records field information
Herrera Environmental Consultants	Dylan Ahearn	Project Manager and Principal-In-Charge	Reviews deliverables. Coordinates with Project Lead and Project Manager.
	James Packman	Assistant Project Manager	Supports Project Manager.
	Stacy Luell	Assistant Project Manager	Supports Project Manager.
	Nicholas Harris	King County Field Lead and Technical Lead	Leads collection of samples in King County and records field information. Installs monitoring equipment. Leads development of data dashboard.
	Sam Nilsson	Clark County Field Lead and Technical Support	Leads collection of samples in Clark County and records field information. Compiles CEC database.
	Nikki VandePutte	Technical Support	Leads development of QAPP. Reviews and manages chemistry data.
	Rebecca Stebbing	GIS Analyst	Compiles and analyzes GIS data.
University of Washington Tacoma	Ed Kolodziej	CEC Expert	Directs CEC lab analyses.
	Kaylee Martin	Pierce County Field Lead	Leads collection of samples in Pierce County and records field information. Performs review of CEC literature.
Aspect Consulting	Bryan Berkompas	Snohomish County and City of Seattle Field Lead	Leads collection of samples in Snohomish County and Seattle and records field information.
Moore Institute for Plastic Pollution Research	Win Cowger	QAPP Review	Reviews QAPP material related to microplastics sampling and analysis.
Virginia Institute of Marine Science	Meredith Seeley	Laboratory Coordinator	Directs Microplastics Lab.
Department of Ecology	Chelsea Morris	Project Manager	Manages this SAM project. Verifies requirements are being met.
	Chris Dudenhoeffer	Quality Assurance Coordinator	Reviews and approves the draft QAPP and final QAPP

GIS = Geographical Information System

CEC = Contaminant of Emerging Concern

QAPP = Quality Assurance Project Plan

A Technical Advisory Committee (TAC) has been formed to provide technical expertise to the project. The TAC is made up of regional and national experts in stormwater management, representing public, private, and educational organizations (Table 3).

Table 3. Technical Advisory Committee (TAC).

Member	Affiliation	Email
Ani Jayakaran	Washington State University - Puyallup	anand.jayakaran@wsu.edu
Bob Hutton	Clark County	Bob.Hutton@clark.wa.gov
Brad Archbold	Washington State Department of Transportation	ArchboB@wsdot.wa.gov
Brandi Lubliner	City of Tacoma	BLubliner@cityoftacoma.org
Carla Milesi	Center for Urban Waters	milesi@uw.edu
Carol Falkenhayn Maloy	Pierce County	carol.falkenhaynmaloy@piercecountywa.gov
Curtis Nickerson	City of Redmond	cnickerson@redmond.gov
Dana DeLeon	City of Tacoma	ddeleon@cityoftacoma.org
David Batts	King County	David.Batts@kingcounty.gov
Dylan Ahearn	Herrera	dahearn@herrerainc.com
Ed Kolodziej	University of Washington - Tacoma	koloj@uw.edu
James Packman	Herrera	jpackman@herrerainc.com
Jennifer Arthur	City of Seattle	Jennifer.Arthur@seattle.gov
Jim Crawford	King County	Jim.Crawford@kingcounty.gov
John Herrmann	Snohomish County	John.Herrmann@co.snohomish.wa.us
Meredith Evans Seeley	Virginia Institute of Marine Science	Meredith.Seeley@vims.edu
Win Cowger	Moore Institute for Plastic Pollution Research	wincowger@gmail.com
Shelby Giltner	Department of Ecology	shelby.giltner@ecy.wa.gov
Madison Hattaway	Department of Ecology	Madison.hattaway@ecy.wa.gov

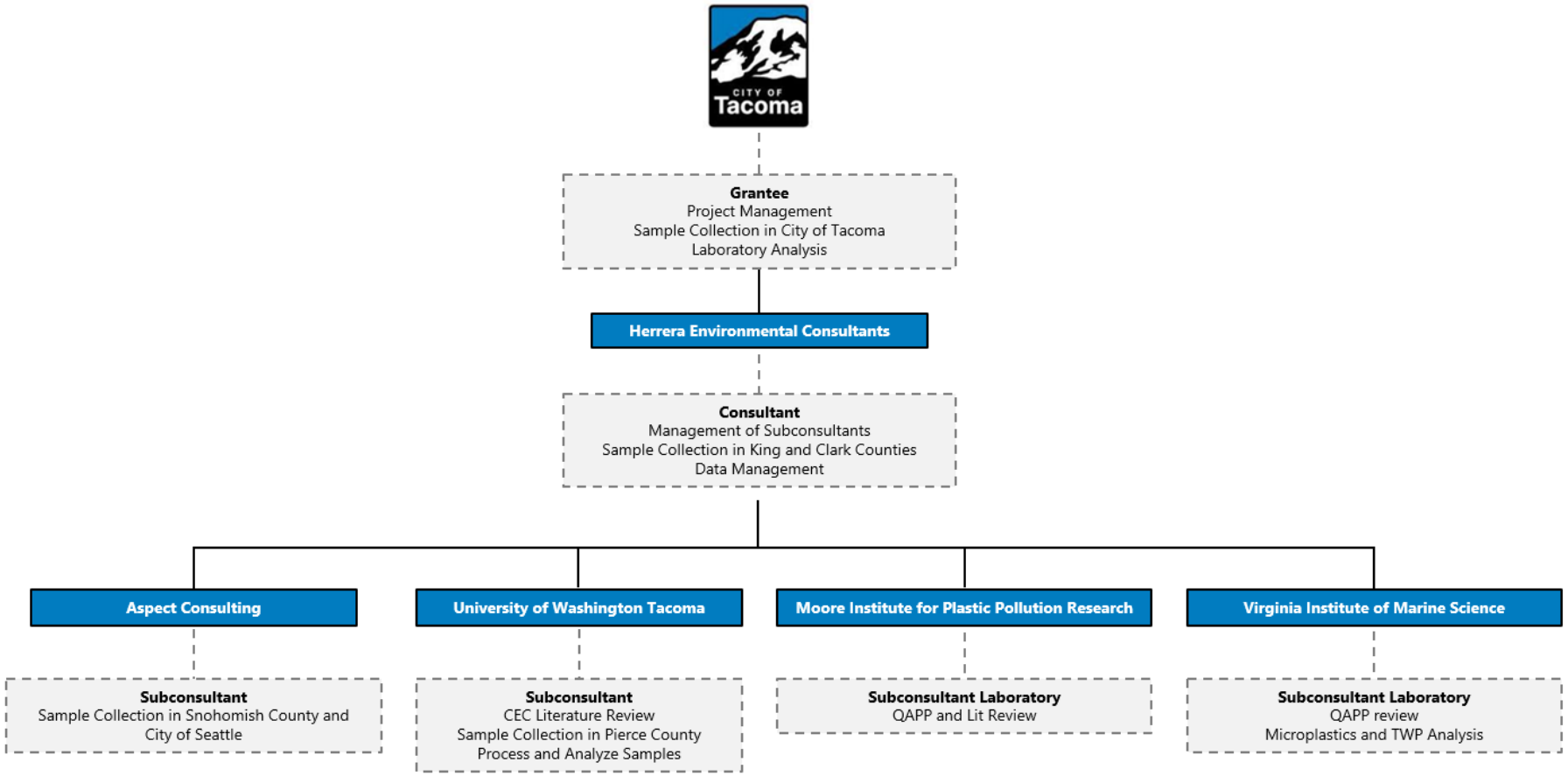
4.2. Special Training and Certifications

Staff responsible for installing and accessing equipment in confined spaces will be required to have confined-space entry certification. All field personnel involved in sample collection will be trained in the procedures outlined in Section 7.2. All field personnel will be trained in adult first aid and CPR and have on-the-job training in traffic control. The City will provide support for intensive traffic control situations within the City of Tacoma.

4.3. Organization Chart

Figure 2 shows the organization of the project team.

Figure 2. Organization Chart.



4.4. Proposed Project Schedule

Table 4 outlines the proposed project schedule. Phase 2 of the project (starting in 2026) has not yet been scoped, so due dates for this phase are subject to change.

Table 4. Proposed Project Schedule.

Task	Project Phase	Due Date	Lead Staff
Planning Activities			
Equipment Purchase	Phase 1	January 2025	Nicholas Harris
Site Reconnaissance Visits	Phase 1	November 2024	Nicholas Harris
Identify Industrial Sites	Phase 1	November 2024	Dylan Ahearn
GIS Analysis	Phase 1	December 2024	Rebecca Stebbing
Draft QAPP and SOPs	Phase 1	December 2024	Nikki VandePutte Stacy Luell
Health & Safety Plan	Phase 1	December 2024	Stacy Luell
Final QAPP and SOPs	Phase 1	March 2025	Nikki VandePutte Stacy Luell
Review of CEC Literature and Available Data			
Completion of CEC Database	Phase 1	March 2025	Sam Nilsson
Draft Literature Review	Phase 1	March 2025	Ed Kolodziej
Final Literature Review	Phase 1	June 2025	Ed Kolodziej
Sample Collection and Monitoring			
Equipment Installation	Phase 1	March 2025	Nicholas Harris Bryan Berkompas
Field Staff Training	Phase 1	February 2025	James Packman Nicholas Harris
Sample Collection	Phase 1	December 2025	Nicholas Harris Sam Nilsson Ed Kolodziej Bryan Berkompas City of Tacoma
	Phase 2	March 2027	
Laboratory Analyses	Phase 1	December 2025	Ed Kolodziej Monica Herbert Meredith Seeley
	Phase 2	March 2027	
Data Management and Analysis			
Laboratory Data Validation	Phase 1	December 2025	Nikki VandePutte
	Phase 2	March 2027	
Internal Technical Dashboard	Phase 1	December 2025	Nicholas Harris
Communications Dashboard	Phase 2	November 2027	Nicholas Harris

Table 4 (continued). Proposed Project Schedule.			
Task	**Project Phase**	**Due Date**	**Lead Staff**
Final Report and Communications			
Communication Plan	Phase 1	November 2024	Dylan Ahearn
Draft Report	Phase 2	October 2027	Dylan Ahearn James Packman Stacy Luell
Final Report	Phase 2	December 2027	Dylan Ahearn James Packman Stacy Luell
Three Presentations	Phase 2	December 2027	Dylan Ahearn James Packman
SAM Fact Sheet	Phase 2	November 2027	Nikki VandePutte

4.5. Budget and Funding

Funding for this project comes from the Stormwater Action Monitoring (SAM) program administered by Ecology. The Stormwater Work Group approved the award to the City of Tacoma (SAM Proposal #FP08) with an overall budget for Phase 1 and 2 of the project of $2,383,786.

5. Quality Objectives

5.1. Data Quality Objectives

The main data quality objective (DQO) for this project is to develop a high-quality dataset that correlates CECs with land use and can be used by Permittees in their modeling and pollutant management efforts. The data will be obtained from a minimum of 144 composite samples collected at eight sites, as well as 288 grab samples and 32 SSPM samples collected at 16 sites.

5.2. Measurement Quality Objectives for Continuous Data

Hydrologic monitoring will involve continuous measurements of water level, water velocity, and precipitation depth. In addition, continuous temperature data will be logged at each monitoring location. Continuous flow monitoring will be conducted at eight of the 16 locations (see Section 6) using area-velocity sensors. Flow measurement errors with these devices can be introduced through error associated with the level sensor or error associated with the velocity sensor. Error associated with precipitation data is derived from either rain gauge miscalibration or placement inaccuracy (e.g., bias from overhanging or nearby trees). Error associated with the thermistors can occur from sensor drift or from locating the sensor in direct sunlight.

The data quality indicators for these measurements are expressed in terms of precision, bias, representativeness, completeness, and comparability. Assessments of precision and bias will be conducted before equipment is deployed in the field and again at the end of the project when the monitoring equipment is retrieved from the field. The measurement quality objectives (MQOs) for continuous data collection are defined below. Note that the quality assurance (QA) procedures for the three City of Tacoma monitoring locations are covered under the Ecology-approved Thea Foss monitoring QAPP (Tacoma 2020). Also note that Herrera is relying on municipal gauges for rainfall for many of the locations, all of which are assessed for accuracy under different programs.

5.2.1. Targets for Precision and Bias

5.2.1.1. Precision

The precision of the water level sensors used will be assessed by submerging the sensors in a 2-liter graduated cylinder covered with foil. The sensor reading will be recorded on a 5-minute time step for 8 to 12 hours at approximately 25 degrees Celsius. Subsequently, the coefficient of variation will be calculated using the following equation:

$$C_v = \frac{\sigma}{\mu} \times 100\%$$

Where: C_v = Coefficient of variation

σ = Standard deviation

μ = The average measured reading

The MQO will be a C_v of no more that 5 percent.

The precision of the velocity sensors cannot be verified without the use of a hydraulics lab with a flume that can produce a constant velocity. The manufacturer of the Starflow QSD and Isco 2150 velocity sensors used in this study claim an accuracy of ±1 to 2 percent.

Rain gauge precision is estimated by (1) repeatedly releasing a known volume of water into the rain gauge to cause the tipping bucket mechanism to tip and (2) recording the number of tips for each run. The process will be repeated three times, and the resultant C_v will be calculated using the above equation. The MQO for rain gauge precision will be 5 percent.

Precision of the temperature sensors will be assessed by submerging all the sensors in a 5-gallon bucket at a constant room temperature and then logging the water temperature over 2 hours. The resultant C_v will be calculated using the above equation. The MQO for thermistor precision will be 5 percent.

5.2.1.2. Bias

Bias will be assessed based on a comparison of monitoring equipment readings to an independently measured "true" value. To assess bias associated with the water level sensors, the sensors will be placed in a 2-liter graduated cylinder. The cylinder will be filled with water to four different known depths (measured with an engineer's scale), and the resultant level sensor readings will be compared with the "true" measured values. Three readings will be recorded at each water level. This test will be conducted at the beginning and end of the study period. The MQO for level measurements will be a difference of no more than 5 percent between the instrument reading and an independently measured level value.

Bias associated with the velocity sensors cannot be determined without access to a known and constant velocity of flowing water. After the sensors are installed on site, a water truck will be used to discharge these flows. The following procedures will assess bias and confirm pipe hydraulics:

- The level sensor in the area-velocity module will be calibrated, so that the water level reads "0" at the structure invert.
- A water truck will be used to discharge flow into the upstream of the sensor, such that flow enters the monitored structure in the same manner it would during a rain event.
- A Master Meter FHM rotameter will be used to estimate discharge from the water truck. Flows will be stabilized at five different flow rates of increasing magnitude, and the associated sensor water level, velocity, and flow rate will be recorded.
- The bias between the "true" rotameter flow—closed channel flow meters are very reliable and accurate—and the measured area-velocity sensor flow will be determined.
- Any bias corrections will be made in the Campbell datalogger, such that real-time estimates of flow are accurate.

Bias in precipitation depth data collected through this study will be assessed based on a comparison of the rain gauge's actual reading to its theoretical reading as specified by the manufacturer. The rain gauge's actual reading will be determined by releasing a known volume of water into the rain gauge. The number of tips recorded will be compared with the theoretical number of tips (assuming the bucket tips with each 0.01 inch of rain). The MQO for precipitation depth will be a difference of no more than 5 percent between the rain gauge's actual reading and the theoretical reading.

Bias associated with the temperature sensors will be assessed by submerging the sensors in a 5-gallon bucket at a constant room temperature and then measuring the water temperature with a calibrated thermometer 3 times over 2 hours. The resultant average difference between the measured temperature and the true temperature will be calculated. The MQO for thermistor bias will be a difference of no more than 5 percent between the true and measured values.

5.2.2. Targets for Comparability, Representativeness, and Completeness

5.2.2.1. Completeness

Completeness will be assessed based on occurrence of gaps in the data record for all monitoring equipment. The associated MQO is less than 10 percent of the total data record missing due to equipment malfunctions or other operational problems. Completeness will be ensured through routine maintenance of all monitoring equipment and the immediate implementation of corrective actions if problems arise.

5.2.2.2. Representativeness

The representativeness of the hydrologic data will be ensured by the proper installation of the monitoring equipment, including primary and secondary devices.

5.2.2.3. Comparability

There is no numeric MQO for this data quality indicator. However, standard monitoring procedures, units of measurement, and reporting conventions will be applied in this study to meet the goal of data comparability.

5.3. Measurement Quality Objectives for Laboratory Data

The overall laboratory measurement quality objective is to ensure that data of a known and acceptable quality are obtained. All laboratory measurements will be performed to yield consistent results that are representative of the media and conditions measured. MQOs for laboratory data are defined by precision, bias, sensitivity, representativeness, completeness, and comparability. Project-specific water and SSPM quality MQOs are provided in Table 5 and Table 6.

Table 5. Measurement Quality Objectives (MQOs) for Water Quality Parameters.							
Analytical Group	**Parameter**	**Laboratory Duplicate (RPD)**	**Field Duplicate (RPD)[a]**	**Method Blank**	**Matrix Spike (% Recovery)**	**Lab Control Standard (% Recovery)**	**Laboratory Reporting Limit**
Conventional	Total Suspended Solids	≤20	≤20	<MDL	NA	85–115	2 mg/L
Metals (EPA 200.8)	Copper (Total and Dissolved)	≤20	≤20	<MDL	70–130	85–115	0.8 ug/L
	Zinc (Total and Dissolved)	≤20	≤20	<MDL	70–130	85–115	5.0 ug/L
Nutrients	Total Phosphorus	≤20	≤20	<MDL	90–110	90–110	0.01 mg/L
	Orthophosphate	≤20	≤20	<MDL	90–110	90–110	0.01 mg/L
	Total Nitrogen [c]	≤20	≤20	<MDL	75–125	90–110	0.10 mg/L
	Nitrate+Nitrite	≤20	≤20	<MDL	90–110	90–110	0.05 mg/L
Petroleum Hydrocarbons	NWTPH-Dx - Deisel	≤50	≤50	<MDL	NA	50–150	0.05 mg/L
	NWTPH-Dx – Motor Oil	≤50	≤50	<MDL	NA	50–150	0.1 mg/L
Emerging Contaminants	6PPDQ	≤20[b]	≤35	<MDL	70–130[b]	70–130	10 ng/L
	Microplastics/TWP [d]	≤35	≤35	<MDL	NA	50–150	1 ug/sample
	Perfluorobutanoic acid (PFBA)	≤35[b]	≤35	<MDL	40–160[b]	70–140	6.4 ng/L
	Perfluoropentanoic acid (PFPeA)	≤35[b]	≤35	<MDL	40–160[b]	65–135	3.2 ng/L
	Perfluorohexanoic acid (PFHxA)	≤35[b]	≤35	<MDL	40–160[b]	70–145	1.6 ng/L
	Perfluoroheptanoic acid (PFHpA)	≤35[b]	≤35	<MDL	40–160[b]	70–150	1.6 ng/L
	Perfluorooctanoic acid (PFOA)	≤35[b]	≤35	<MDL	40–160[b]	70–150	1.6 ng/L
	Perfluorononanoic acid (PFNA)	≤35[b]	≤35	<MDL	40–160[b]	70–150	1.6 ng/L
	Perfluorodecanoic acid (PFDA)	≤35[b]	≤35	<MDL	40–160[b]	70–140	1.6 ng/L
	Perfluoroundecanoic acid (PFUnA)	≤35[b]	≤35	<MDL	40–160[b]	70–145	1.6 ng/L
	Perfluorododecanoic acid (PFDoA)	≤35[b]	≤35	<MDL	40–160[b]	70–140	1.6 ng/L
	Perfluorotridecanoic acid (PFTrDA)	≤35[b]	≤35	<MDL	40–160[b]	65–140	1.6 ng/L
	Perfluorotetradecanoic acid (PFTeDA)	≤35[b]	≤35	<MDL	40–160[b]	60–140	1.6 ng/L
	Perfluorobutanesulfonic acid (PFBS)	≤35[b]	≤35	<MDL	40–160[b]	60–145	1.6 ng/L
	Perfluoropentanesulfonic acid (PFPeS)	≤35[b]	≤35	<MDL	40–160[b]	65–140	1.6 ng/L

Table 5 (continued). Measurement Quality Objectives (MQOs) for Water Quality Parameters.							
Analytical Group	**Parameter**	**Laboratory Duplicate (RPD)**	**Field Duplicate (RPD)[a]**	**Method Blank**	**Matrix Spike (% Recovery)**	**Lab Control Standard (% Recovery)**	**Laboratory Reporting Limit**
Emerging Contaminants (continued)	Perfluorohexanesulfonic acid (PFHxS)	≤35[b]	≤35	<MDL	40–160[b]	65–145	1.6 ng/L
	Perfluoroheptanesulfonic acid (PFHpS)	≤35[b]	≤35	<MDL	40–160[b]	70–150	1.6 ng/L
	Perfluorooctanesulfonic acid (PFOS)	≤35[b]	≤35	<MDL	40–160[b]	55–150	1.6 ng/L
	Perfluorononanesulfonic acid (PFNS)	≤35[b]	≤35	<MDL	40–160[b]	65–145	1.6 ng/L
	Perfluorodecanesulfonic acid (PFDS)	≤35[b]	≤35	<MDL	40–160[b]	60–145	1.6 ng/L
	Perfluorododecanesulfonic acid (PFDoS)	≤35[b]	≤35	<MDL	40–160[b]	50–145	1.6 ng/L
	1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)	≤35[b]	≤35	<MDL	40–160[b]	70–145	6.4 ng/L
	1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	≤35[b]	≤35	<MDL	40–160[b]	65–155	45 ng/L
	1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)	≤35[b]	≤35	<MDL	40–160[b]	60–150	6.4 ng/L
	Perfluorooctanesulfonamide (PFOSA)	≤35[b]	≤35	<MDL	40–160[b]	70–145	1.6 ng/L
	N-methylperfluorooctane sulfonamide (NMeFOSA)	≤35[b]	≤35	<MDL	40–160[b]	60–150	1.6 ng/L
	N-ethylperfluorooctane sulfonamide (NEtFOSA)	≤35[b]	≤35	<MDL	40–160[b]	65–145	1.6 ng/L
	N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	≤35[b]	≤35	<MDL	40–160[b]	50–140	1.6 ng/L
	N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	≤35[b]	≤35	<MDL	40–160[b]	70–145	1.6 ng/L
	N-methylperfluorooctane sulfonamidoethanol (NMeFOSE)	≤35[b]	≤35	<MDL	40–160[b]	70–145	16.0 ng/L
	N-ethylperfluorooctane sulfonamidoethanol (NEtFOSE)	≤35[b]	≤35	<MDL	40–160[b]	70–135	16.0 ng/L
	Hexafluoropropylene Oxide Dimer Acid (HFPO-DA)	≤35[b]	≤35	<MDL	40–160[b]	70–140	6.4 ng/L
	4,8-Dioxa-3H-perfluorononanoic acid (ADONA)	≤35[b]	≤35	<MDL	40–160[b]	65–145	6.4 ng/L
	Perfluoro-3-methoxypropanoic acid (PFMPA)	≤35[b]	≤35	<MDL	40–160[b]	55–140	3.2 ng/L
	Perfluoro-4-methoxybutanoic acid (PFMBA)	≤35[b]	≤35	<MDL	40–160[b]	60–150	3.2 ng/L
	Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	≤35[b]	≤35	<MDL	40–160[b]	50–150	3.2 ng/L
	9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	≤35[b]	≤35	<MDL	40–160[b]	70–155	6.4 ng/L

Table 5 (continued). Measurement Quality Objectives (MQOs) for Water Quality Parameters.							
Analytical Group	**Parameter**	**Laboratory Duplicate (RPD)**	**Field Duplicate (RPD)[a]**	**Method Blank**	**Matrix Spike (% Recovery)**	**Lab Control Standard (% Recovery)**	**Laboratory Reporting Limit**
Emerging Contaminants (continued)	11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	≤35[b]	≤35	<MDL	40–160[b]	55–160	6.4 ng/L
	Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	≤35[b]	≤35	<MDL	40–160[b]	70–140	3.2 ng/L
	3-Perfluoropropylpropanoic acid (3:3 FTCA)	≤35[b]	≤35	<MDL	40–160[b]	65–130	8.0 ng/L
	3-Perfluoropentylpropanoic acid (5:3 FTCA)	≤35[b]	≤35	<MDL	40–160[b]	70–135	40 ng/L
	3-Perfluoroheptylpropanoic acid (7:3 FTCA)	≤35[b]	≤35	<MDL	40–160[b]	50–145	40 ng/L

[a] If one or both results are within 5 times the reporting limit, the difference between results will be less than 2 times the reporting limit.

[b] Laboratory duplicates and matrix spikes for 6PPDQ and PFAS will only be analyzed upon request.

[c] Total Kjeldahl nitrogen will be calculate as Total Nitrogen – Nitrate+Nitrite.

[d] Plastics will be identified in each of three size classes: >250, 53-250, and <53 microns, as tire wear particles (TWP), polyethylene (PE), Polypropylene (PP), Polystyrene (PS), Polyethylene terephthalate (PET), polycarbonate (PC), and Polymethyl methacrylate (PMMA).

RPD = relative percent difference
MDL = method detection limit
PAHs = Polycyclic aromatic hydrocarbons
PCBs = Polychlorinated biphenyls
TWP = tire wear particles
mg/L = milligrams per liter
PFAS = per- and polyfluoroalkyl substances
ug/L = micrograms per liter
ng/L = nanograms per liter

Table 6. Measurement Quality Objectives (MQOs) for SSPM Parameters.							
Analytical Group	**Parameter**	**Laboratory Duplicate (RPD)**	**Field Duplicate (RPD)[a]**	**Method Blank**	**Matrix Spike (% Recovery)**	**Lab Control Standard (% Recovery)**	**Laboratory Reporting Limit**
Conventional	Total Solids	≤35	≤35	<MDL	NA	NA	1%
	Total Organic Carbon	≤35	≤35	<MDL	75–125	85–115	200 mg/Kg
	Grain Size	≤20	≤20	<MDL	NA	NA	NA
Metals (6020B)	Arsenic	≤20	≤20	<MDL	75–125	80–120	0.25 mg/Kg dry
	Cadmium	≤20	≤20	<MDL	75–125	80–120	0.25 mg/Kg dry
	Copper	≤20	≤20	<MDL	75–125	80–120	2.5 mg/Kg dry
	Lead	≤20	≤20	<MDL	75–125	80–120	0.25 mg/Kg dry
	Mercury	≤35	≤35	<MDL	80–120	80–120	0.025 mg/Kg dry
	Zinc	≤20	≤20	<MDL	75–125	80–120	10 mg/Kg dry
Petroleum Hydrocarbons	NWTPH-Dx - Deisel	≤50	≤50	<MDL	NA	50–150	5 mg/Kg
PAHs	2-Methylnaphthalene	≤50	≤50	<MDL	58–120	58–120	12 ug/Kg
	Acenaphthylene	≤50	≤50	<MDL	61–120	61–120	10 ug/Kg
	Acenaphthene	≤50	≤50	<MDL	61–120	61–120	13.5 ug/Kg
	Anthracene	≤50	≤50	<MDL	61–120	61–120	10 ug/Kg
	Benzo(a)anthracene	≤50	≤50	<MDL	73–120	73–120	10 ug/Kg
	Benz(a)pyrene	≤50	≤50	<MDL	63–120	63–120	12 ug/Kg
	Benzo(g,h,i)perylene	≤50	≤50	<MDL	53–126	53–126	10 ug/Kg
	Benzo(b,j,k)fluoranthenes	≤50	≤50	<MDL	50–121	50–121	25 ug/Kg
	Chrysene	≤50	≤50	<MDL	67–120	67–120	10 ug/Kg
	Dibenzo(a,h)anthracene	≤50	≤50	<MDL	60–127	60–127	20 ug/Kg
	Fluoranthene	≤50	≤50	<MDL	67–120	67–120	10 ug/Kg
	Fluorene	≤50	≤50	<MDL	66–120	66–120	10 ug/Kg
	Indeno(1,2,3-cd)pyrene	≤50	≤50	<MDL	66–120	66–120	15 ug/Kg
	Naphthalene	≤50	≤50	<MDL	61–120	61–120	13.7 ug/Kg

Table 6 (continued). Measurement Quality Objectives (MQOs) for SSPM Parameters.							
Analytical Group	**Parameter**	**Laboratory Duplicate (RPD)**	**Field Duplicate (RPD)[a]**	**Method Blank**	**Matrix Spike (% Recovery)**	**Lab Control Standard (% Recovery)**	**Laboratory Reporting Limit**
PAHs (continued)	Phenanthrene	≤50	≤50	<MDL	65–120	65–120	10 ug/Kg
	Pyrene	≤50	≤50	<MDL	66–120	66–120	10 ug/Kg
Phthalates	Di-ethyl phthalate	≤50	≤50	<MDL	55–120	55–120	35.4 ug/Kg
	Butyl benzyl phthalate	≤50	≤50	<MDL	73–120	73–120	13.7 ug/Kg
	Bis(2-ethylhexyl) phthalate	≤50	≤50	<MDL	76–120	76–120	51.1 ug/Kg
	Di-n-butyl phthalate	≤50	≤50	<MDL	60–120	60–120	30.9 ug/Kg
	Di-n-octyl phthalate	≤50	≤50	<MDL	70–120	70–120	15 ug/Kg
	Di-methyl phthalate	≤50	≤50	<MDL	67–120	67–120	18.6 ug/Kg
Phenolics	Pentachlorophenol	≤50	≤50	<MDL	30–120	30–120	35 ug/Kg
	p-Cresol	≤50	≤50	<MDL	35–120	35–120	10 ug/Kg
	o-Cresol	≤50	≤50	<MDL	26–123	26–123	10 ug/Kg
PCBs	Aroclor 1016	≤35	≤35	<MDL	NA	NA	5 ug/Kg
	Aroclor 1221	≤35	≤35	<MDL	NA	NA	5 ug/Kg
	Aroclor 1232	≤35	≤35	<MDL	NA	NA	5 ug/Kg
	Aroclor 1242	≤35	≤35	<MDL	NA	NA	5 ug/Kg
	Aroclor 1248	≤35	≤35	<MDL	NA	NA	5 ug/Kg
	Aroclor 1254	≤35	≤35	<MDL	40–132	40–132	5 ug/Kg
	Aroclor 1260	≤35	≤35	<MDL	NA	NA	5 ug/Kg
	Aroclor 1262	≤35	≤35	<MDL	NA	NA	5 ug/Kg
	Aroclor 1268	≤35	≤35	<MDL	NA	NA	5 ug/Kg
Pesticides	Bifenthrin	≤50	≤50	<MDL	45–130	45–130	0.5 ug/Kg
	Dichlobenil	≤50	≤50	<MDL	55–120	55–120	1 ug/Kg
Emerging Contaminants	6PPDQ	≤20[b]	≤20	<MDL	70–130[b]	70–130	5 ng/g
	Microplastics/TWP	≤35	≤35	<MDL	NA	60–140	1 ug/sample

Table 6 (continued). Measurement Quality Objectives (MQOs) for SSPM Parameters.							
Analytical Group	**Parameter**	**Laboratory Duplicate (RPD)**	**Field Duplicate (RPD)[a]**	**Method Blank**	**Matrix Spike (% Recovery)**	**Lab Control Standard (% Recovery)**	**Laboratory Reporting Limit**
Emerging Contaminants (continued)	Perfluorobutanoic acid (PFBA)	≤35[b]	≤35	<MDL	40–160[b]	70–140	1.6 ng/g
	Perfluoropentanoic acid (PFPeA)	≤35[b]	≤35	<MDL	40–160[b]	60–150	4 ng/g
	Perfluorohexanoic acid (PFHxA)	≤35[b]	≤35	<MDL	40–160[b]	65–140	0.4 ng/g
	Perfluoroheptanoic acid (PFHpA)	≤35[b]	≤35	<MDL	40–160[b]	65–145	0.4 ng/g
	Perfluorooctanoic acid (PFOA)	≤35[b]	≤35	<MDL	40–160[b]	70–150	0.4 ng/g
	Perfluorononanoic acid (PFNA)	≤35[b]	≤35	<MDL	40–160[b]	70–155	0.4 ng/g
	Perfluorodecanoic acid (PFDA)	≤35[b]	≤35	<MDL	40–160[b]	70–155	0.4 ng/g
	Perfluoroundecanoic acid (PFUnA)	≤35[b]	≤35	<MDL	40–160[b]	70–155	0.4 ng/g
	Perfluorododecanoic acid (PFDoA)	≤35[b]	≤35	<MDL	40–160[b]	70–150	0.4 ng/g
	Perfluorotridecanoic acid (PFTrDA)	≤35[b]	≤35	<MDL	40–160[b]	65–150	0.4 ng/g
	Perfluorotetradecanoic acid (PFTeDA)	≤35[b]	≤35	<MDL	40–160[b]	65–150	0.4 ng/g
	Perfluorobutanesulfonic acid (PFBS)	≤35[b]	≤35	<MDL	40–160[b]	65–145	0.4 ng/g
	Perfluoropentanesulfonic acid (PFPeS)	≤35[b]	≤35	<MDL	40–160[b]	55–160	0.5 ng/g
	Perfluorohexanesulfonic acid (PFHxS)	≤35[b]	≤35	<MDL	40–160[b]	60–150	0.5 ng/g
	Perfluoroheptanesulfonic acid (PFHpS)	≤35[b]	≤35	<MDL	40–160[b]	65–155	0.4 ng/g
	Perfluorooctanesulfonic acid (PFOS)	≤35[b]	≤35	<MDL	40–160[b]	65–160	1 ng/g
	Perfluorononanesulfonic acid (PFNS)	≤35[b]	≤35	<MDL	40–160[b]	55–140	0.4 ng/g
	Perfluorodecanesulfonic acid (PFDS)	≤35[b]	≤35	<MDL	40–160[b]	40–155	0.4 ng/g
	Perfluorododecanesulfonic acid (PFDoS)	≤35[b]	≤35	<MDL	40–160[b]	25–160	0.4 ng/g
	1H,1H,2H,2H-Perfluorohexane sulfonic acid (4:2 FTS)	≤35[b]	≤35	<MDL	40–160[b]	60–150	0.8 ng/g
	1H,1H,2H,2H-Perfluorooctane sulfonic acid (6:2 FTS)	≤35[b]	≤35	<MDL	40–160[b]	55–200	0.8 ng/g
	1H,1H,2H,2H-Perfluorodecane sulfonic acid (8:2 FTS)	≤35[b]	≤35	<MDL	40–160[b]	70–150	0.8 ng/g
	Perfluorooctanesulfonamide (PFOSA)	≤35[b]	≤35	<MDL	40–160[b]	70–140	0.5 ng/g

Table 6 (continued). Measurement Quality Objectives (MQOs) for SSPM Parameters.							
Analytical Group	**Parameter**	**Laboratory Duplicate (RPD)**	**Field Duplicate (RPD)[a]**	**Method Blank**	**Matrix Spike (% Recovery)**	**Lab Control Standard (% Recovery)**	**Laboratory Reporting Limit**
Emerging Contaminants (continued)	N-methylperfluorooctane sulfonamide (NMeFOSA)	≤35[b]	≤35	<MDL	40–160[b]	70–155	0.5 ng/g
	N-ethylperfluorooctane sulfonamide (NEtFOSA)	≤35[b]	≤35	<MDL	40–160[b]	70–140	0.5 ng/g
	N-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA)	≤35[b]	≤35	<MDL	40–160[b]	65–155	0.5 ng/g
	N-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA)	≤35[b]	≤35	<MDL	40–160[b]	65–165	0.5 ng/g
	N-methylperfluorooctane sulfonamidoethanol (NMeFOSE)	≤35[b]	≤35	<MDL	40–160[b]	70–140	5 ng/g
	N-ethylperfluorooctane sulfonamidoethanol (NEtFOSE)	≤35[b]	≤35	<MDL	40–160[b]	70–135	5 ng/g
	Hexafluoropropylene Oxide Dimer Acid (HFPO-DA)	≤35[b]	≤35	<MDL	40–160[b]	70–145	2 ng/g
	4,8-Dioxa-3H-perfluorononanoic acid (ADONA)	≤35[b]	≤35	<MDL	40–160[b]	70–160	0.8 ng/g
	Perfluoro-3-methoxypropanoic acid (PFMPA)	≤35[b]	≤35	<MDL	40–160[b]	30–140	1 ng/g
	Perfluoro-4-methoxybutanoic acid (PFMBA)	≤35[b]	≤35	<MDL	40–160[b]	60–150	1 ng/g
	Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	≤35[b]	≤35	<MDL	40–160[b]	60–155	1 ng/g
	9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	≤35[b]	≤35	<MDL	40–160[b]	70–150	2 ng/g
	11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	≤35[b]	≤35	<MDL	40–160[b]	45–160	2 ng/g
	Perfluoro (2-ethoxyethane) sulfonic acid (PFEESA)	≤35[b]	≤35	<MDL	40–160[b]	70–140	1 ng/g
	3-Perfluoropropylpropanoic acid (3:3 FTCA)	≤35[b]	≤35	<MDL	40–160[b]	45–130	4 ng/g
	3-Perfluoropentylpropanoic acid (5:3 FTCA)	≤35[b]	≤35	<MDL	40–160[b]	60–130	20 ng/g
	3-Perfluoroheptylpropanoic acid (7:3 FTCA)	≤35[b]	≤35	<MDL	40–160[b]	60–150	20 ng/g

[a] If one or both results are within 5 times the reporting limit, the difference between results will be less than 2 times the reporting limit.

[b] Laboratory duplicates and matrix spikes for 6PPDQ and PFAS will only be analyzed upon request.

RPD = relative percent difference
MDL = method detection limit
PAHs = Polycyclic aromatic hydrocarbons
PCBs = Polychlorinated biphenyls
TWP = tire wear particles
mg/Kg = milligrams per kilogram
PFAS = per- and polyfluoroalkyl substances
ug/Kg = micrograms per kilogram
ng/g = nanograms per gram
NA = Not applicable

5.3.1. Targets for Precision, Bias, and Sensitivity

5.3.1.1. Precision

Precision will be assessed based on the analyses of laboratory and field duplicates. One laboratory duplicate will be analyzed with each batch of samples, and one field duplicate will be analyzed at the frequency described in the Quality Control section.

Specific MQOs for laboratory and field duplicates are defined by the analysis methods in Table 5 and Table 6.

5.3.1.2. Bias

Bias will be assessed based on analyses of equipment blanks, field blanks, method blanks, matrix spikes (MS), and laboratory control samples (LCS). Blanks will be analyzed at the frequency described in the Quality Control section. Bias in blanks will be quantified by comparison to the detection limit. Bias in MS and LCS will be quantified based on percent recovery or the average (arithmetic mean) of the percent recovery. Specific MQOs for MS and LCS are defined in Table 5 and Table 6.

5.3.1.3. Sensitivity

Sensitivity, as a measure of the capability of a method to detect a substance, is described as the Method Reporting Limit (MRL) defined by the laboratory based on analytical confidence. Concentrations reported as greater than the method detection limit (MDL) but less than the MRL will be checked and potentially qualified by the laboratory as an estimated value.

5.3.2. Targets for Comparability, Representativeness, and Completeness

5.3.2.1. Comparability

Comparability of the data will be assessed relative to the methods and standard operating procedures (SOPs) used for the project. Standard sampling procedures, analytical methods, units of measurement, and reporting limits will be applied in this study to meet the goal of data comparability.

5.3.2.2. Representativeness

Sample representativeness will be ensured by employing consistent and standard sampling procedures identified in this QAPP.

To help assure that the chemistry results from the eight automated sampler locations are representative of the event mean concentrations (EMC), the same criteria that were used in the 2007 S8.D study will be followed:

- Automatic samplers shall be programmed to begin sampling as early in the runoff event as practical and to continue sampling past the longest estimated time of concentration for the tributary area.
- For storm events lasting less than 24 hours, samples shall be collected for at least 75 percent of the storm event hydrograph.
- For storm events lasting longer than 24 hours, samples shall be collected for at least 75 percent of the hydrograph of the first 24 hours of the storm.
- Each composite sample must consist of at least 10 aliquots. Composite samples with 7 to 9 aliquots are acceptable if they meet the other sampling criteria and help achieve a representative balance of wet season/dry season events and storm sizes.

To help assure that the grab sample dataset is representative of average storm concentrations, the timing of grab sample collection shall be distributed across all portions of the storm hydrographs throughout the study, such that summary statistics will be representative of the entire storm event. Water level sensors installed at each monitoring location will be used to assess if the grab sample was collected on the rising, peak, or falling limb of the event.

To help assure that the chemistry data are representative of typical storm events in the Pacific Northwest the same criteria that were used in the 2007 S8.D study will be followed:

- Rainfall depth: ≥0.2 inch
- Rainfall duration: no requirement
- Inter-event dry period: 6 hours
- Antecedent dry period:
    - Wet Season (October 1 through April 30): ≤ 0.02 inch of rain in the previous 24 hours
    - Dry Season (May 1 through September 30): ≤0.02 inch of rain in the previous 72 hours

5.3.2.3. Completeness

Completeness will be assessed based on the percentage of specified samples (listed in this QAPP) collected. The completeness goal will be 95 percent. Completeness for acceptable data is defined as the percentage of acceptable data out of the total amount of data generated. Acceptable data are either data that pass all quality control (QC) criteria or data that may not pass all QC criteria but have appropriate corrective actions taken. Completeness will be calculated by dividing the number of valid values by the total number of values. If completeness is less than 95 percent, then samples will be reanalyzed or recollected if possible.

5.4. Acceptance Criteria for Quality of Existing Data

The criteria that will be used to assess quality and usability of the existing data include the data quality and measurement quality objectives, as explained above.

5.5. Model Quality Objectives

There is no modeling associated with this project.

6. Study Design

6.1. Study Boundaries

Figure 1 in Section 2 shows the study area of the project. Figures in the following sections show basin boundaries for each monitoring location.

6.2. Field Data Collection

6.2.1. Sampling Locations and Frequency

Monitoring locations were chosen from locations sampled during the original S8.D study, with two new locations in the City of Seattle. Primary land use upstream of each sampling location is one of four types: low-density residential (LDR), high-density residential (HDR), commercial (COM), or industrial (IND). The new locations were added to have equal representation of industrial basins relative to other land uses. Locations are summarized in Table 7 and described in depth in the following subsections. Table 8 summarizes basin characteristics for each monitoring location for both 2015 and 2023. The equipment deployed at the monitoring location is described in this section, and cutsheets are presented in Appendix B.

Water samples will be collected during nine storm events per year for 2 years, for a total of 18 events. The frequency of sample collection will depend on the occurrence of qualifying storm events, but efforts will be made to spread the sampling through the year.

SSPM samples will be collected via three Norton style sediment traps at each monitoring location. A total of two annual SSPM sample collections and analyses are planned over the course of the project.

Table 7. Summary of Monitoring Locations.

Jurisdiction	Land Use	Water Quality Sample Type	Continuous Flow Monitoring?	Continuous Level and Temperature?	Inline SSPM Sampling?	Original Location ID	ID for this Study	Location Description	Latitude	Longitude
Snohomish County	LDR	Automated and grab	Yes	Yes	Yes	SNO_LDR	SNO_LDR	100 meters south of the intersection of Broadway Avenue and Connelly Road on the West side of Broadway Avenue in Snohomish	47.86894	-122.09929
	HDR	Grab	No	Yes	Yes	SNO_HDR	SNO_HDR	North of 168th Street Southeast at the intersection with 28th Drive Southeast in Bothell	47.84607	-122.19396
	COM	Grab	No	Yes	Yes	SNO_COM	SNO_COM	4th Avenue West between 128th Street Southwest and 132nd Street Southwest, adjacent to I-5 in Everett	47.88110	-122.23902
King County	LDR	Automated and grab	Yes	Yes	Yes	KICLDRS8D_OUT	KING_LDR	Off 148th Avenue Southeast in Renton	47.511083	-122.143241
	HDR	Automated and grab	Yes	Yes	Yes	KICHDRS8D_OUT	KING_HDR	Corner of 335th Place Southeast & 44th Lane in Fall City	47.565304	-121.894560
	COM	Automated and grab	Yes	Yes	Yes	KICCOMS8D_OUT	KING_COM	Corner of Redmond-Fall City Road Southeast and Preston-Fall City Road Southeast	47.56737	-121.88840
City of Seattle	IND	Automated and grab	Yes	Yes	Yes	SEAIS8D_OUT	SEA_IND1	Near 10023 Martin Luther King Jr Way South in Seattle	47.50997	-122.28249
	IND	Grab	No	Yes	Yes	New site in 2024	SEA_IND2	On South Front Street between 5th and 6th Avenue	47.54807	-122.32729
	IND	Grab	No	Yes	Yes	New site in 2024	SEA_IND3	On South Nevada Street near East Marginal Way South	47.56581	-122.34092
Pierce County	LDR	Grab	No	Yes	Yes	PIELORES_OUT	PIER_LDR	400 feet to the northeast of the corner of 81st Street Avenue Northwest and 68th Street Court Northwest in Gig Harbor	47.32064	-122.64584
Pierce County (continued)	HDR	Grab	No	Yes	Yes	PIEHIRES_OUT	PIER_HDR	Near the intersection of 128th Street East and 82nd Avenue Court East, at the end of the cul-de-sac on 82nd Avenue Court East near Puyallup	47.13805	-122.31954
	COM	Grab	No	Yes	Yes	PIECOMM_OUT	PIER_COM	Near the intersection of 112th St. East and Canyon Road East near Tacoma	47.15460	-122.35388
City of Tacoma	HDR	Automated and grab	Yes	Yes	Yes	TFWD1-OF237B	TAC_HDR	2300 block of East "C" Street, in the City's Dock Street Pump Station Yard	47.2408	-122.4315
	COM	Automated and grab	Yes	Yes	Yes	TAC001S8D_OF235	TAC_COM	South 21st Street and Dock Street	47.24333	-122.43365
	IND	Automated and grab	Yes	Yes	Yes	TAC003S8D_OF245	TAC_IND	East 19th Street and East "D" Street in parking stall at Johnny's Dock Restaurant	47.24561	-122.43073
Clark County	LDR	Grab	No	Yes	Yes	LDR010	CLRK_LDR	Headwater of tributary of Packard Creek; 120 feet west of Northwest 11th Avenue and Northwest 184th Street	45.75432	-122.68301

LDR = Low density residential HDR = High density residential COM = Commercial IND = Industrial

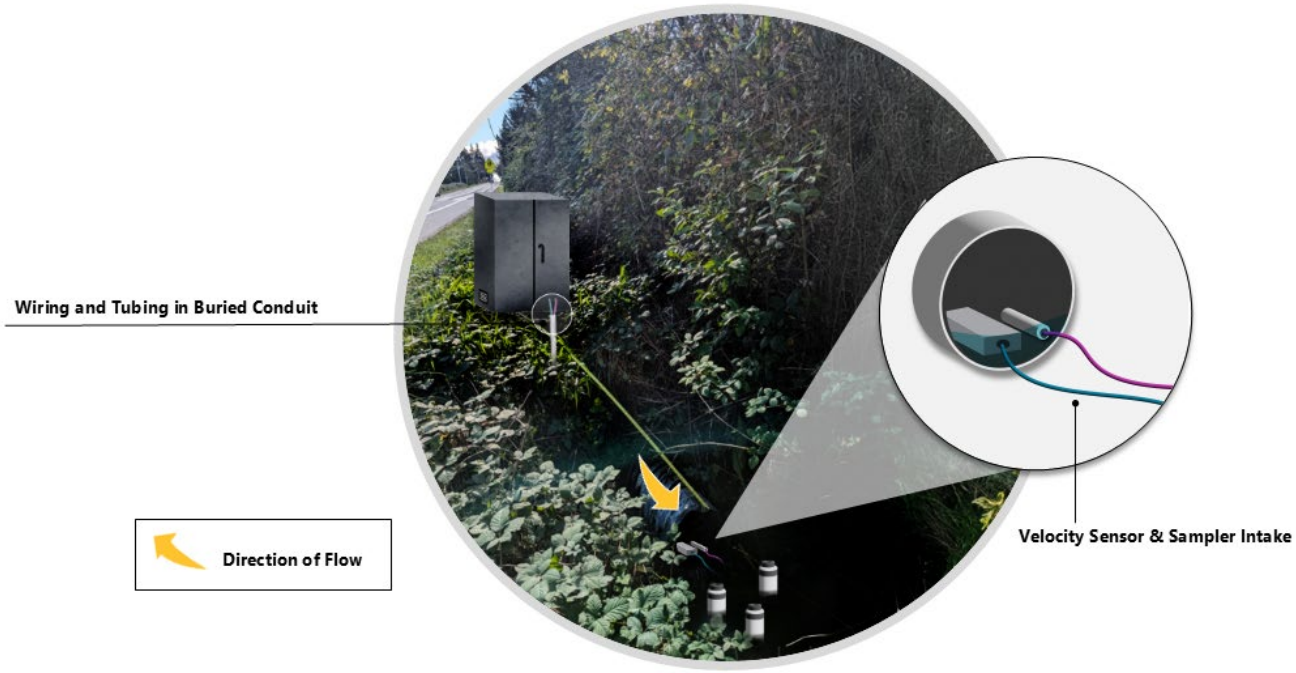
Table 8. Monitoring Location Basin Characteristics.										
	Historic Characteristics (2015)					Current Characteristics (2023)				
Location ID	Basin Size (acres)	% Impervious Area	% Canopy Cover	Number of Buildings	Buildings per acre	Basin Size (acres)	% Impervious Area	% Canopy Cover	Number of Buildings	Buildings per acre
SNO_LDR	81.30	25	28	110	1.35	81.30	25	27	120	1.48
SNO_HDR	20.35	48	33	86	4.23	20.35	49	32	90	4.42
SNO_COM	27.78	70	20	72	2.59	27.78	70	20	72	2.59
KING_LDR	25.56	15	57	43	1.68	25.56	17	56	48	1.88
KING_HDR	6.96	37	22	25	3.59	6.96	39	20	27	3.88
KING_COM	5.86	74	13	17	2.90	5.86	75	13	18	3.07
SEA_IND1	156.72	42	45	174	1.11	156.72	42	45	186	1.19
SEA_IND2	16.19	91	6	15	0.93	16.19	90	6	15	0.93
SEA_IND3	2.63	95	1	1	0.38	2.63	95	1	1	0.38
PIER_LDR	132.32	9	78	83	0.63	132.32	11	75	114	0.86
PIER_HDR	1739.51	30	38	2883	1.66	1739.51	32	38	3185	1.83
PIER_COM	32.88	80	11	26	0.79	32.88	79	11	26	0.79
TAC_HDR	2112.26	45	22	9208	4.36	2112.26	46	21	9714	4.60
TAC_COM	110.49	71	7	120	1.09	110.49	74	7	117	1.06
TAC_IND	29.55	92	2	13	0.44	29.55	92	1	12	0.41
CLRK_LDR	43.34	13	23	38	0.88	43.34	16	21	42	0.97

6.2.1.1. Snohomish County Monitoring Locations

SNO_LDR

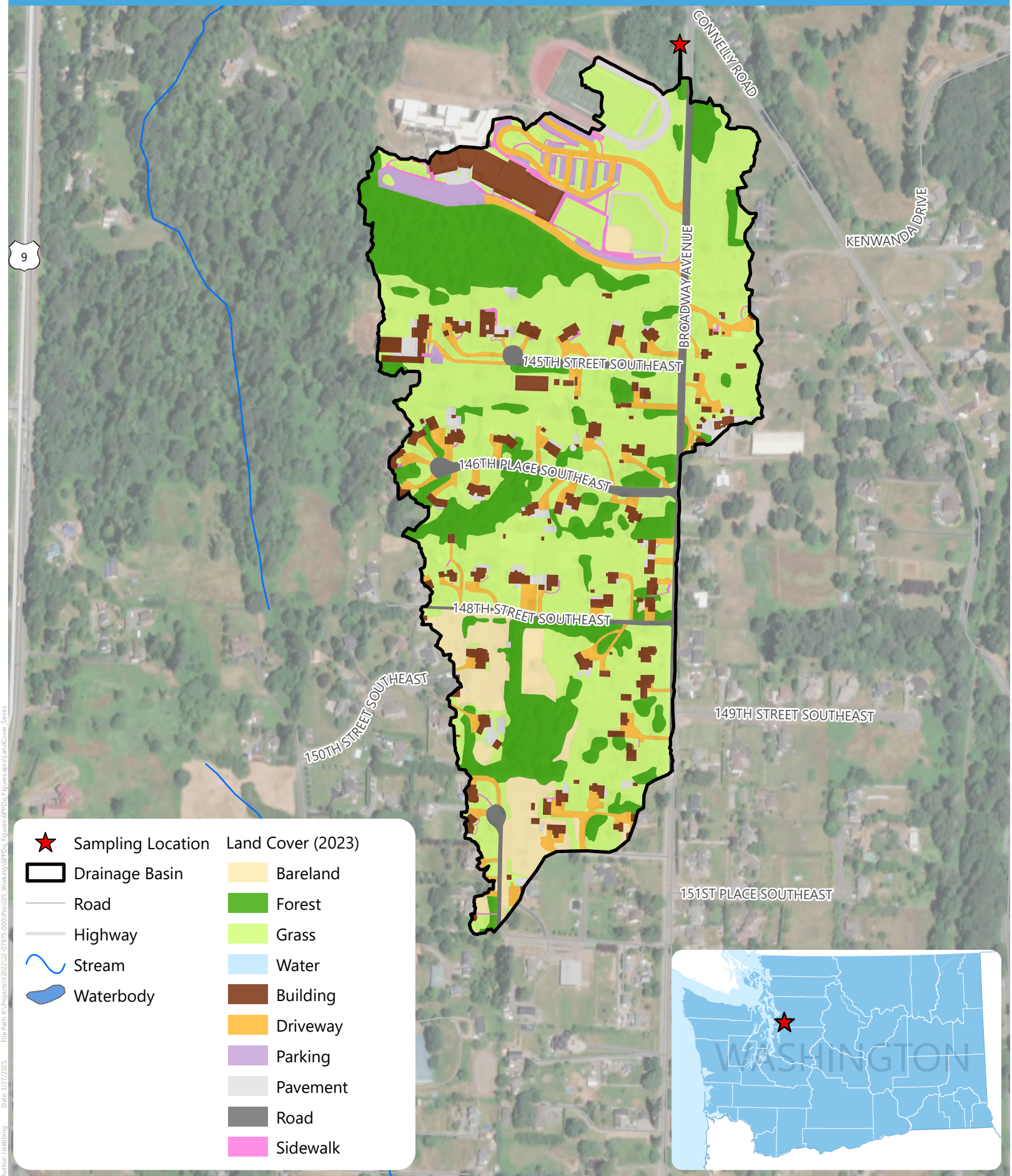
The low-density residential monitoring location (SNO_LDR) for Snohomish County is located in Snohomish, Washington, off of Broadway Avenue, adjacent to Valley View Middle School (Figure 4). The monitoring location is an outfall from an 18-inch diameter corrugated metal culvert that empties into a roadside ditch. The outfall flows north towards the Snohomish River valley within the Snohomish River-Frontal Possession Sound Hydrologic Unit Code (HUC-12) Watershed. Flow from the outfall will be monitored using an area-velocity sensor (Isco 2150). An automated sampler (Isco 6712) and a datalogger with a modem (Campbell Scientific CR310) will be installed approximately 10 feet south of the outfall. A Snohomish County rain gauge, which is located 1.5 miles to the northeast at the French Slough Pump Station, will be used to measure precipitation. The autosampler intake will be installed in the outfall pipe, and the SSPM traps will be installed in the ditch under the outfall spill zone (Figure 3).

Figure 3. SNO_LDR Monitoring Location Design.



HERRERA
Science + Planning + Design

Figure 4.
Snohomish County Low-Density Residential.



SNO_HDR

The high-density residential monitoring location (SNO_HDR) for Snohomish County is located in Bothell, Washington, northeast of the intersection of 168th Street Southeast and 28th Drive Southeast (Figure 6). The monitoring location is an outfall into a stormwater pond that receives flow from surrounding neighborhoods. It is located within the North Creek HUC-12 Watershed. The storm drain enters the pond through a 24-inch diameter corrugated plastic pipe and through a rubber "duckbill" backflow preventer. Only grab and SSPM samples will be collected at this location. Precipitation will be measured by King County rain gauge MNCR (North Creek Maltby I&I), 1.5 miles to the south of the monitoring location. The SSPM traps will be installed in the spill zone of the outfall (Figure 5).

Figure 5. SNO_HDR Monitoring Location Design.

HERRERA
Science + Planning + Design

Figure 6.
Snohomish County High-Density Residential.

SNO_COM

The commercial monitoring location (SNO_COM) for Snohomish County is located in the Southeast corner of the Washington State Employees Credit Union parking lot near the corner of 128th Street Southwest and 4th Avenue West in Everett, Washington (Figure 8). The monitoring location is a catch basin that receives flow from the commercial parking lots to the west. It is located within the North Creek HUC-12 Watershed. The storm drain enters the catch basin from the southwest via an 18-inch diameter corrugated metal pipe and exits to the north through a 24-inch diameter concrete pipe. Only grab and SSPM samples will be collected at this location. Precipitation will be measured by the Snohomish County rain gauge located at the Silver Lake Water District office, 1.7 miles to the east of the monitoring location. The SSPM traps will be installed in the spill zone of the outfall (Figure 7).

Figure 7. SNO_COM Monitoring Location Design.

HERRERA
Science + Planning + Design

Figure 8.
Snohomish County Commercial Basin.

Produced by Herrera Environmental Consultants (herrerainc.com) | Sources: Ecopia Vector Maps © [2025] Ecopia Tech Corporation, ESRI (Aerial, 2023), NHD, WSDOT

0 130 260 520 Feet

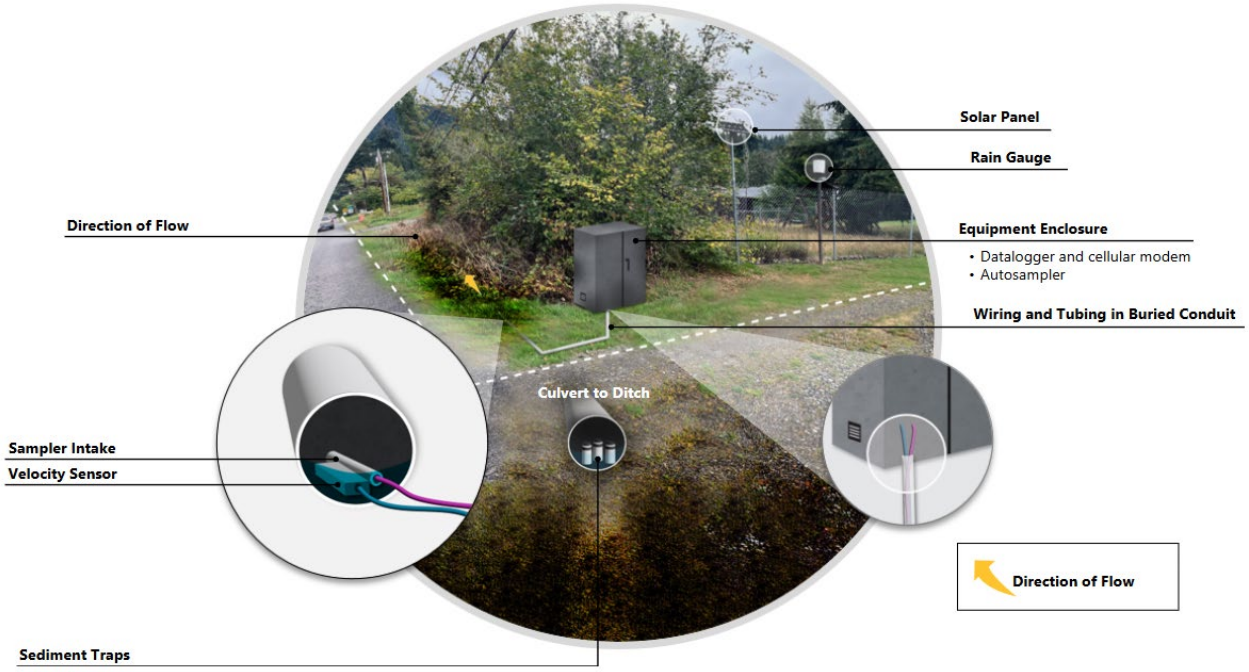
File Path: K:\Projects\Y2022\22-07975-000\Pro\GIS_Working\6PPDq_Figures\6PPDq_Figures.aprx\LandCover_Series
Date: 3/27/2025
Author: rstebbing

6.2.1.2. King County Monitoring Locations

KING_LDR

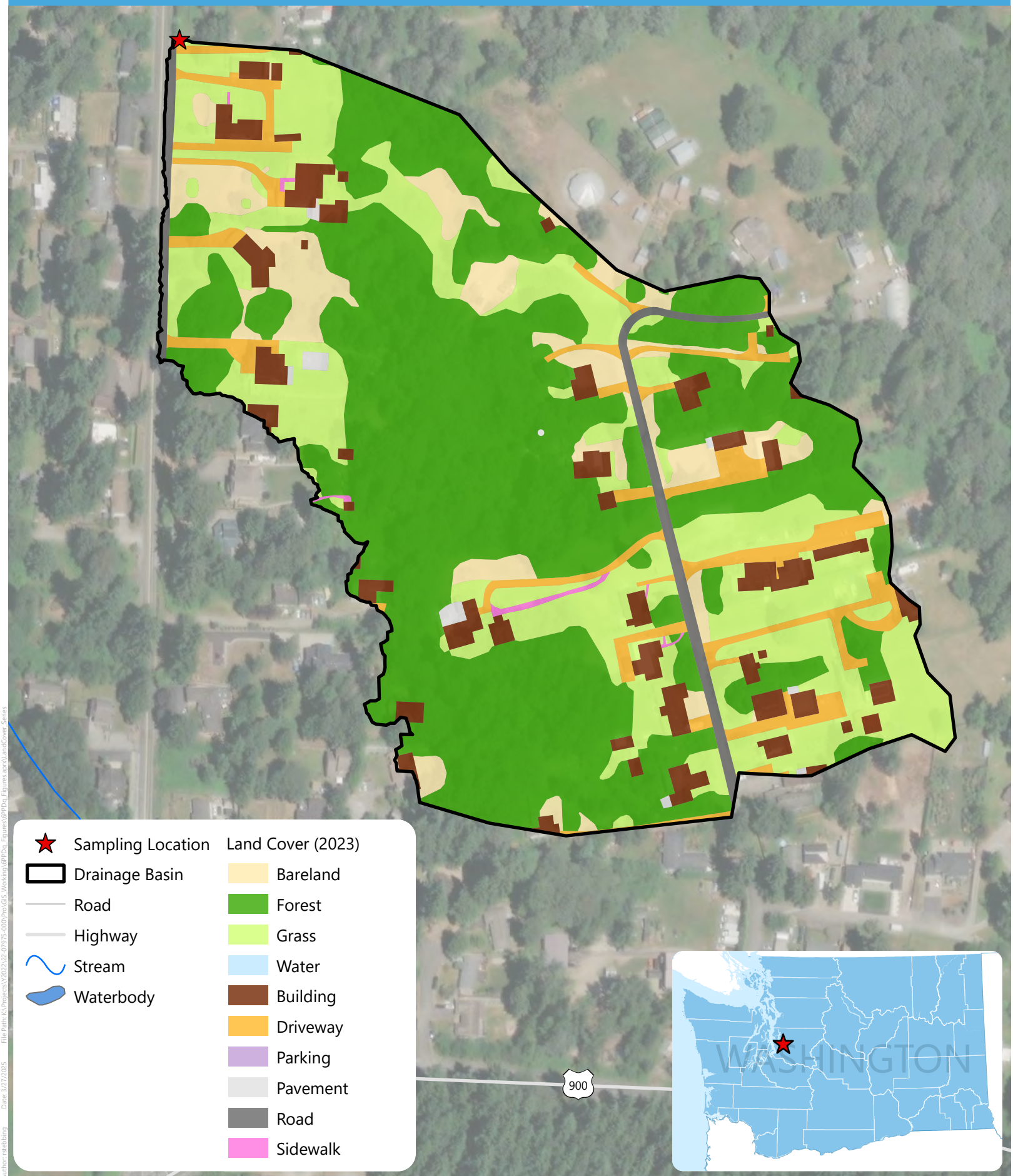
The low-density residential monitoring location (KING_LDR) for King County is located in Renton, Washington, off of 148th Avenue Southeast (Figure 10). The monitoring location is a roadside ditch that flows north towards May Creek, located within the Lake Washington-Sammamish River HUC-12 Watershed. Flow from the roadside ditch will be monitored using an area-velocity sensor (Isco 2150). An automated sampler (Isco 6712) and a datalogger with a modem (Campbell CR310) will be installed approximately 10 feet to the east of the ditch. An adjacent onsite King County rain gauge will be used to measure precipitation. The autosampler intake and SSPM traps will be installed in the pipe (Figure 9).

Figure 9. KING_LDR Monitoring Location Design.



HERRERA
Science + Planning + Design

Figure 10.
King County Low-Density Residential Basin.



Produced by Herrera Environmental Consultants (herrerainc.com) | Sources: Ecopia Vector Maps © [2025] Ecopia Tech Corporation, ESRI (Aerial, 2023), NHD, WSDOT

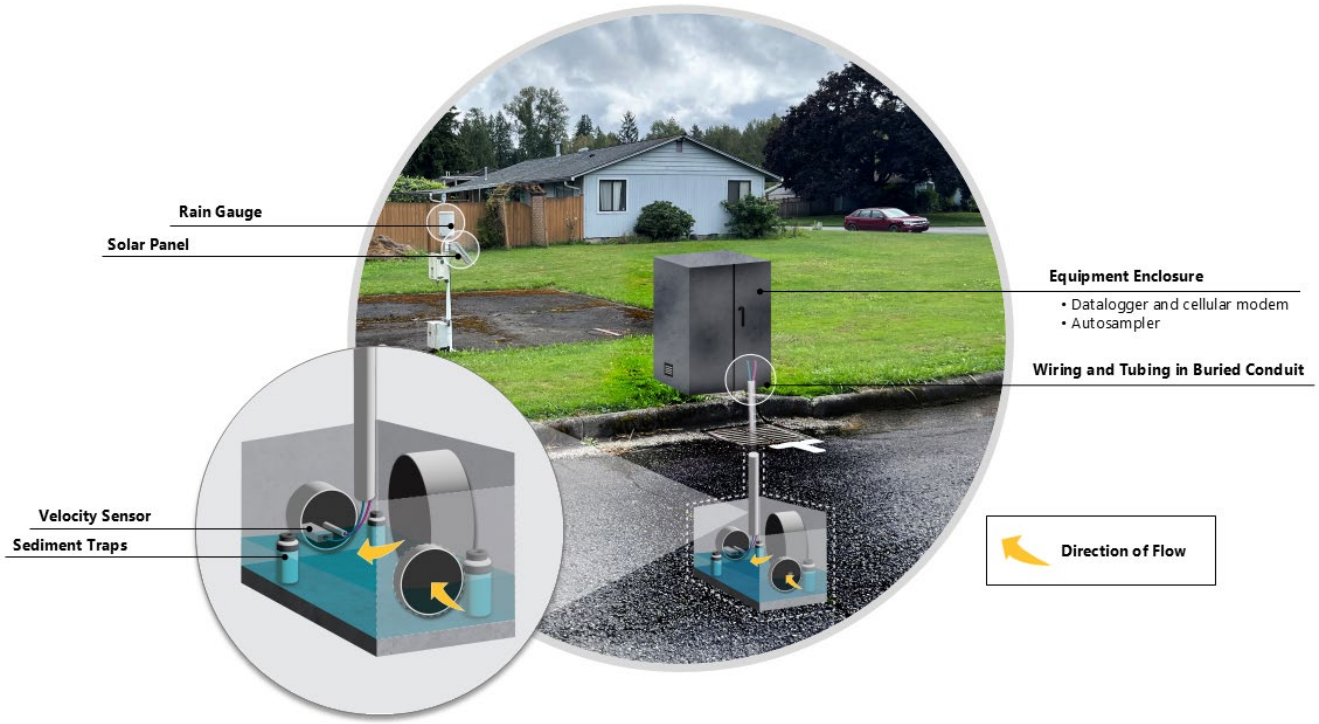
0 80 160 320 Feet

File Path: K:\Projects\Y2022\22-07975.000\Pro\GIS_Working\6PPDq_Figures.aprx\LandCover_Series
Date: 3/27/2025
Author: rstebbing

KING_HDR

The high-density residential monitoring location (KING_HDR) for King County is located in Fall City, Washington, near the corner of 335th Place Southeast and Southeast 44th Lane (Figure 12). The monitoring location is upstream of a detention vault that receives flow from the southeast. The monitoring location is located within the Patterson Creek-Snoqualmie River HUC-12 Watershed. Water enters the detention vault through a 12-inch inlet pipe. Flow will be measured in this pipe using a Starflow QSD area-velocity sensor. An Isco 6712 automated sampler and Campbell CR310 datalogger/cellular modem will be installed approximately 20 feet to the west of the detention vault, with equipment wiring and sampler tubing running through a catch basin grate. A King County rain gauge (19U), located on site, will be used to measure precipitation. The autosampler intake will be installed in the inlet pipe, downstream of the flow sensor. (Figure 11). The SSPM traps will be installed in the sump just upstream of the 12-inch pipe (Figure 11).

Figure 11. KING_HDR Monitoring Location Design.



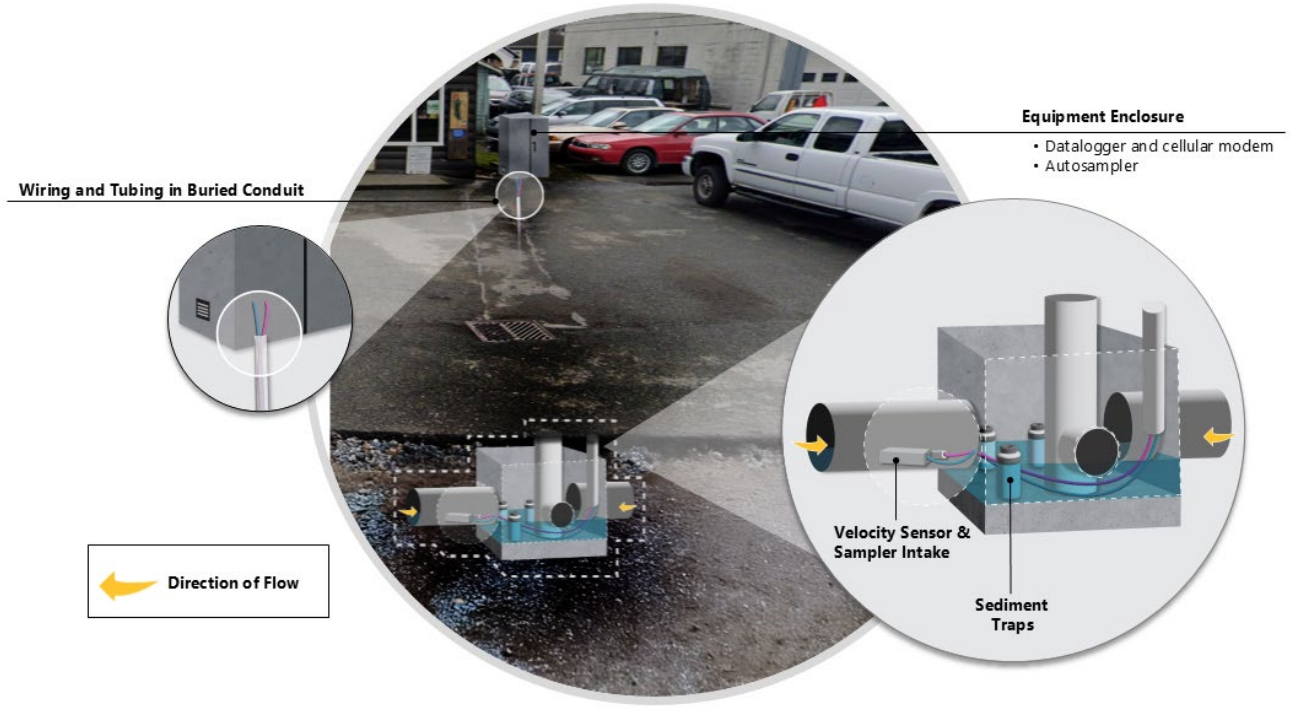
HERRERA
Science + Planning + Design

Figure 12.
King County High-Density Residential Basin.

KING_COM

The commercial monitoring location (KING_COM) for King County is located in Fall City, Washington, on the corner of Redmond-Fall City Road Southeast and Preston-Fall City Road Southeast (Figure 14). The monitoring location is a catch basin that receives water from the southeast and outfalls into the Snoqualmie River This site is located within the Patterson Creek-Snoqualmie River HUC-12 Watershed. Flow will be measured using a Starflow QSD area-velocity sensor installed in the 12-inch inlet pipe to the structure. An Isco 6712 automated sampler and Campbell CR310 datalogger/cellular modem will be installed approximately 40 feet to the south of the structure, with equipment wiring and sampler tubing running through buried conduit. The King County rain gauge (19U) located at KING_HDR, 1,000 feet to the southwest, will be used to measure precipitation. The autosampler intake will be installed in the inlet pipe, downstream of the flow sensor (Figure 13). The SSPM traps will be installed in the sump just downstream of the pipe where flow measurement will occur (Figure 13).

Figure 13. KING_COM Monitoring Location Design.



HERRERA
Science + Planning + Design

Figure 14.
King County Commercial Basin.

Produced by Herrera Environmental Consultants (herrerainc.com) | Sources: Ecopia Vector Maps © [2025] Ecopia Tech Corporation, ESRI (Aerial, 2023), NHD, WSDOT

0 80 160 320 Feet

File Path: K:\Projects\Y2022\22-07975.000\Pro\GIS_Working\6PPDq_Figures.aprx\LandCover_Series
Date: 3/27/2025
Author: rstebbing

6.2.1.3. City of Seattle Monitoring Locations

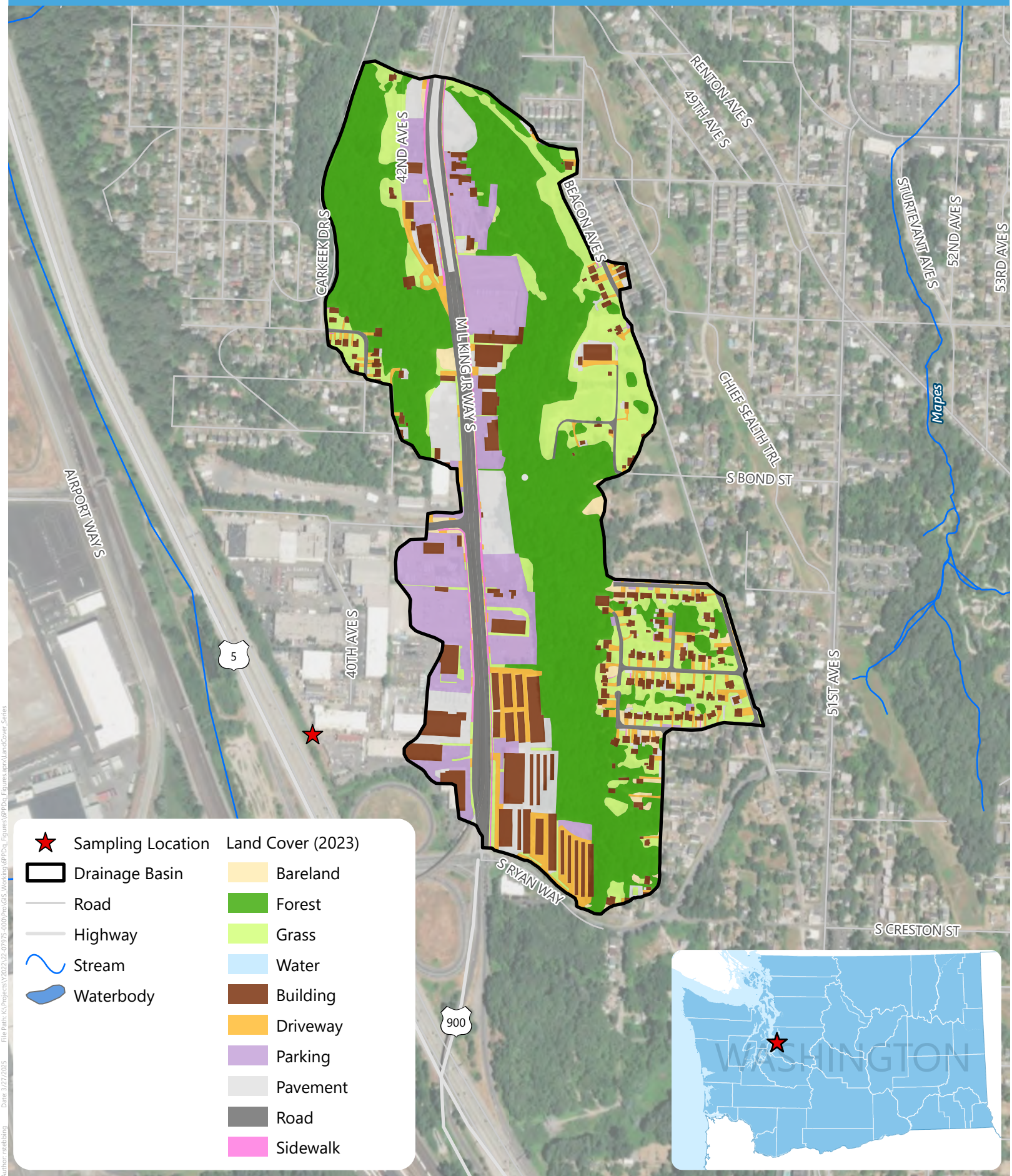
SEA_IND1

The first industrial monitoring location in the City of Seattle (SEA_IND1) is located between Martin Luther King Jr. Way and the Washington Department of Transportation (WSDOT) ditch located on the east side of Interstate 5 (Figure 16). It is located in the Lake Washington-Sammamish River HUC-12 Watershed and drains under I-5, to the west into the Duwamish waterway. The monitoring location is located within a pipe and flow diversion structure vault. This location was monitored previously for the S8.D monitoring. The equipment configuration will match the previous setup except for the addition of two more sediment traps (for a total of three sediment traps). Flow will be measured using an Isco 2150 area-velocity sensor installed in the 64-inch inlet pipe to the structure. An Isco 6712 automated sampler and Campbell CR310 datalogger/cellular modem will be installed approximately 40 feet to the south of the structure, with equipment wiring and sampler tubing running through buried conduit. The SSPM traps will be installed in the diversion structure (Figure 15). Seattle Public Utilities (SPU) rain gauge RG30 will be used to measure rainfall. The rain gauge is approximately 0.4 mile northeast of the monitoring location.

Figure 15. SEA_IND1 Monitoring Location Design.

HERRERA
Science + Planning + Design

Figure 16.
City of Seattle Industrial Basin #1.



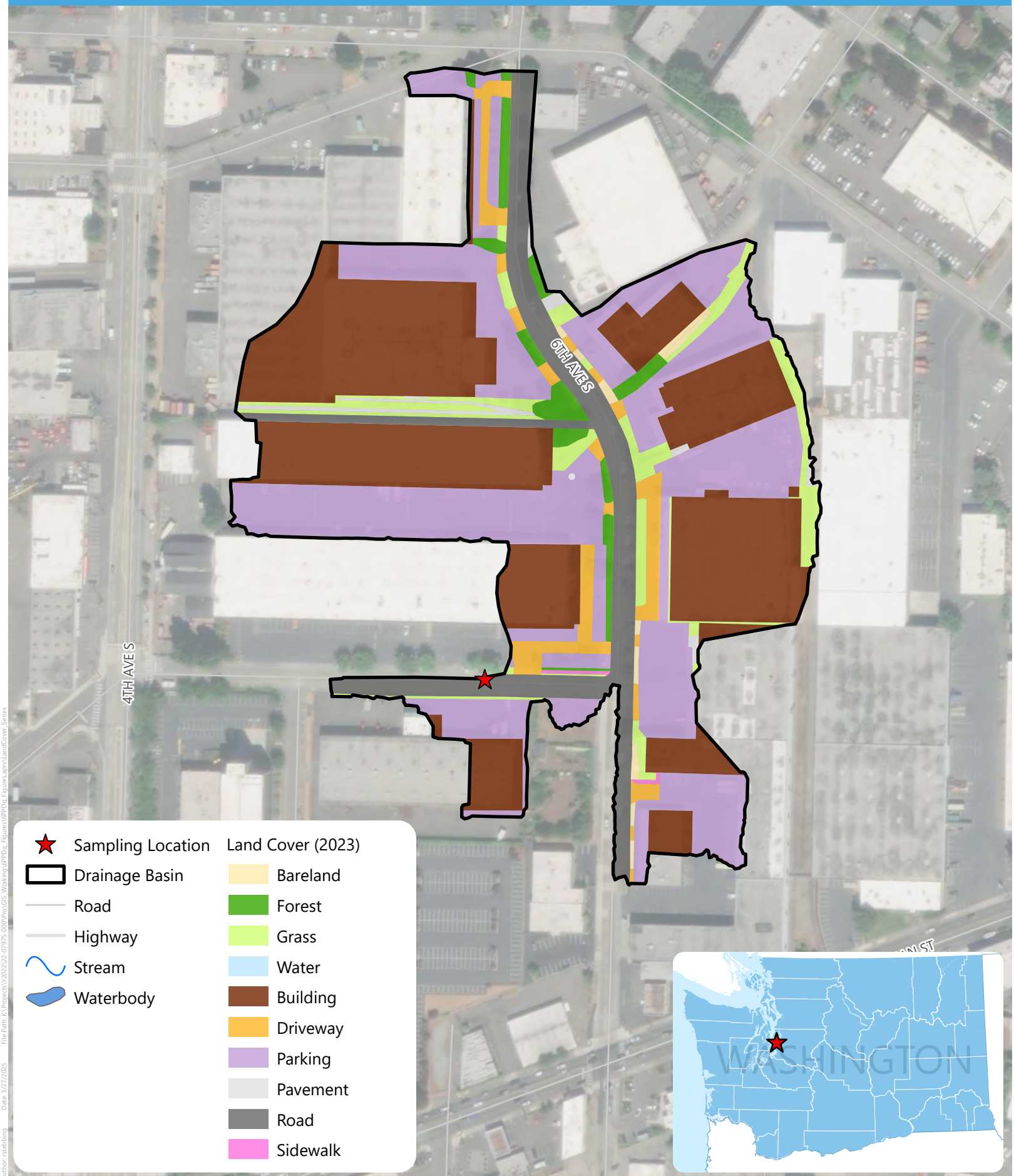
SEA_IND2

The second industrial monitoring location in the City of Seattle (SEA_IND2) is located on South Front Street between 5th and 6th Avenue South, about 260 feet west of 6th Avenue South (Figure 18), in the Green River HUC-12 Watershed. This is a new monitoring location that was not previously monitored for S8.D compliance. The monitoring location is in a maintenance hole in the street. Minor traffic control will be needed for equipment install and sample collection. The grab samples will be collected from the 21-inch pipe entering the structure from the east (Figure 17). The SSPM traps will be installed in the pipe through the maintenance hole (Figure 17). SPU rain gauge RG16 will be used to measure rainfall. The rain gauge is approximately 0.8 mile southeast of the monitoring location.

Figure 17. SEA_IND2 Monitoring Location Design.

HERRERA
Science + Planning + Design

Figure 18.
City of Seattle Industrial Basin #2.



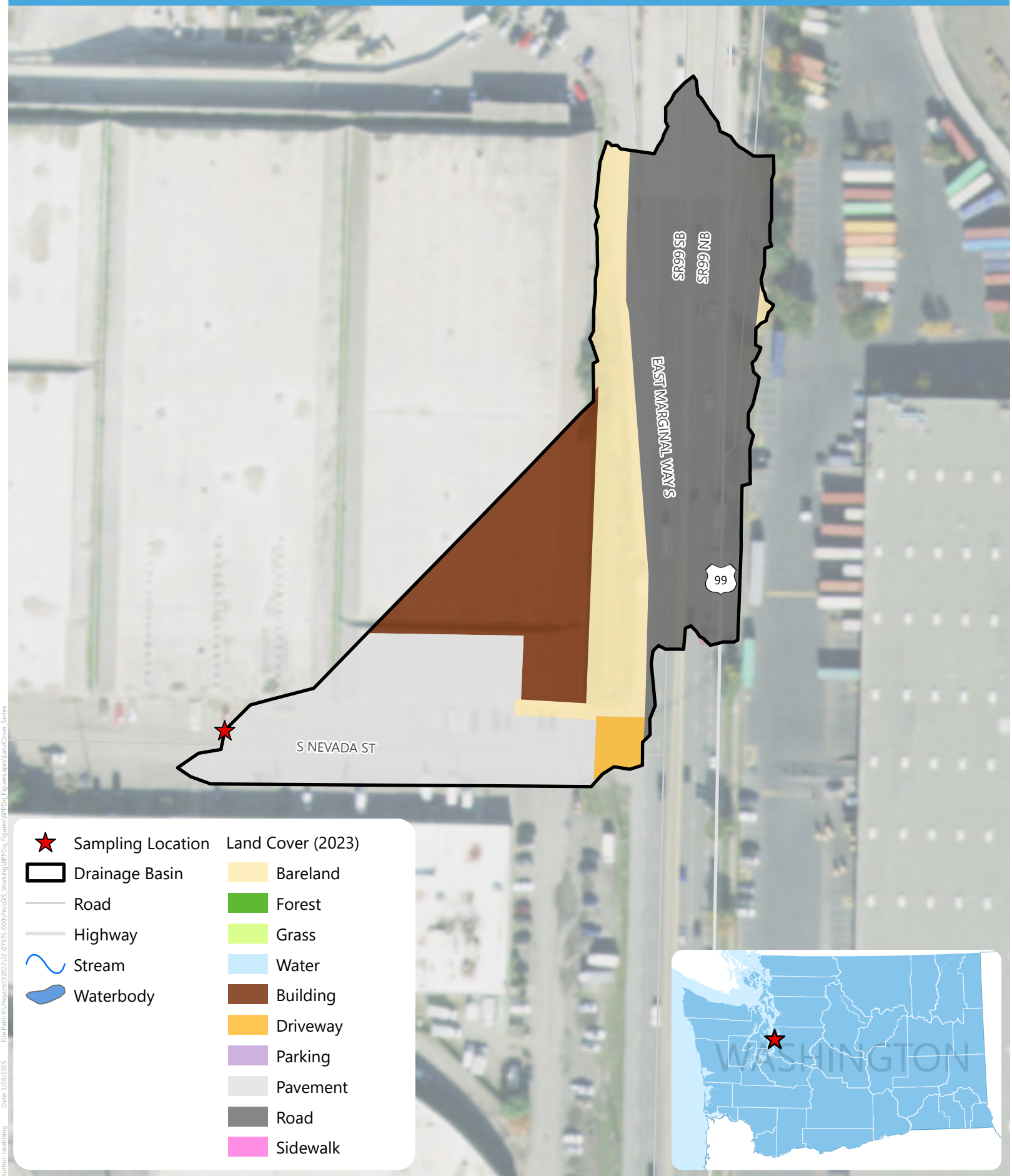
SEA_IND3

The third industrial monitoring location in the City of Seattle (SEA_IND3) is located on South Nevada Street, east of East Marginal Way South (Figure 20), in the Green River HUC-12 Watershed. This is a new monitoring location that was not previously monitored for S8.D compliance. The monitoring location is in a maintenance hole in the street about 350 feet west of East Marginal Way South. Minor traffic control will be needed for equipment install and sample collection. The grab samples will be collected from the 15-inch pipe entering the maintenance hole structure from the east (Figure 19). The SSPM traps will be installed in the pipe through the maintenance hole (Figure 19). SPU rain gauge RG15 will be used to measure rainfall. The rain gauge is approximately 1,000 feet south of the monitoring location.

Figure 19. SEA_IND3 Monitoring Location Design.

HERRERA
Science + Planning + Design

Figure 20.
City of Seattle Industrial Basin #3.



Produced by Herrera Environmental Consultants (herrerainc.com) | Sources: Ecopia Vector Maps © [2025] Ecopia Tech Corporation, ESRI (Aerial, 2023), NHD, WSDOT

0 40 80 160 Feet

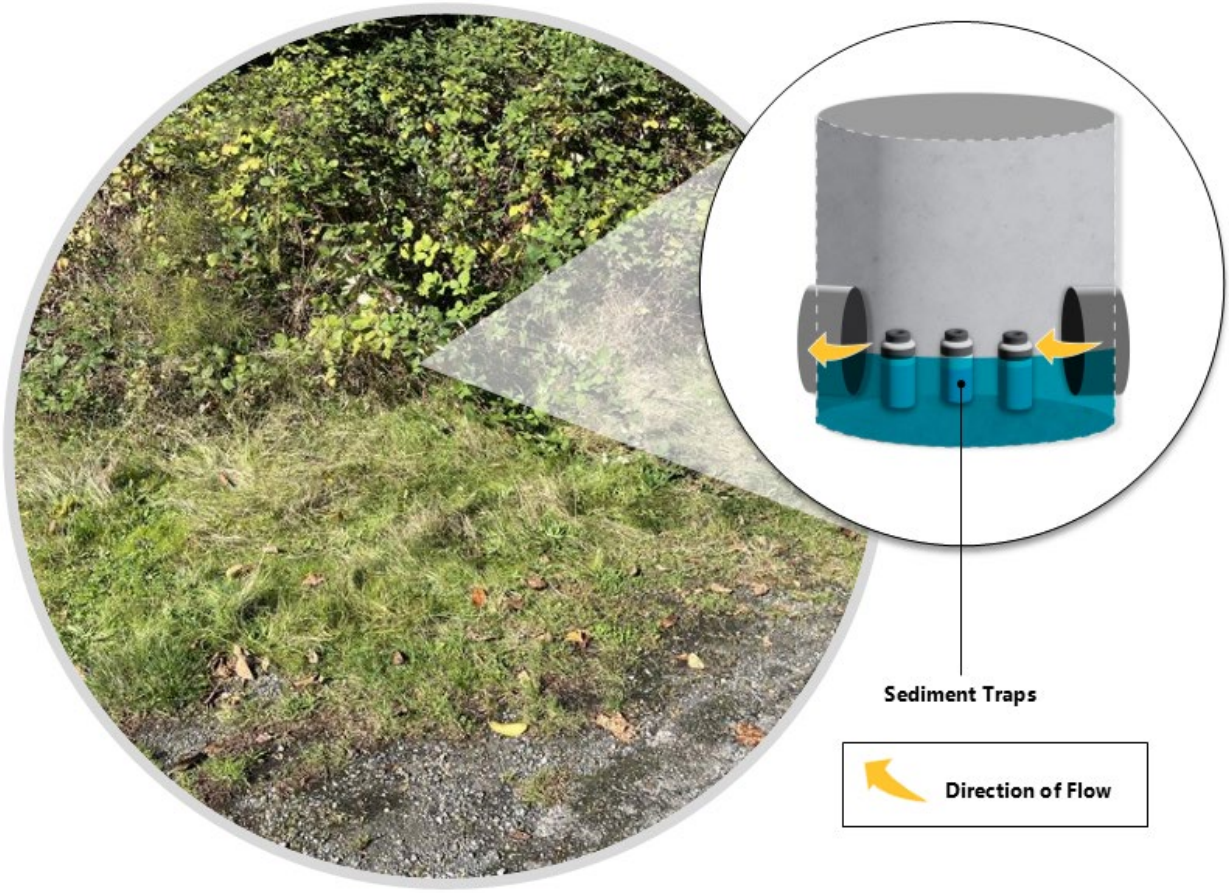
File Path: K:\Projects\Y2022\22-07975.000\ProjGIS_Working\6PPDq_Figures\6PPDq_Figures.aprx\LandCover_Series
Date: 3/28/2025
Author: rstebbing

6.2.1.4. Pierce County Monitoring Locations

PIER_LDR

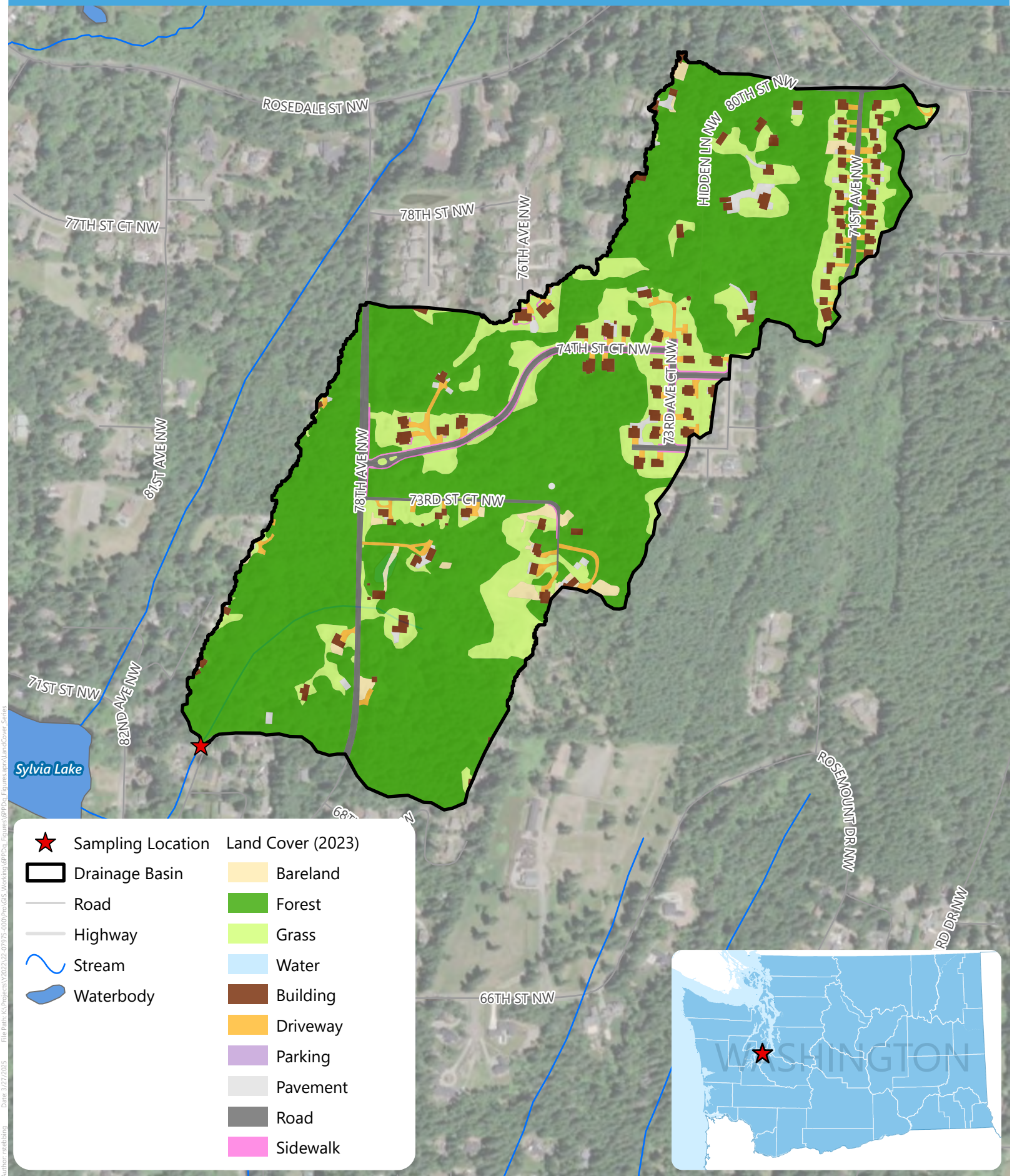
The low-density residential monitoring location (PIER_LDR) for Pierce County is located in Gig Harbor, Washington, near Lake Sylvia (Figure 22). This location is in the Burley Creek-Frontal Carr Inlet HUC-12 Watershed. The specific outfall monitoring location is in a stormwater maintenance hole, southwest of the corner of 81st Street Avenue Northwest and 70th Street Northwest. Only SSPM samples and grab samples will be collected at this location. The SSPM traps will be installed in the maintenance hole sump. Grab samples will be collected from the pipe entering the structure from the northeast (Figure 21). Pierce County's Gig Harbor rain gauge will be used to measure rainfall. The rain gauge is approximately 3.5 miles east of the monitoring location.

Figure 21. PIER_LDR Monitoring Location Design.



HERRERA
Science + Planning + Design

Figure 22.
Pierce County Low-Density Residential Basin.



File Path: K:\Projects\Y2022\22-07975-000\Pro\GIS_Working\6PPDq_Figures.aprx\LandCover_Series
Date: 3/27/2025
Author: rstebbing

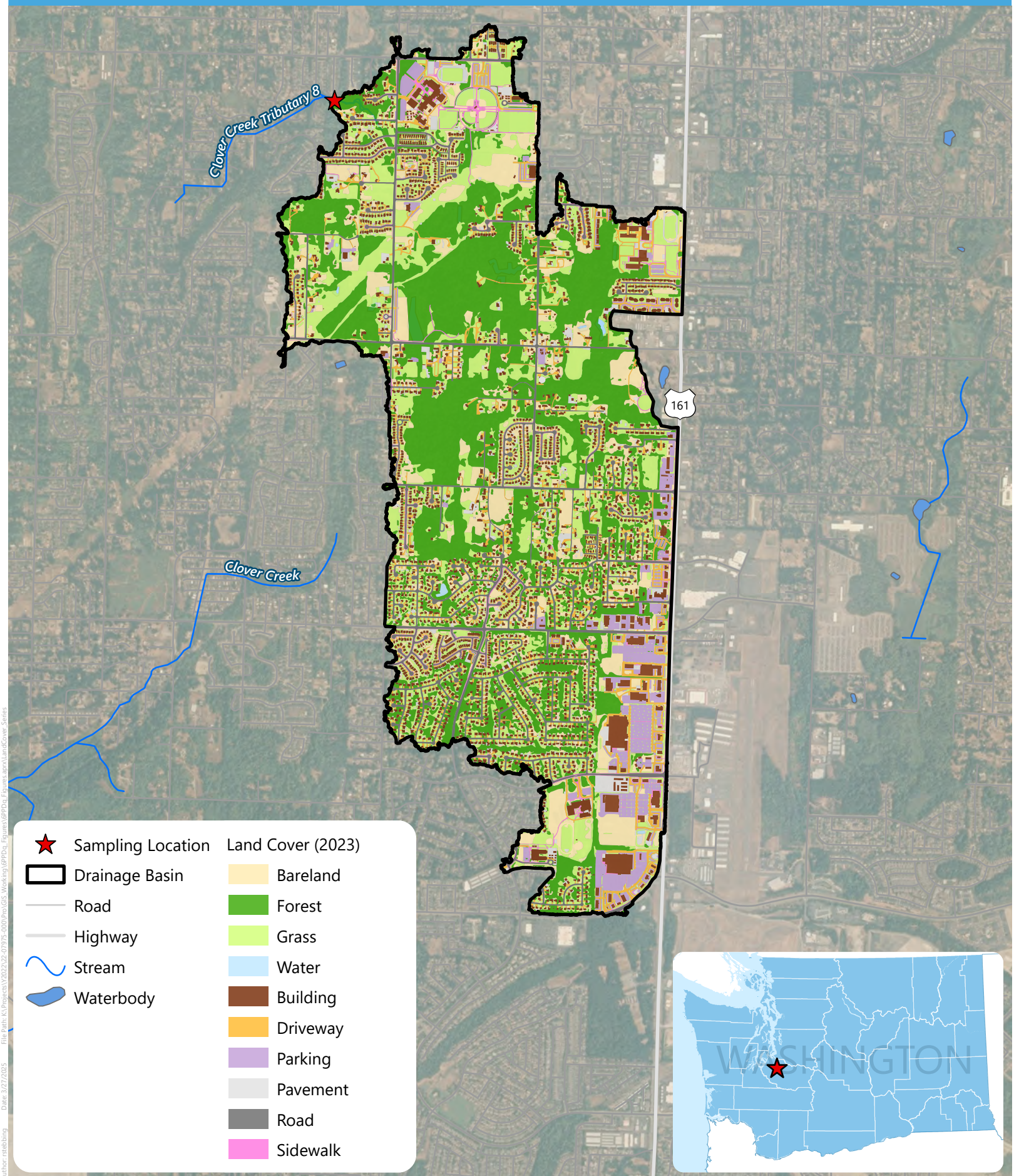
PIER_HDR

The high-density residential monitoring location (PIER_HDR) for Pierce County is located in Puyallup, Washington (Figure 24), within the Puyallup River HUC-12 Watershed. The specific outfall monitoring location is near the intersection of 128th Street East and 82nd Avenue Court East, at the end of the cul-de-sac on 82nd Avenue Court East. Only SSPM samples and grab samples will be collected at this location. The SSPM traps will be installed in the channel adjacent to the overflow structure. Grab samples will be collected from the same channel (Figure 23). Pierce County's Woodland Pond rain gauge will be used to measure rainfall. The rain gauge is approximately 1.8 miles southeast of the monitoring location.

Figure 23. PIER_HDR Monitoring Location Design.

HERRERA
Science + Planning + Design

Figure 24.
Pierce County High-Density Residential Basin.



Produced by Herrera Environmental Consultants (herrerainc.com) | Sources: Ecopia Vector Maps © [2025] Ecopia Tech Corporation, ESRI (Aerial, 2023), NHD, WSDOT

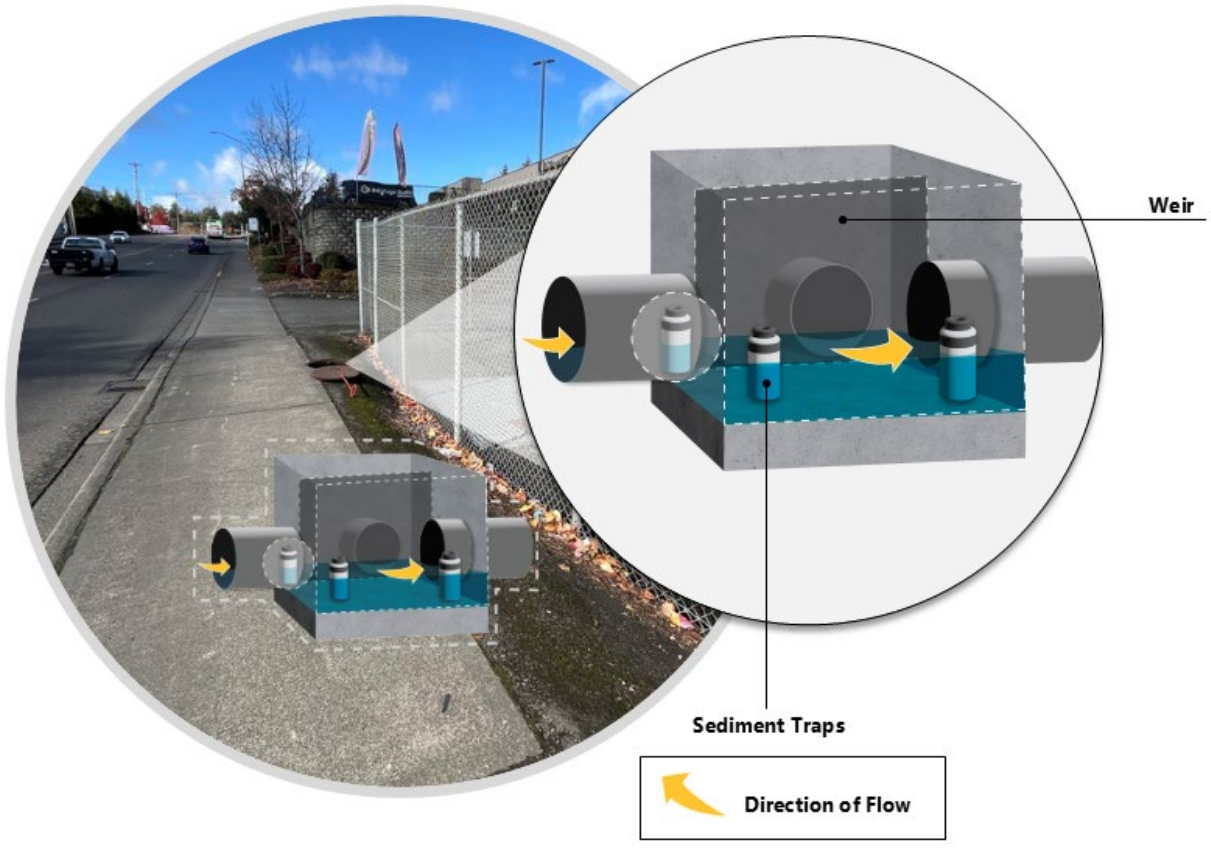
0 5001,000 2,000 Feet

File Path: K:\Projects\Y2022\22-07975-000\ProjGIS_Working\6PPDq_Figures.aprx\LandCover_Series
Date: 3/27/2025
Author: rstebbing

PIER_COM

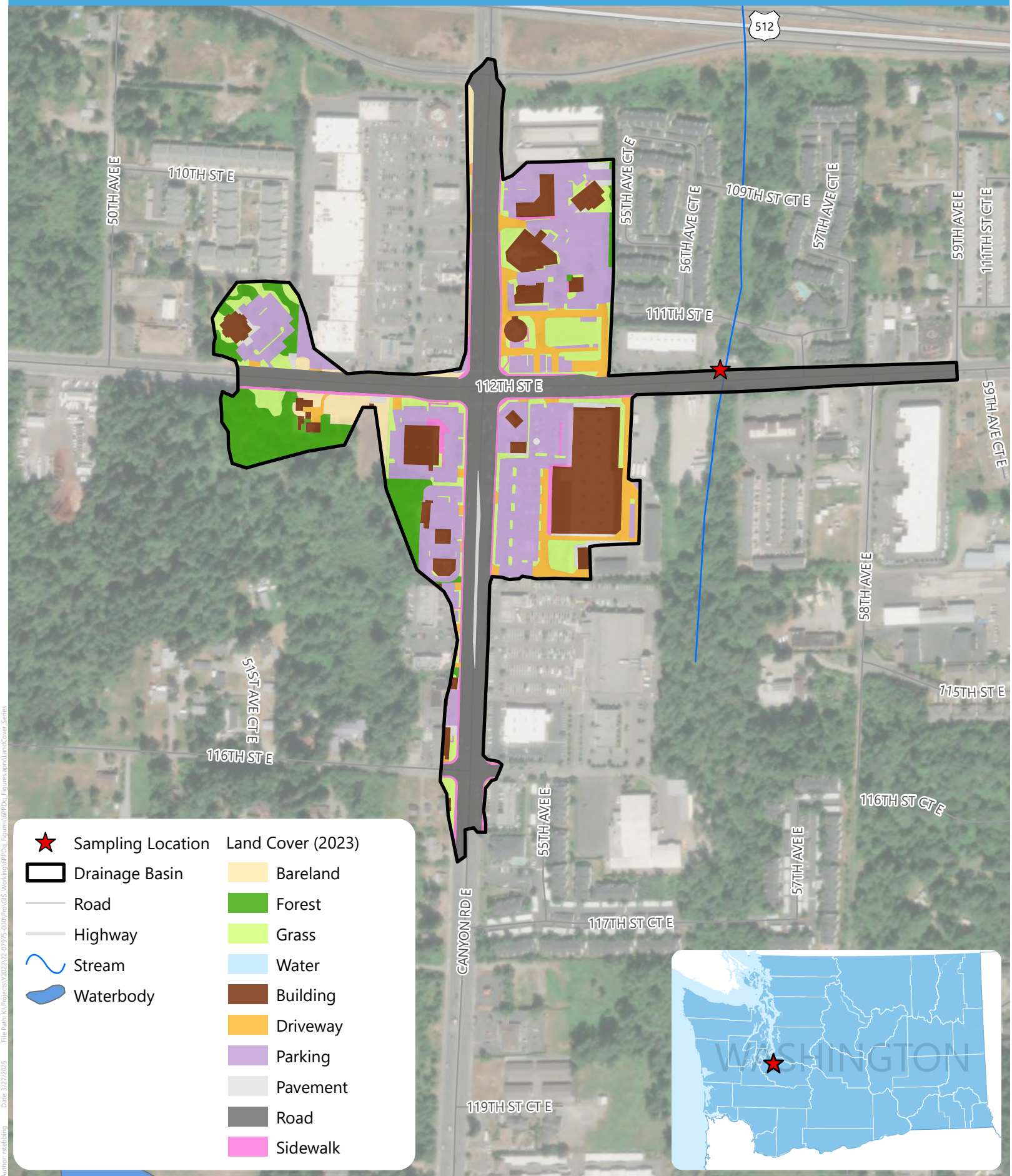
The commercial monitoring location (PIER_COM) for Pierce County is located in Puyallup, Washington (Figure 26), within the Puyallup River HUC-12 Watershed. The specific outfall monitoring location is adjacent to a culverted stream near the northeast corner of 112th Street East and 56th Avenue Court East. Only SSPM samples and grab samples will be collected at this location. The SSPM traps will be installed in the sump of the diversion structure, which routes flow to an adjacent stormwater filter. Grab samples will be collected from the pipe entering the structure from the south (Figure 25). Pierce County's Woodland Pond rain gauge will be used to measure rainfall. The rain gauge is approximately 1 mile northeast of the monitoring location.

Figure 25. PIER_COM Monitoring Location Design.



HERRERA
Science + Planning + Design

Figure 26.
Pierce County Commercial Basin.



6.2.1.5. City of Tacoma Monitoring Locations

TAC_HDR

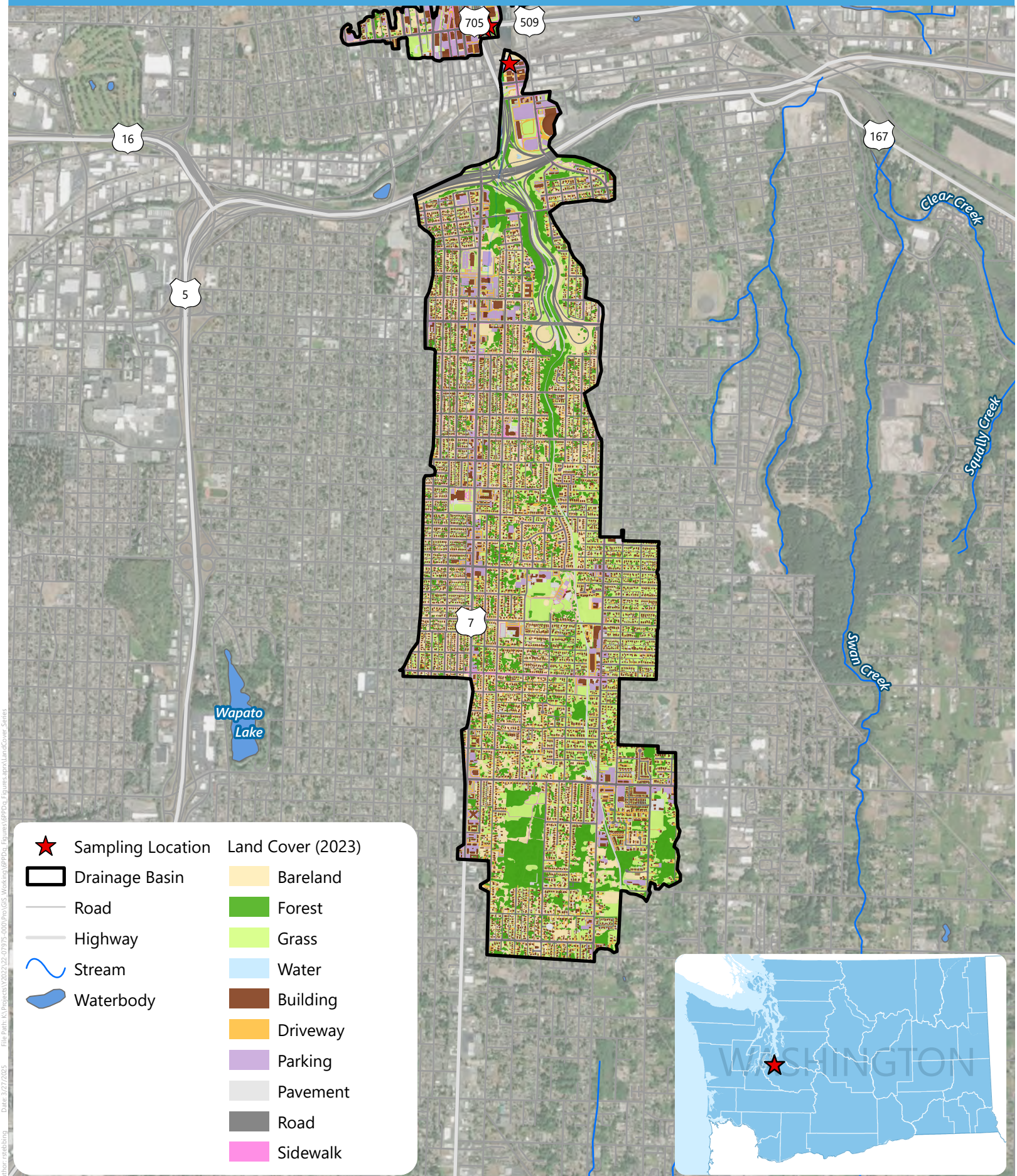
The high-density residential monitoring location (TAC_HDR) for the City of Tacoma is located at the City's Dock Street Pump Station Yard in the 2300 block of East "C" Street (Figure 28). This location is in the City of Tacoma-Frontal Commencement Bay HUC-12 Watershed. The City of Tacoma has monitored this location regularly since the 2007 S8.D study, and equipment is already installed. The equipment is housed within a maintenance hole located in the southeast section of the asphalt-paved yard (Figure 27). The SSPM traps are existing traps used for the City's own monitoring. The SSPM traps are located upstream of the stormwater monitoring location.

The monitoring location is generally above tidal influence. However, at tides 12 feet above mean lower low water (MLLW) and greater, the monitoring location may be influenced by tidal waters. To avoid tidal influence, the autosampler will collect water in 12 separate 1-liter glass bottles. If the tide reaches 12 feet MLLW, samples collected during that time will be discarded.

Figure 27. TAC_HDR Monitoring Location Design.

HERRERA
Science + Planning + Design

Figure 28.
City of Tacoma High-Density Residential Basin.



Produced by Herrera Environmental Consultants (herrerainc.com) | Sources: Ecopia Vector Maps © [2025] Ecopia Tech Corporation, ESRI (Aerial, 2023), NHD, WSDOT

0 1,000 2,000 4,000 Feet

File Path: K:\Projects\Y2022\22-07975.000\Pro\GIS_Working\6PPDq_Figures\6PPDq_Figures.aprx\LandCover_Series
Date: 3/27/2025
Author: rstebbing

TAC_COM

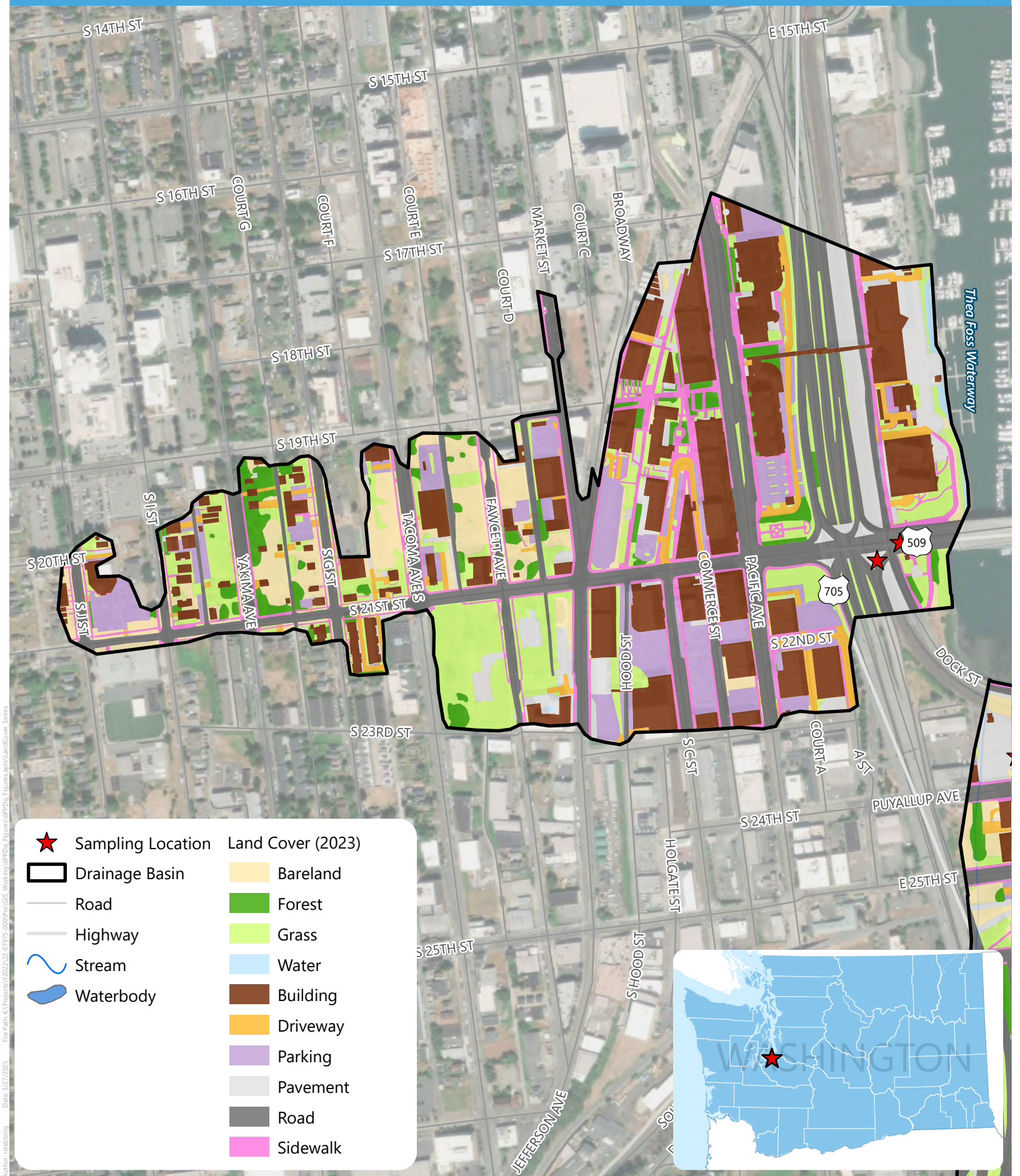
The commercial monitoring location (TAC_COM) for the City of Tacoma is located directly under the SR 509 bridge, on the east side of Thea Foss Waterway, at South 21st Street and Dock Street (Figure 30). This location is in the City of Tacoma-Frontal Commencement Bay HUC-12 Watershed. The City of Tacoma has monitored this location regularly since the previous study, and equipment is already installed. The equipment housing and automated sampler intake are located in the southeast corner of a parking lot. One SSPM trap exists upstream of the stormwater monitoring location, which is used for the City's own monitoring. SSPM sample material will come from the existing trap and from two additional traps that will be installed. Access to this location is on the curb of Dock Street (Figure 31).

The location is tidally influenced. Depending on tidal height, portions of the pipe are inundated with marine water twice per day. Average tidal fluctuations vary from 0 feet MLLW to 11 feet MLLW. Extreme tides, which occur in June and December, range from approximately -4.0 feet MLLW to 14.5 feet MLLW. To avoid tidal influence, the autosampler will collect water in 12 separate 1-liter glass bottles. Samples collected while the pipe is inundated with marine water will be discarded.

Figure 29. TAC_COM Monitoring Location Design.

HERRERA
Science + Planning + Design

Figure 30.
City of Tacoma Commercial Basin.

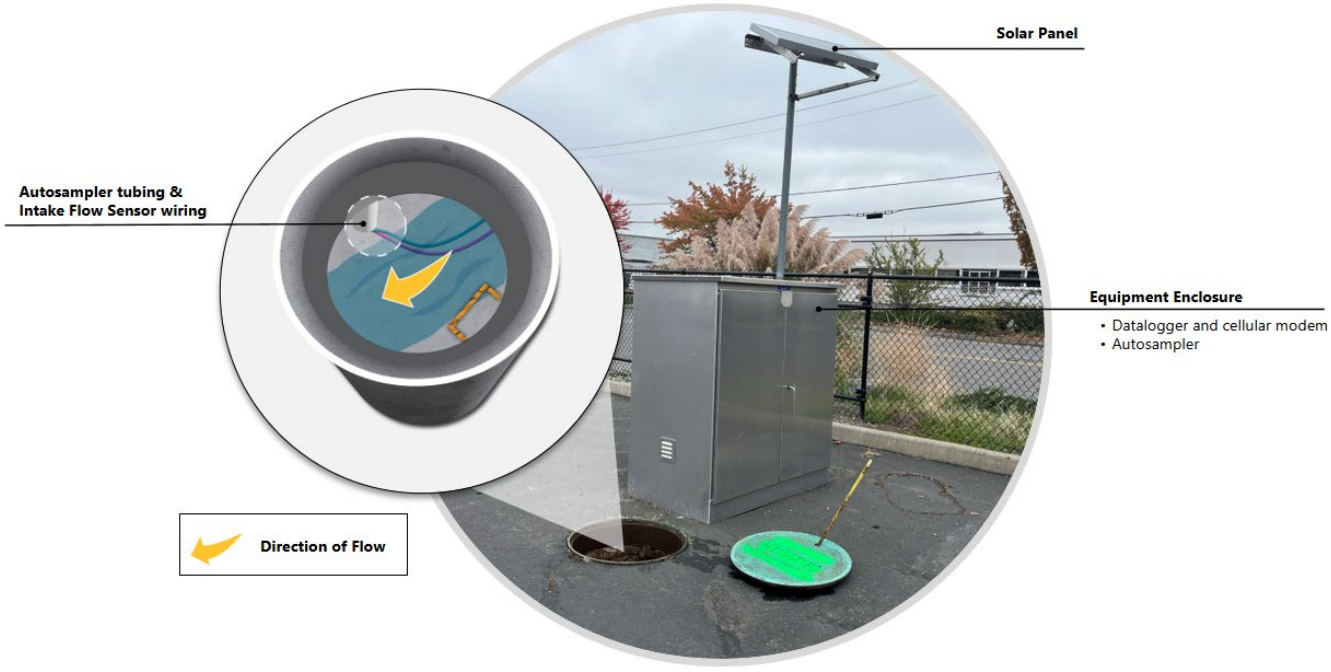


TAC_IND

The industrial monitoring location (TAC_IND) for the City of Tacoma is located at East 19th Street and East "D" Street in the former Johnny's Restaurant parking lot (Figure 32). This location is in the City of Tacoma-Frontal Commencement Bay HUC-12 Watershed. The monitoring location is a deep bottom sump maintenance hole, located in an abandoned parking lot. The City of Tacoma has monitored this location regularly since the previous study, and equipment is already installed. There are no SSPM traps at this location. SSPM samples will be collected by Ponar grab from the sump (Figure 31).

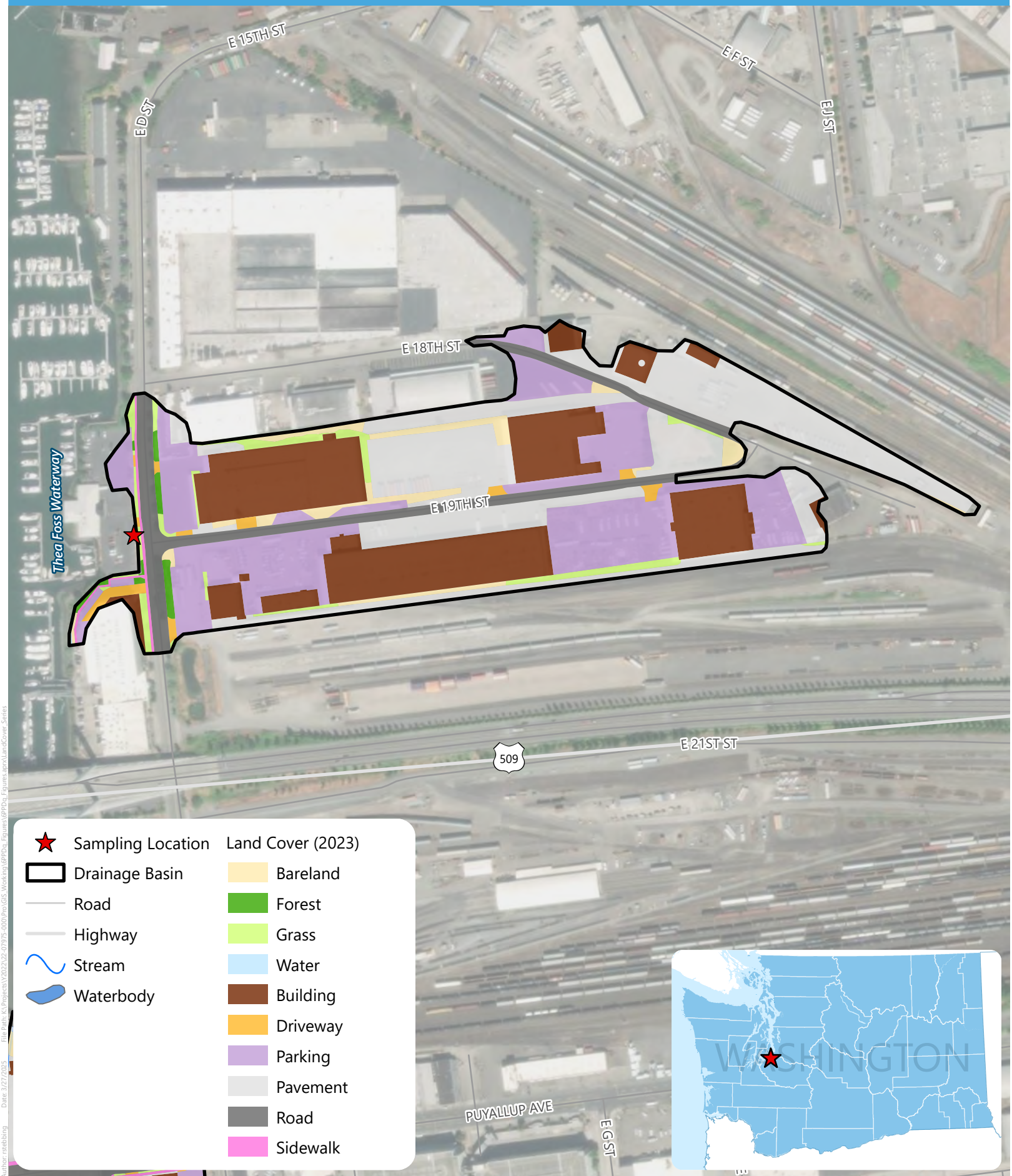
The monitoring location is tidally influenced. Depending on tidal height, portions of the outfall pipe and sump are inundated with marine water twice per day. Average tidal fluctuations vary from 0 feet MLLW to 11 feet MLLW. Extreme tides, which occur in June and December, range from approximately -4.0 feet MLLW to 14.5 feet MLLW. To avoid tidal influence, the autosampler will collect water in 12 separate 1-liter glass bottles. Samples collected while the pipe is inundated with marine water will be discarded.

Figure 31. TAC_IND Monitoring Location Design.



HERRERA
Science + Planning + Design

Figure 32.
City of Tacoma Industrial Basin.



Produced by Herrera Environmental Consultants (herrerainc.com) | Sources: Ecopia Vector Maps © [2025] Ecopia Tech Corporation, ESRI (Aerial, 2023), NHD, WSDOT

0 145 290 580 Feet

6.2.1.6. Clark County Monitoring Location

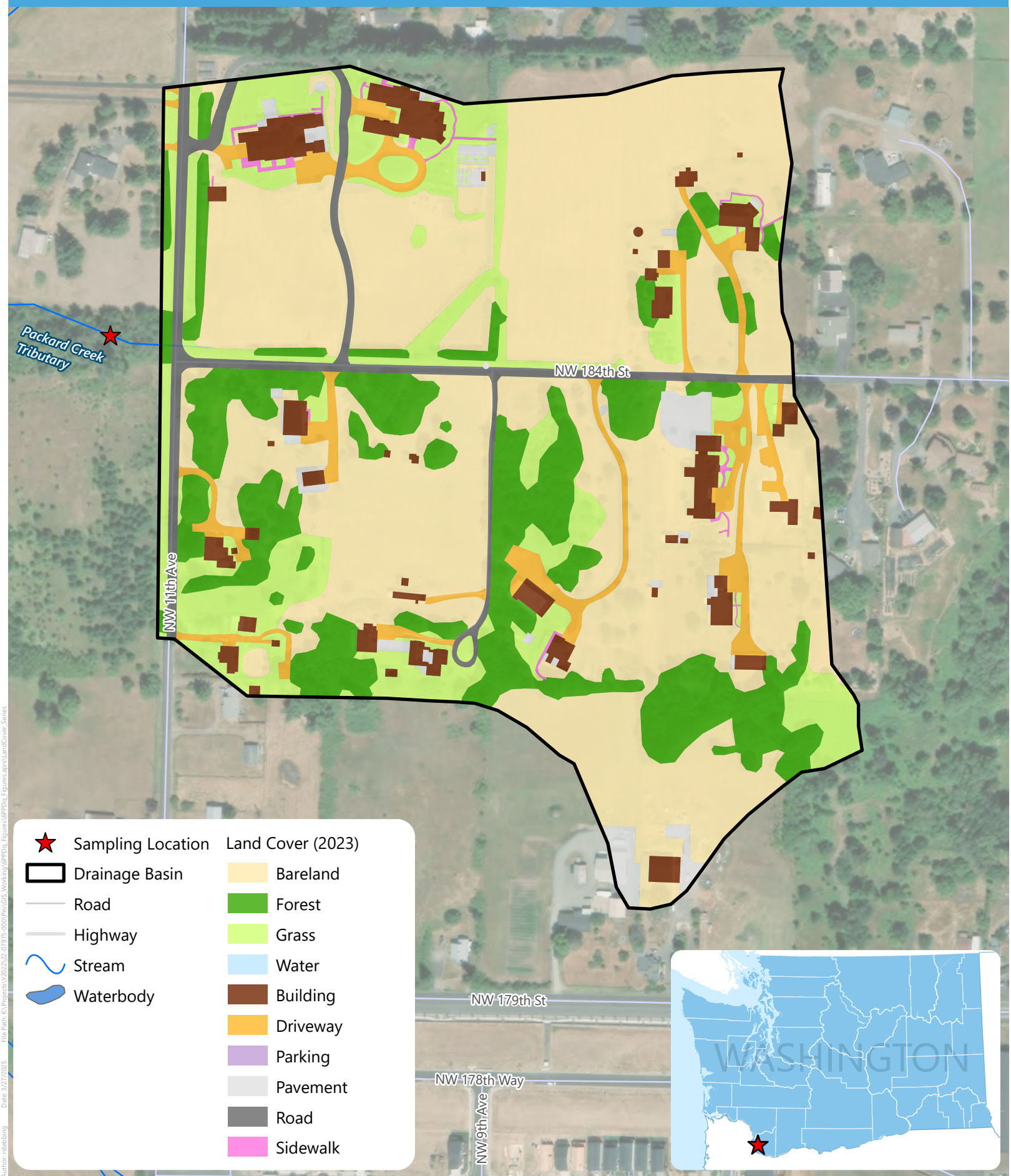
CLRK_LDR

The low-density residential station (CLRK_LDR) for Clark County is located in Ridgefield, Washington, approximately 200 feet west of the intersection of Northwest 184th Street and Northwest 11th Avenue (Figure 34). The monitoring station is a small tributary that flows west towards Packard Creek, located within the Lake River-Frontal Columbia River HUC-12 Watershed. Only SSPM samples and grab samples will be collected at this location. The SSPM traps will be installed in the channel, upstream of the flume (continuous flow is not being measured at this location under this study; there is just coincidentally an old flume at the monitroing location). Grab samples will be collected at the mouth of the flume (Figure 33). Clark County's LDR010 rain gauge will be used to measure rainfall. The rain gauge is collocated with the monitoring location.

Figure 33. CLRK_LDR Monitoring Location Design.

HERRERA
Science + Planning + Design

Figure 34.
Clark County Low-Density Residential Basin.



Produced by Herrera Environmental Consultants (herrerainc.com) | Sources: Ecopia Vector Maps © [2025] Ecopia Tech Corporation, ESRI (Aerial, 2023), NHD, WSDOT

0 100 200 400 Feet

6.2.2. Field Parameters and Laboratory Analytes to be Measured

Flow-weighted composite samples will be collected at all King County locations, all City of Tacoma locations, and SEA_IND1 and SNO_LDR during each storm event. These samples will be analyzed for the following parameters:

- Total suspended solids
- Total phosphorus
- Orthophosphate
- Total nitrogen
- Nitrate+nitrite as N
- Total and dissolved metals (copper and zinc)
- 6PPDQ

At these same locations the following continuous data will be collected:

- Rainfall
- Water level
- Water velocity
- Flow rate
- Water temperature

Grab samples will be collected at all 16 locations and analyzed for the following parameters:

- Total petroleum hydrocarbons (diesel range)
- 6PPDQ
- Microplastics/TWP
- PFAS

The following plastics will be identified in this project in each of three size classes: >250, 53-250, and <53 microns: tire wear particles (TWP), polyethylene (PE), Polypropylene (PP), Polystyrene (PS), Polyethylene terephthalate (PET), polycarbonate (PC), and Polymethyl methacrylate (PMMA).

Note that 6PPDQ analysis will be performed on both grab samples and automated composite samples. This is because 6PPDQ is of particular importance for the region and the science is not yet settled on if a grab sampling approach is adequate to meet the objective of identifying typical or maximal concentrations from the target land uses in the study. Grab samples will be collected from all monitoring locations, composite samples at only a subset of locations. In addition, a 1-liter HRMS grab sample will be collected from all locations and a 1-liter split will be taken from the composite sample bottle (when enough volume is available and project constraints allow) for the eight automated sampler locations.

SSPM samples will be collected at all 16 locations using in-line SSPM traps. SSPM samples will be analyzed for the following parameters:

- Total solids
- Total organic carbon
- Grain size
- Total recoverable metals
- PAHs
- Phthalates
- Phenolics
- PCBs
- Bifenthrin
- Dichlobenil
- 6PPDQ
- Microplastics/TWP
- PFAS

The continuous data to be collected at all 16 monitoring locations will consist of the following:

- Rainfall (either at the station or from a nearby gauge)
- Water level
- Water temperature

6.3. Modeling and Analysis Design

There is no modeling associated with this project.

6.4. Assumptions Underlying Design

Assumptions underlying the study design include the following:

- There has been minimal change in land use and stormwater infrastructure in the monitoring basins, so monitoring results will be comparable to the S8.D results. If changes have occurred, those changes will be determined by calculating the differences in imperviousness, tree cover, number of structures, etc. for each basin. The TAC will then convene to review the basins and confirm they are still representative of their land use type.
- At least nine qualifying storm events will occur each year, with appropriate timing for field staff to target them.
- Sample collection and delivery can be completed by one to two staff members in one day.

- Using glass bottles for SSPM sample collection will not bias PFAS in sediment results. Ideally, PFAS would be collected in HDPE bottles, but those bottles would impact the accuracy of Microplastics/TWP and 6PPDQ results.
- The SSPM sample collection method will use three variations on the Norton sediment trap. However, the sampling method—passive settling of solids into the collection bottle as water flows over the bottle mouth—is effectively the same among all of the trap design variations and is the same as under the previous S8D program. Other sediment trap types and sampling methods were considered, and the variations on the Norton trap were selected for this study to be consistent with previous collection method and provide comparable data.

6.5. Possible Challenges and Contingencies

6.5.1. Logistical Problems

Samples will be collected across a large geographical area. Samples will need to be delivered to the laboratory in Tacoma, with enough time for analyses to be completed within holding times. The shortest holding time is 24 hours for filtration of orthophosphate and dissolved metals. The laboratory closes for the weekend on Fridays at 4:00 p.m. To meet holding time requirements, Friday and weekend storms generally will not be targeted.

At the writing of this QAPP, the project team does not anticipate any challenges that could prevent sampling at any of the locations described above. However, if something did arise, such as permitting issues or loss of access, a replacement location will be identified, and steps will be taken to gain access and install equipment. The new location will have similar basin characteristics to the original location. Finding and characterizing an additional site would delay the project by 4 to 8 months.

6.5.2. Practical Constraints

EPA requires filtering for dissolved metals and orthophosphate within 15 minutes of the collection of the last aliquot. This goal is not practical to meet when conducting flow-weighted sampling. A more practical proxy goal of 24 hours from the last sample aliquot has been adopted for this study, and both goals will be reported with the data.

To ensure the safety of field personnel, grab samples will only be collected during daylight hours. If sampling after dark is determined to be necessary to meet project objectives, two staff members will be required for sampling.

6.5.3. Schedule Limitations

Several factors may affect project schedule. The timing of the start of sampling may be affected by factors such as the following:

- QAPP and SOP review schedule by Ecology
- Identifying sampling sites and gaining access
- Obtaining and installing equipment

Once sampling is underway, the timing of sampling events is dependent on weather conditions. Storm sampling will target qualifying storm events as defined in Section 6.2.1.

7. Field Procedures

7.1. Invasive Species Evaluation

Field staff will follow Ecology's SOP to *Minimize the Spread of Invasive Species* (Ecology 2024b).The main considerations for this project include the following:

- Select equipment that can be easily inspected and cleaned to avoid spreading invasive species.
- Conduct field activities to minimize contact between equipment and potential sources of invasive species. This can include the following:
  - Sample from least to most contaminated areas, for example, sample upstream to downstream or from areas of less weed growth to areas of dense weed growth.
  - Avoid getting plants or sediment inside sampling gear.
  - Avoid driving or walking through areas of mud and high aquatic plant growth.
- Before leaving the site, if possible, inspect and clean all equipment that contacted sediment, vegetation, or water. If conducted after leaving the site, ensure that no debris will leave the equipment and potentially spread invasive species during transit or cleaning.

7.2. Measurement and Sampling Procedures

7.2.1. Grab Sample Protocol

In general, grab sample collection will follow Ecology's SOP for *Collecting Grab Samples from Stormwater Discharges* (Ecology 2024c). Special requirements for sampling for CECs are outlined below and described in detail in the SOPs located in Appendix C.

Due to the variable access requirements of each of the 16 locations and the large sample volume required for microplastics/TWP analyses, a high flow rate peristaltic pump with 3/8-inch ID silicone tubing may be used to collect the grab samples for microplastics/TWP, PFAS, and 6PPDQ. Total petroleum hydrocarbons (TPH) grab samples will be collected by dipping a glass bottle. For pumped samples, either the pump in the autosampler or a PM6000RB Portable Battery Peristaltic Pump will be used to draw water from the flowing pipe or ditch. Field staff will be careful to position the end of the intake in the water to avoid entraining deposited sediments. For sampling locations that already have an automated sampler, the sampler will be programed to grab sample mode and the sampler pump will be used to collect the sample.

All grab samples (except microplastics/TWP) will be stored on ice immediately after collection and during transport to the laboratory. Field staff will deliver all samples to the laboratory, with the exception of

Clark County samples. Samples collected at the Clark County location will be shipped to the laboratory on ice overnight.

To help assure that grab samples are, in aggregate, representative of the average storm concentration, In-Situ Rugged Troll 100 sensors will be deployed at each monitoring location. These sensors contain both a pressure transducer for water level and a thermistor to measure temperature. The sensors will be installed in perforated PVC tubes to protect and shade them from sunlight. Data will be downloaded approximately quarterly from these sensors, and then a post-hoc analysis will be conducted to assess the hydrograph position of the grab samples collected since the last download.

7.2.1.1. Microplastics/TWP

Upon arrival at the project site, field staff will establish a sample collection staging area for stormwater samples. The staging area will be free from potential or known sources of plastics and rubber that could contact the sample. Field staff will use fresh powder-free nitrile gloves during sample collection for other parameters but will rinse gloves with microplastics/TWP-free water prior to collecting the microplastics/TWP sample.

Procedures for collecting stormwater grab samples for microplastics/TWP analysis are generally consistent with the typical stormwater grab sampling procedures described in Ecology's SOP (Ecology 2024c), but the laboratories advise a larger quantity (typically greater than 10 liters) of sample to prevent non-detection results. A 20-liter grab sample will be collected by peristaltic pump into a laboratory-clean, 22-liter stainless steel vessel.

Additional considerations are needed to avoid cross-contamination due to the widespread uses of plastics, which include the following:

- Sampling equipment
- Field clothing and personal protective equipment (PPE)
- Cosmetic products
- Food packaging

It is recommended, when possible, to exclude materials from sampling equipment if those materials are known to contain plastics (with the exception of silicone tubing). Field staff will review the list of prohibited supplies in the SOP (Appendix C) prior to sampling.

One equipment rinsate blank will be collected from one location during each of the four microplastics sampling events. The rinsate blank will be collected by using the sampling equipment on site to discharge laboratory-provided microplastics/TWP-free DI water into the laboratory-provided sample container.

In general, field staff will wear clothing without plastic or rubber. Field staff may wear plastic or rubber shoes/boots that are not breaking down or shedding material. When possible, field staff will wear natural materials (cotton, wool) or particularly avoid polyester clothing—as this is a common contaminant for polyethylene terephthalate (a targeted microplastic). Immediately prior to sample collection, field staff

will avoid handling plastic or rubber containers or wrappers and ensure the sampling area is clear of potential contaminant sources, pens, and plastic binders or clipboards. Any plastic that is present during sampling will be documented in field notes (e.g., reflective vest for safety working in or near roads) (Appendix C).

7.2.1.2. PFAS

Upon arrival at the project site, field staff will establish a sample staging area and a decontamination area. The staging area will be free from potential or known sources of PFAS contamination. The decontamination area may include sampling equipment or rinse water but no other potential sources of PFAS contamination. Field sampling equipment will be decontaminated, as described in Section 7.4.3, and moved to the staging area. Field staff will change to fresh powder-free nitrile gloves after decontaminating equipment, when re-entering the staging area, and as needed to prevent contamination.

It is recommended, when possible, to exclude materials known to contain PFAS. Field staff will review the list of prohibited supplies in the SOP (Appendix C) prior to sampling. However, PFAS are prevalent in materials used within the sampling environment. These materials include the following:

- Sampling equipment
- Field clothing and personal protective equipment (PPE)
- Sun and biological protection products
- Personal hygiene and personal care products (PCPs)
- Food packaging

To minimize PFAS contamination, field staff will wear well-laundered (washed at least six times without fabric softener) clothing made from natural fibers and avoid clothing that contains GoreTex or Tyvek or have been treated with waterproofing chemicals. On the day of sampling, field staff will avoid use of certain personal care products including deodorant, floss, moisturizer, and makeup. Sunscreen and insect repellants prescreened to be PFAS-free may be used if necessary. Immediately prior to sample collection, field staff will avoid handling food packaged in containers or wrappers and ensure the sampling area is clear of potential contaminant sources including chemical ice packs, felt tip pens, sticky notes, and plastic binders or clipboards. Only powder-free nitrile gloves will be worn by the field staff during sampling area preparation and sample collection.

Sample collection entails filling two 250-mL and one 125-mL HDPE sample bottles for each sample by either directly dipping the bottles or pumping sample into the bottles with a peristaltic pump and clean silicone tubing. Each bottle will be triple rinsed with sample prior to sample collection. The bottle will not be overfilled (Appendix C).

During one sampling round, a field blank will be collected from each location in the sample staging area prior to field sample collection by pouring laboratory-provided PFAS-free water into the laboratory-provided sample container using the sampling equipment on site.

Samples will be stored together in a plastic bag inside of a sample cooler. Wet ice will be bagged and used to keep the sample cool until delivery to the analytical laboratory. PFAS grab samples will not be stored with other samples (Appendix C).

7.2.1.3. 6PPDQ

6PPDQ stormwater grab sample collection procedures are generally consistent with Ecology's grab sample SOP (Ecology 2024c). However, certain precautions must be taken to ensure that the sample is handled properly and that the correct sampling materials are used.

These precautions include using only laboratory-provided amber glass sample containers for stormwater grab samples, minimizing headspace in the sample container, minimizing exposure to light, and selecting appropriate materials (e.g., stainless steel) if intermediate sampling equipment is necessary. General sampling procedures include the following:

- Wear a fresh pair of powder-free nitrile gloves prior to sample collection.
- Remove the lid from the sample container immediately prior to sample collection, with care not to contaminate the sample container or lid.
- Fill two 250-mL amber glass bottle to overfilling and cap with no headspace.
- Store the sample container according to method requirements, out of direct sunlight, and on ice (Appendix C).

During one sampling round, a field blank will be collected from each location by discharging laboratory-provided DI water into the laboratory-provided sample container using the sampling equipment on site.

7.2.1.4. TPH

TPH water samples cannot be collected by peristaltic pump, due to adhesion of oils to the silicone tubing. Instead, two 1-liter amber glass bottles will be dipped into the flowing water and allowed to fill to 80 percent full. Field staff will take care not to overfill the bottle, which would bias the sample. The bottle will be dipped into the fastest and most well mixed portion of the cross section to help assure that a uniform solution is sampled. To the extent feasible, the bottle will be dipped but not submerged. This will allow floating oils to enter the mouth of the bottle. Some locations will require a sampling pole; at other locations, the bottle can be hand dipped. No field blanks are required for TPH analysis.

7.2.2. Automated Composite Sample Collection

Flow-weighted composite sampling will follow Ecology's SOP for *Automatic Sampling for Stormwater Monitoring* (Ecology 2024d).

To facilitate the collection of flow-weighted composite samples, Isco 6712 automated samplers will be installed at eight of the 16 monitoring locations (Table 7). Automated samplers will be housed in a secure

enclosure and will be battery powered. The dataloggers will be programmed to record hydrological measurements and to trigger the automated samplers at predefined pacing intervals.

The Campbell Scientific CR350 datalogger will be programmed to trigger the autosampler and pace sample collection off the measured flows at each monitoring location. Once triggered, the automated sampler will collect a 200-milliliter (mL) aliquot for compositing into a 20-liter glass bottle, or 12 separate 1-liter glass bottles (City of Tacoma locations only). Samplers will be programmed to begin sample collection once water level at the sensor has reached a preset threshold. The initial sample will be collected immediately. The autosampler will continuously collect additional aliquots based off of the pacing volume until the end of storm criteria has been met.

Poly(tetrafluoroethylene) (PTFE)-lined sample tubing will be routed via conduit from the automated samplers in the enclosure to the monitoring locations. Care will be taken to ensure the tubing is installed with a constant linear grade, so that water completely drains through the sample tube during rinse, purge, and sampling cycles. The sampler intakes will be carefully positioned at each location to also ensure the homogeneity and representativeness of the samples. Specifically, sampler intakes will be installed to ensure that an adequate depth will be available for sampling. This will also help to avoid the capture of litter, debris, and bed load that may be present.

Weather forecasts will be monitored via the Internet, and a determination will be made as to whether to target an approaching storm. Before each targeted storm event, field staff will conduct site visits to set up the automated samplers at all monitoring locations. During these pre-storm site visits, field staff will perform the following activities:

- Remove any blockages in the rain gauge and velocity sensors (if present on site).
- Calibrate the pressure transducers or area-velocity sensors, as needed.
- Backflush the sample lines with deionized water.
- Check the state of the desiccant associated with the equipment.
- Place a clean sample bottle in the samplers.
- Pack ice around the sample bottles within each sampler.

  Note: Ice is estimated to keep the interior of the samplers cool for 48 hours; therefore, ice will be added to the samplers not more than 24 hours before a targeted storm event.

Sample pacing for the automated samplers will be set based on the size of the forecasted storm event. The samplers will be configured such that between 30 and 70 aliquots are collected across the entire hydrograph.

When the first aliquot is collected during a targeted storm event, the datalogger will send an alarm via text message to alert field personnel that the sampling routine has begun.

Flow-weighted composite sampling criteria will be assessed, before post-storm sample retrieval, by accessing sampling data with a remote cellular link. If sampling criteria are not met, the samples will be retrieved before the next storm event. If sampling criteria are met, field personnel will return to the site, make visual and operational checks of the system, and collect detailed field notes using standardized field forms. Field personnel will then remove the 20-liter glass bottles from each automated sampler and transport them on ice to the laboratory within the allowable limits for sample holding times (see Table 9). For quality assurance purposes, additional samples will also be collected through the course of the performance verification (e.g., 14 field duplicates and 24 equipment rinsate blanks).

In general, the laboratory will be given prior notice of a pending sampling event to ensure that adequate laboratory staff will be available to process the incoming samples. This will include subsampling from the 20-liter composite bottles to fill sample bottles for the required analyses (Table 9).

7.2.3. SSPM Sample Collection

SSPM samples will be collected following Ecology's SOP for *Collection of Stormwater Solids Using In-Line Traps* (Ecology 2024e). The trap type that will be used is a "Norton" style sediment trap. Three traps will be used at each SSPM monitoring location. The Norton trap utilizes a bottle to collect SSPM, which is typically polyethylene or Teflon. For this study, however, glass bottles will be used to minimize potential contamination for analysis of PFAS and microplastics/TWP.

The traps will be secured at each site by either attaching to mounted bracket, anchored directly into concrete, or by attaching to rebar that is driven into open channel beds. When possible, the sediment traps will be installed such that stormwater runoff overtops the collection bottles during rain events. For maintenance holes without a sump, the sediment traps will be installed in the structure at the lowest practical elevation where runoff will occur. The general configuration of the installed sediment traps is shown in the monitoring location design figures in Section 6.2.1, and the exact installation locations will be determined in the field. Field staff will check that the traps are functioning properly and for damage during water sampling activities.

SSPM samples will be retrieved annually, each year of the study. The bottles will be taken to the laboratory to homogenize the samples. The sample material will be removed from the bottles by pouring into a clean stainless-steel bowl. Sample material will be homogenized, during which coarse organic material will be manually removed. The sample and water will then rest in the bowl for 1 hour to settle the solids. Then, the excess water will be decanted by being poured out slowly from the bowl. The decanted water will be filtered for microplastics/TWP. The sample material will then be placed into appropriate sample jars.

Decontamination of equipment and processing of samples will follow requirements for sampling CECs, as described in Section 7.4 and in the SOPs in Appendix C.

7.3. Containers, Preservation, and Holding Times

Table 9 and Table 10 summarize the required containers, preservation methods, and holding times for each matrix and analyte. Sample containers will be provided by the laboratory and prepared with any required chemical preservatives. Samples will be placed on ice immediately after collection and remain on ice during transportation to the laboratory.

Table 9. Sample Containers, Preservation, and Holding Times for Water Quality Parameters.						
Parameter	**Matrix**	**Minimum Quantity Required**	**Field Container**	**Laboratory Container**	**Sample Preservation**	**Maximum Holding Time**
Total Suspended Solids	Water	1 L	20-L Glass jug[a]	1-L HDPE bottle	Cool ≤6°C	7 days
Zinc, Total	Water	100 mL		250-mL HDPE bottle	Cool ≤6°C, HNO3 to pH <2	180 days
Copper, Total	Water					
Zinc, Dissolved	Water	100 mL		125-mL HDPE bottle	Cool ≤6°C, HNO3 to pH <2	Filter ≤24 hours[b,c], 180 days
Copper, Dissolved	Water					
Orthophosphate	Water	75 mL		125-mL WM HDPE Amber bottle	Cool ≤6°C	Filter ≤24 hours[b,c], 48 hours
Total Phosphorus	Water	325 mL		500-mL BR Amber Glass bottle	Cool ≤6°C, H2SO4 to pH <2	28 days
Total Nitrogen[c]	Water					
Nitrate+Nitrite	Water					
NWTPH-Dx	Water	1 L	2x 1-L BR Amber Glass bottle	2x 1-L BR Amber Glass bottle	Cool ≤6°C, HCl to pH <2	7 days extraction; 40 days analytical
6PPDQ	Water	250 mL	20-L Glass jug[a] (composite)/2x 250-mL Amber Glass bottle (grab)	2x 250-mL Amber Glass bottle	Cool ≤6°C. Minimize head space, do not freeze	14 days extraction; 28 days analytical
Microplastics/TWP	Water	20 L	20-L stainless steel jug	1-L glass jar	None	30 days extraction; 90 analytical
PFAS	Water	250 mL	2x 250-mL HDPE bottles 1x 125-mL HDPE bottle	2x 250-mL HDPE bottles 1x 125-mL HDPE bottle	Cool ≤6°C	28 days extraction; 90 days analytical

[a] Composite samples will be churn-split into subsamples at the laboratory.

[b] EPA requires filtering for dissolved metals and orthophosphate within 15 minutes of the collection of the last aliquot. This goal is difficult to meet when conducting flow-weighted sampling. A more practical proxy goal of 24 hours has been adopted for this study, both goals will be reported with the data.

[c] A 0.45-micron fiber nylon filter will be used for dissolved metals (copper and zinc) and orthophosphate filtration.

[d] Total Kjeldahl nitrogen will be calculated as Total Nitrogen – Nitrate+Nitrite.

An additional 1-liter grab sample collected from each location and, when feasible, a 1-liter split from each composite sample will be analyzed for additional parameters by HRMS.

HDPE = High-density polyethylene
HCl = Hydrochloric acid
L = liter
BR = Boston Round
HNO3 = Nitric acid
WM = Wide mouth
°C = degrees Celsius
H2SO4 = Sulfuric acid
mL = milliliter

Table 10. Sample Containers, Preservation, and Holding Times for SSPM Parameters.						
Parameter	**Matrix**	**Minimum Quantity Required[a]**	**Field Container**	**Laboratory Container**	**Sample Preservation**	**Maximum Holding Time**
Total Solids	Solid	25 g	3x 1-L glass bottle[b]	8-oz glass jar	Cool ≤6°C	7 days
Total Organic Carbon	Solid	10 g				28 days
PAHs	Solid	10 g				14 days extraction; 40 days analytical
Phthalates	Solid					
Phenolics	Solid					
Pesticide: Bifenthrin	Solid					
Herbicide: Dichlobenil	Solid					
PCBs	Solid	10 g				365 days
Grain Size	Solid	100 g		8-oz glass jar	Cool ≤6°C	180 days
Total Recoverable Metals	Solid	5 to 10 g		4-oz glass jar	Cool ≤6°C	180 days
6PPDQ	Solid	5 to 10 g		8-oz amber glass jar	Cool ≤6°C	14 days extraction; 28 days analytical 1 year extraction if frozen
Microplastics/TWP	Solid	200 to 400 g		8-oz amber glass jar	None	180 days
PFAS	Solid	5 to 10 g		250-mL WM HDPE	Cool ≤6°C, fill no more than 3/4 full	90 days

[a] Minimum quantities are dry weights

[b] Sample material from the three bottles will be homogenized at the laboratory

PAHs = Polycyclic aromatic hydrocarbons PCBs = Polychlorinated biphenyls TWP = tire wear particles
PFAS = per- and polyfluoroalkyl substances g = grams oz = ounces °C = degrees Celsius
WM = Wide mouth

7.4. Equipment Decontamination

Equipment decontamination will follow the procedures outlined in the SOPs for invasive species removal and water sampling equipment (cited in Sections 7.1 and 7.2). The following subsections outline special considerations for SSPM sampling equipment and equipment for sampling CECs.

7.4.1. SSPM

The field team will decontaminate SSPM sampling equipment prior to initial installation and in between sampling collection periods. SSPM traps and any stainless-steel materials used during sampling will be decontaminated with the following procedure, which is adapted from an SOP developed by the City of Seattle for Storm Drain Sediment Sampling (SPU 2008):

1. Phosphate free detergent wash such as Alconox® or Liquinox®, followed by tap water rinse
2. Deionized water rinse
3. Methanol rinse
4. Air dry

If equipment is not immediately being reinstalled following decontamination procedures, it will be wrapped in aluminum foil prior to deployment.

7.4.2. Microplastics/TWP

Special considerations for the equipment used for microplastics/TWP sampling are detailed in the SOPs in Appendix C and are summarized below:

- Only use scrub brushes or sponges made with natural materials.
- Use laboratory-provided, microplastics-free deionized water during all decontamination steps, or triple rinse with laboratory-provided, microplastics-free deionized water if standard deionized water was used during earlier decontamination steps. FEP squeeze bottles will be used in place of HDPE squeeze bottles, if needed in the lab.
- Decontaminated equipment may be stored in aluminum foil for a short time prior to use.

7.4.3. PFAS

Special considerations for the equipment used for PFAS sampling are detailed in the SOPs in Appendix C and are summarized below:

- Only use scrub brushes with polyethylene or PVC bristles.
- Use PFAS-free detergent, such as Alconox®, Liquinox®, or Citranox®.

- Use laboratory-provided, PFAS-free deionized water during all decontamination steps, or triple rinse with laboratory-provided, PFAS-free deionized water if standard deionized water was used during earlier decontamination steps.
- Decontaminated equipment may be stored in clean plastic bags for short periods of time prior to use.

7.5. Sample Identification

The sample identification (ID) system will include the jurisdiction, land use type, eight-digit date, composite (C) or grab (G), and sample number. The sample number starts at 1 for each unique location and date.

- TAC = City of Tacoma
- SNO = Snohomish County
- CLRK = Clark County
- PIER = Pierce County
- KING = King County
- SEA = City of Seattle

For example, the first grab sample collected at Tacoma's commercial site on March 20, 2025, would be labelled "TAC_COM_G1_20250320" and the first composite sample collected on the same date would be labelled "TAC_COM_C1_20250320". If a second grab sample was collected on this date, the ID would be "TAC_COM_G2_20250320". A second sample would only be collected on the same date if there was a sufficient break in rain before a second storm began.

Field duplicates will be labelled "DUP" and field blanks will be labelled "QA," followed by the sample type, number, and date (e.g., "QA_G1_20250320").

7.6. Chain of Custody

A chain-of-custody (COC) form will be delivered along with each sample batch to the laboratory. Example COC forms for water and sediment are included in Appendix D. The COC form will include sample IDs, date and time of collection, location, matrix, and the analyses that will be requested by the laboratory. Special instructions will be noted on the COC if required. The COC form will be signed by the person relinquishing the samples and by the person receiving the samples at the laboratory.

7.7. Field Log Requirements

A field log will be maintained for each sampling event. A field log will be presented as an appendix in any report in which it appears. An example field log is included in Appendix D. Field logs may be updated throughout the project, as necessary. Field logs will include information such as the following:

- Name and location of project
- Field personnel
- Sequence of events
- Any changes or deviations from the QAPP or SOPs
- Environmental conditions
- Date, time, location, ID, and description of each sample
- Identity of QC samples collected
- Unusual circumstances that might affect interpretation of results

7.8. Other Activities

Not applicable.

8. Laboratory Procedures

8.1. Laboratory Procedures Table

Laboratory procedures used for analyzing samples are summarized in Table 11 and Table 12.

Table 11. Laboratory Measurement Methods for Water Quality Parameters.

Parameter Group	Analyte	Sample Matrix	Detection or Reporting Limit	Sample Prep Method	Analytical Method
Conventional	Total Suspended Solids	Water	2 mg/L	NA	SM 2540 D
Metals	Copper (Total and Dissolved)	Water	0.8 ug/L	200.8	EPA 200.8
	Zinc (Total and Dissolved)	Water	5.0 ug/L	200.8	EPA 200.8
Nutrients	Total Phosphorus	Water	0.01 mg/L	SM 4500 B	SM 4500-P F
	Orthophosphate	Water	0.01 mg/L	NA	SM 4500-P F
	Total Nitrogen[a]	Water	0.10 mg/L	NA	Combustion-Chemiluminescence
	Nitrate+Nitrite	Water	0.05 mg/L	NA	EPA 353.2
Petroleum Hydrocarbons	NWTPH-Dx - Deisel	Water	0.05 mg/L	NWTPH-Dx	NWTPH-Dx
	NWTPH-Dx - Motor Oil	Water	0.1 mg/L	NWTPH-Dx	NWTPH-Dx
Emerging Contaminants	6PPDQ	Water	25 ng/L	EPA 1634 (draft) or TESL 5040[b]	EPA 1634 (draft) or TESL 5040[b]
	Microplastics/TWP	Water	1 ug/sample	Intercal v4.03	PYGCMS and Raman/FTIR[c]
	PFAS	Water	1.6 – 45.0 ng/L	EPA 1633	EPA 1633

[a] Total Kjeldahl nitrogen will be calculate as Total Nitrogen – Nitrate+Nitrite.

[b] Tacoma Environmental Services Laboratory (TESL) has submitted an accreditation request to Ecology to use TESL 5040 for 6PPDQ analysis. EPA method 1634 (draft) allows for modifications while meeting the method criteria which have been described in TESL 5040.

[c] Pyrolysis GCMS following Seeley and Lynch (2023) and More et al. (2023).

PAHs = Polycyclic aromatic hydrocarbons
PFAS = per- and polyfluoroalkyl substances
EPA = US Environmental Protection Agency
PCBs = Polychlorinated biphenyls
mg/L = milligrams per liter
SM = Standard Methods
TWP = tire wear particles
ug/L = micrograms per liter
ng/L = nanograms per liter

Table 12. Laboratory Measurement Methods for SSPM Parameters.					
Parameter Group	**Analyte**	**Sample Matrix**	**Detection or Reporting Limit**	**Sample Prep Method**	**Analytical Method**
Conventional	Total Solids	Solids	1%	NA	SM 2540G-2011
	Total Organic Carbon	Solids	200 mg/Kg	NA	EPA 9060A
	Grain Size	Solids	NA	NA	PSEP 1986 or D-422
Metals	Arsenic	Solids	0.25 mg/Kg dry	3051A	EPA 6020B
	Cadmium	Solids	0.25 mg/Kg dry	3051A	EPA 6020B
	Copper	Solids	2.5 mg/Kg dry	3051A	EPA 6020B
	Lead	Solids	0.25 mg/Kg dry	3051A	EPA 6020B
	Mercury	Solids	0.025 mg/Kg dry	7471B	EPA 7471B
	Zinc	Solids	10 mg/Kg dry	3051A	EPA 6020B
PAHs	2-Methylnaphthalene	Solids	12 ug/Kg	3546	EPA 8270E SIM
	Acenaphthylene	Solids	10 ug/Kg	3546	EPA 8270E SIM
	Acenaphthene	Solids	13.5 ug/Kg	3546	EPA 8270E SIM
	Anthracene	Solids	10 ug/Kg	3546	EPA 8270E SIM
	Benz[a]anthracene	Solids	10 ug/Kg	3546	EPA 8270E SIM
	Benz(a)pyrene	Solids	12 ug/Kg	3546	EPA 8270E SIM
	Benzo(g,h,i)perylene	Solids	10 ug/Kg	3546	EPA 8270E SIM
	Total Benzofluoranthenes	Solids	25 ug/Kg	3546	EPA 8270E SIM
	Chrysene	Solids	10 ug/Kg	3546	EPA 8270E SIM
	Dibenzo(a,h)anthracene	Solids	20 ug/Kg	3546	EPA 8270E SIM
	Fluoranthene	Solids	10 ug/Kg	3546	EPA 8270E SIM
	Fluorene	Solids	10 ug/Kg	3546	EPA 8270E SIM
	Indeno(1,2,3-cd)pyrene	Solids	15 ug/Kg	3546	EPA 8270E SIM
	Naphthalene	Solids	13.7 ug/Kg	3546	EPA 8270E SIM
	Phenanthrene	Solids	10 ug/Kg	3546	EPA 8270E SIM
	Pyrene	Solids	10 ug/Kg	3546	EPA 8270E SIM
Phthalates	Di-ethyl phthalate	Solids	35.4 ug/Kg	3546	EPA 8270E SIM
	Butyl benzyl phthalate	Solids	13.7 ug/Kg	3546	EPA 8270E SIM
	Bis(2-ethylhexyl) phthalate	Solids	51.1 ug/Kg	3546	EPA 8270E SIM
	Di-n-butyl phthalate	Solids	30.9 ug/Kg	3546	EPA 8270E SIM
	Di-n-octyl phthalate	Solids	15 ug/Kg	3546	EPA 8270E SIM
	Di-methyl phthalate	Solids	18.6 ug/Kg	3546	EPA 8270E SIM
Phenolics	Pentachlorophenol	Solids	35 ug/Kg	3546	EPA 8270E SIM
	p-Cresol	Solids	10 ug/Kg	3546	EPA 8270E SIM
	o-Cresol	Solids	10 ug/Kg	3546	EPA 8270E SIM

Table 12 (continued). Laboratory Measurement Methods for SSPM Parameters.					
Parameter Group	**Analyte**	**Sample Matrix**	**Detection or Reporting Limit**	**Sample Prep Method**	**Analytical Method**
PCBs[a]	Aroclor 1016	Solids	5 ug/Kg	3546	EPA 8270E
	Aroclor 1221	Solids	5 ug/Kg	3546	EPA 8270E
	Aroclor 1232	Solids	5 ug/Kg	3546	EPA 8270E
	Aroclor 1242	Solids	5 ug/Kg	3546	EPA 8270E
	Aroclor 1248	Solids	5 ug/Kg	3546	EPA 8270E
	Aroclor 1254	Solids	5 ug/Kg	3546	EPA 8270E
	Aroclor 1260	Solids	5 ug/Kg	3546	EPA 8270E
	Aroclor 1262	Solids	5 ug/Kg	3546	EPA 8270E
	Aroclor 1268	Solids	5 ug/Kg	3546	EPA 8270E
Pesticides	Bifenthrin	Solids	0.5 ug/Kg	NA	EPA 8270E SIM
	Dichlobenil	Solids	1 ug/Kg	NA	EPA 8270E SIM
Emerging Contaminants	6PPDQ	Solids	5 ng/g	TESL SOP 5041[b]	EPA 1634 draft or TESL 5040[c]
	Microplastics/TWP	Solids	1 ug/sample	Intercal v4.03	PYGCMS and Raman/FTIR[c]
	PFAS	Solids	0.4–20 ng/g	EPA 1633	EPA 1633

[a] Tacoma Environmental Services Laboratory (TESL) has approval from Ecology to use method 8270E for PCB analysis, as it is comparable to method 8082A.

[b] TESL has approval from Ecology to use TESL method 5041 for 6PPDQ extraction from sediment matrices.

[c] Tacoma Environmental Services Laboratory (TESL) has submitted an accreditation request to Ecology to use TESL 5040 for 6PPDQ analysis. EPA method 1634 (draft) allows for modifications while meeting the method criteria which have been described in TESL 5040.

[d] Pyrolysis GCMS following Seeley and Lynch (2023) and More et al. (2023).

PAHs = Polycyclic aromatic hydrocarbons
PFAS = per- and polyfluoroalkyl substances
EPA = US Environmental Protection Agency
PSEP = Puget Sound Estuary Program
PCBs = Polychlorinated biphenyls
mg/Kg = milligrams per kilogram
SM = Standard Methods
TWP = tire wear particles
ug/Kg = micrograms per kilogram
ng/g = nanograms per gram

8.2. Sample Preparation Methods

Preparation of samples for chemical analysis will include steps from the standard methods used for each analyte, with exceptions for CECs described in Section 8.3.

8.3. Special Method Requirements

There is no established extraction or analytical method for 6PPDQ in sediments. The City's laboratory, Tacoma Environmental Services Laboratory (TESL), has developed an SOP for extraction of 6PPDQ from sediments and received approval from Ecology. In addition, TESL also developed an SOP for 6PPDQ

extraction and analysis from water samples. This SOP is based on Draft EPA Method 1634. The two TESL SOPs are included in Appendix C.

The microplastics/TWP sample preparation procedure was developed by a consortium of plastic pollution researchers. The consortium produced a guidance document for microplastics/TWP measurement in multiple types of media, including clean water, dirty water, fish tissue, and sediments (SCCWRP 2020). The guidance is provided in Appendix C. The preparation for stormwater samples will include condensing the sample into a concentrated slurry, followed by filtering at selected particle size ranges prior to the microplastics/TWP analysis.

If required for cleanups, the laboratory will perform extract cleanups according to the applicable analytical method to lessen matrix interference. The same cleanup procedures will be performed on the associated quality control samples, including method blanks, laboratory control samples (LCS), matrix spike (MS), and matrix spike duplicate (MSD).

The same guidance document used for microplastics/TWP sample preparation will be used for microplastics/TWP analysis (SCCWRP 2020). The guidance is provided in Appendix C.

8.4. Laboratories Accredited for Methods

TESL will analyze samples for all parameters except for microplastics/TWP. TESL is accredited by Ecology for all the parameters that they are analyzing except for PCB Aroclors 1262 and 1268, PFAS and 6PPDQ for which they are seeking accreditation or waiver. If the waiver is denied, an accredited lab will be used.

Virginia Institute of Marine Sciences (VIMS) will analyze samples for microplastics/TWP. VIMS is not accredited by Ecology, as Ecology does not currently accredit for microplastics analysis.

An accreditation waiver will be submitted for the laboratories and parameters that are not accredited by Ecology.

9. Quality Control

9.1. Table of Field and Laboratory Quality Controls

Laboratory QC procedures involve the use of several types of QC samples that are outlined in Table 13 and Table 14. QC samples collected in the field will go through the same procedures as the standard samples.

Table 13. Quality Control Samples, Types, and Frequency for Water Quality Parameters.

Parameter	Sample Method	Number of Samples	Number of Field Duplicates	Equipment Rinsate/Field Blanks	Total Number of Samples	Laboratory Control Sample	Laboratory Method Blanks	Laboratory Duplicates	Laboratory Matrix Spikes
Total Suspended Solids	Automated	144	14[a]	24	182	1 per batch	1 per batch	1–2 per batch	1–2 per batch
Copper (Total and Dissolved)	Automated	144	14[a]	24	182	1 per batch	1 per batch	1–2 per batch	1–2 per batch
Zinc (Total and Dissolved)	Automated	144	14[a]	24	182	1 per batch	1 per batch	1–2 per batch	1–2 per batch
Total Phosphorus	Automated	144	14[a]	24	182	1 per batch	1 per batch	1–2 per batch	1–2 per batch
Orthophosphate	Automated	144	14[a]	24	182	1 per batch	1 per batch	1–2 per batch	1–2 per batch
Total Nitrogen[c]	Automated	144	14[a]	24	182	1 per batch	1 per batch	1–2 per batch	1–2 per batch
Nitrate+Nitrite	Automated	144	14[a]	24	182	1 per batch	1 per batch	1–2 per batch	1–2 per batch
NWTPH-Dx	Grab	144	14	0	158	1 per batch	1 per batch	1–2 per batch	1–2 per batch
6PPDQ	Grab	288	29	16	333	1 per batch	1 per batch	1–2 per batch[b]	1–2 per batch[b]
	Automated	144	14[a]	24	182				
Microplastics/TWP	Grab	64	6	4	74	1 per batch	1 per batch	1–2 per batch	NA
PFAS	Grab	288	29	16	333	1 per batch	1 per batch	1–2 per batch[b]	1–2 per batch[b]

[a] Field duplicates for composite samples will be churn-split from the original sample at the laboratory.

[b] Laboratory duplicates and matrix spikes for 6PPDQ and PFAS will only be analyzed upon request.

[c] Total Kjeldahl nitrogen will be calculate as Total Nitrogen – Nitrate+Nitrite.

PAHs = Polycyclic aromatic hydrocarbons PCBs = Polychlorinated biphenyls TWP = tire wear particles PFAS = per- and polyfluoroalkyl substances NWTPH-Dx = Northwest Total Petroleum Hydrocarbon Semivolatile

Table 14. Quality Control Samples, Types, and Frequency for SSPM Parameters.

Parameter	Sample Method	Number of Samples	Number of Field Duplicates	Equipment Rinsate/Field Blanks	Total Number of Samples	Laboratory Control Sample	Laboratory Method Blanks	Laboratory Duplicates	Laboratory Matrix Spikes
Total Solids	Grab	32	4	0	36	NA	NA	1–2 per batch	NA
Total Organic Carbon	Grab	32	4	0	36	1 per batch	1 per batch	1–2 per batch	1–2 per batch
Grain Size	Grab	32	4	0	36	1 per batch	1 per batch	1–2 per batch	1–2 per batch
Total Recoverable Metals	Grab	32	4	0	36	1 per batch	1 per batch	1–2 per batch	1–2 per batch
PAHs	Grab	32	4	0	36	1 per batch	1 per batch	1–2 per batch	1–2 per batch
Phthalates	Grab	32	4	0	36	1 per batch	1 per batch	1–2 per batch	1–2 per batch
Phenolics	Grab	32	4	0	36	1 per batch	1 per batch	1–2 per batch	1–2 per batch
PCBs	Grab	32	4	0	36	1 per batch	1 per batch	1–2 per batch	1–2 per batch
Herbicides	Grab	32	4	0	36	1 per batch	1 per batch	1–2 per batch	1–2 per batch
Pesticides	Grab	32	4	0	36	1 per batch	1 per batch	1–2 per batch	1–2 per batch
6PPDQ	Grab	32	4	0	36	1 per batch	1 per batch	1–2 per batch[a]	1–2 per batch[a]
Microplastics/TWP	Grab	32	4	0	36	1 per batch	1 per batch	1–2 per batch	NA
PFAS	Grab	32	4	0	36	1 per batch	1 per batch	1–2 per batch[a]	1–2 per batch[a]

[a] Laboratory duplicates and matrix spikes for 6PPDQ and PFAS will only be analyzed upon request.

PAHs = Polycyclic aromatic hydrocarbons PCBs = Polychlorinated biphenyls TWP = tire wear particles PFAS = per- and polyfluoroalkyl substances NWTPH-Dx = Northwest Total Petroleum Hydrocarbon Semivolatile

9.2. Corrective Action Processes

Should results become inconsistent with what is outlined in the QAPP, corrective action will be taken to ensure that proper sampling procedures are being followed. These actions may include the following:

- Collecting new samples using the method described in the approved QAPP
- Reanalyzing laboratory samples that do not meet QC criteria (analytical methods often state what to do when QC criteria are not met)
- Convening project personnel and technical experts (e.g., the project TAC) to decide on the next steps that need to be taken to improve model performance

10. Data Management Procedures

10.1. Data Recording and Reporting Requirements

Data from the telemetered dataloggers will be remotely transferred on a daily basis. The continuous data from each monitoring location will be imported into a database (Aquarius™ Data Management software) for subsequent analysis and archiving purposes. These data will be immediately checked for evidence of any equipment malfunction or other operational problem. Gaps in the continuous data may need to be interpolated; if this occurs, data will be stored and presented in a manner that makes it clear which data are from measurement, and which have been interpolated. The database will be used to produce event-based hydrologic summary statistics (e.g., location runoff volume, storm precipitation total, and storm duration) for each applicable location. These summary statistics will ultimately be stored in a database with other water quality data collected through the project (see description below).

With the exception of the HRMS data, analytical laboratory data will be stored and managed in ESdat, the consultant's online chemistry database. ESdat serves as a central repository for laboratory data related to soil, groundwater, surface water, stormwater, gas, and other environmental matrices. ESdat integrates with laboratory partners by providing an email address to which they send electronic data deliverables (EDDs).

Once an EDD is in ESdat, the data undergo an initial review and then a data verification/validation review. For the initial review, the data manager will check high-level, basic information related to the EDD before accepting the EDD. This will include checking the sample names and laboratory methods against the chain-of-custody form. Once data are accepted and the EDD is uploaded into the ESdat database, the results are available with an "unapproved" status. Within 2 weeks, Herrera staff will perform a more detailed check of the data to confirm that the laboratory's analyses conform to the MQOs. If the laboratory failed to meet the MQOs, corrective steps will be identified and the project data manager will work with the laboratory to either re-analyze the samples, flag the data appropriately, or both. At the conclusion of this step, the data are "approved" for use in ESdat.

Based on the data review, values associated with minor quality control problems will be considered estimates and assigned *J* qualifiers. Values associated with major quality control problems will be rejected and qualified *R*. Estimated values may be used for evaluation purposes, whereas rejected values will not be used. Qualifiers and usage are defined in Table 15.

The HRMS data are in the form of spectrographic charts and tables and thus cannot be stored in ESdat. Instead, these data will be temporarily stored on the project SharePoint site for the duration of the project and then transferred to the project site on the SAM webpage at the conclusion of the study.

Table 15. Data Qualifier Definitions and Usage Criteria.		
Data Qualifier	**Definition**	**Criteria for Use**
J	Value is an estimate based on analytical results.	MQOs for field duplicates, laboratory duplicates, matrix spikes, laboratory control samples, holding times, or blanks have not been met.
R	Value is rejected based on analytical results.	Major quality control problems with the analytical results.
U	Value is below the detection and/or reporting limit.	Based on laboratory method detection and reporting limits.
UJ	Value is below the reporting limit and is an estimate based on analytical results	Based on laboratory method reporting limit; MQOs for analytical results have not been met.

10.2. Laboratory Data Package Requirements

The Ecology-certified analytical laboratories will generate data packages analogous to EPA Level II documentation during this investigation. This level of documentation is generally considered legally defensible and consists of the following:

- Holding times
- Laboratory method blank data
- Sample data
- Matrix/surrogate spike data
- Duplicate sample data

Completed, final data reports will be provided as a PDF.

10.3. Electronic Transfer Requirements

All laboratory results, including QC sample results, will also be provided as an EDD in Excel or comma-separated value (CSV) format.

10.4. Data Upload Procedures

All data collected for this project will be uploaded to Herrera's Cloud database through Aquarius (continuous flow data) and ESdat (chemistry results). The data will be made publicly available through communications dashboards.

All data compiled for this project will be uploaded to the Washington State Open Data Portal (data.wa.gov) at the conclusion of the project. HRMS data will be uploaded to the SAM project webpage.

10.5. Model Information Management

There is no modeling associated with this project.

11. Audits and Reports

The following section describes the procedures used to ensure that this QAPP is implemented correctly, that the data generated is of sufficient quality to meet the project objectives, and that corrective actions, if necessary, are implemented in a timely manner. The procedures include revisions, audits and response actions, corrective actions, and data quality assurance reporting.

If significant changes to this monitoring plan are required prior to the completion of the study, a revised version of the document (with changes tracked) or an addendum describing the changes will be prepared and submitted to the City of Tacoma and Ecology for review. Significant changes include monitoring station relocation, laboratory analysis method changes, but not minor equipment changes. The approved version of the monitoring plan will remain in effect until the revised version has been approved. Justifications, summaries, and details of expedited changes to the monitoring plan will be documented in the monitoring report.

11.1. Audits

For this project, one audit is planned to occur after the first wet season of monitoring and prior to the second wet season. The audit will include elements to determine if the technical methods are consistent with this QAPP and to help assess quality. The field audit will include verification of field sampling methods, including preparation, collection, and in-field processing of samples. The data management audit will include verifying the procedures for recording data in the field, uploading data to Aquarius and ESdat, and preparing and uploading data to the Data.wa.gov.

A laboratory audit is not planned for this project. The Ecology-accredited laboratories being used for this project undergo audits from Ecology's Laboratory Accreditation Unit (LAU) every 3 years.

11.2. Responsible Personnel

The Herrera project manager will conduct the field and data audits in cooperation with the City project manager and laboratory manager. Ecology's LAU is responsible for regular audits of accredited laboratories.

11.3. Frequency and Distribution of Reports

A final project report will be prepared at the end of the study. In addition, Herrera provides monthly progress reports to the City, and the City provides semi-annual project reports to Ecology.

11.4. Responsibility for Reports

The final report for the project will be authored primarily by Herrera, with contributions from the City and laboratories.

12. Data Verification

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 for conformance to project requirements. This data will then be validated against the data quality objectives, which are listed in Section 5. Only those data that are supported by appropriate quality control data and meet the measurement performance specification defined for this project will be considered acceptable and used in the project.

12.1. Field Data Verification, Requirements, and Responsibilities

Field data will be hand-digitized from notes, as necessary. Data will then be peer reviewed for both accuracy and reasonableness. Review for reasonableness will include identifying any data that are noticeably different from nearby samples or previous samples at the same location. Any questionable data points will be relayed to the project manager, who will discuss the questionable data with field staff. A decision will then be made on whether to keep, flag, or discard the data in question. The project manager or a designated staff member will periodically (i.e., at minimum once per quarter) review field data for completeness and legibility.

Roles and responsibilities are as follows:

- The Field Sampler is responsible for ensuring that field data are properly reviewed and verified for integrity.
- The Data Manager is responsible for entering the data in the project database.
- The Project Manager is responsible for ensuring that all data are properly reviewed, verified, and submitted in the required format to the project database. The Project Manager is responsible for validating the data, and with the concurrence of the City of Tacoma Project Manager, is responsible for ensuring that all data to be reported meet the objectives of the project and are suitable for reporting.

12.2. Laboratory Data Verification

Analytical laboratories will perform internal data verification before releasing data to the project manager. The lab will report to the project manager if holding times are exceeded or if preservation temperatures exceed method requirements. In these cases, the project manager will decide whether samples should be analyzed. If the samples are analyzed, a data flag will be applied.

The roles and responsibilities are as follows:

- The **Laboratory Manager** is responsible for ensuring that laboratory data are scientifically valid, defensible, of acceptable precision and accuracy, and reviewed for integrity.
- The **Data Manager** is responsible for entering the data in the project database.
- The **Project Manager** is responsible for ensuring that all data are properly reviewed, verified, and submitted in the required format to the project database. The Project Manager is responsible for ensuring that all data to be reported meet the objectives of the project and are suitable for reporting.

Data will be reviewed and audited within 14 business days of receiving the results from the field or laboratory. This review will be performed to ensure that all data are consistent, correct, and complete, and that all required quality control information has been provided. Specific quality control elements for the data will also be examined to determine if the MQOs for the project have been met. Values associated with minor quality control problems will be considered estimates and assigned *J* qualifiers. Values associated with major quality control problems will be rejected and qualified *R*. Estimated values may be used for evaluation purposes, while rejected values will not be used. The following sections describe in detail the data validation procedures for these quality control elements:

- Completeness
- Methodology
- Holding times
- Blanks
- Reporting limits
- Duplicates
- Matrix spikes and matrix spike duplicates
- Calibration and control standards
- Sample representativeness

12.2.1. Completeness

Completeness will be assessed by comparing valid sample data with this QAPP and the chain-of-custody records. Completeness will be calculated by dividing the number of valid values by the total number of values. If less than 95 percent of the samples submitted to the laboratory are judged to be valid, then more samples will be collected until at least 95 percent are judged to be valid. If less than 95 percent of the collected flow data is complete, additional monitoring will be implemented until 95 percent of the flow record has been collected.

12.2.2. Methodology

Methodologies for analytical procedures will follow methods specified in Table 11 and Table 12. Field procedures will follow the methodologies described in this quality assurance project plan. Any deviations from these methodologies must be approved by Ecology and documented in an addendum to this QAPP. The database will include a field for identifying analytical method. Deviations that are deemed unacceptable will result in rejected values (*R*) and will be corrected for future analyses.

12.2.3. Holding Times

Holding times for each analytical parameter in this study are summarized in Table 9 and Table 10. Holding time compliance will be assessed by comparing sample collection dates and times to filtration (pre-filtration) and analytical dates and times (post-filtration or total). Automated sampler sample collection times will be based on the date and time that the last aliquot was collected, but date and time of start of sampling will be recorded as well.

12.2.3.1. Pre-Filtration Holding Times

Samples requiring filtration should be filtered within 24 hours of collection of the last aliquot. The EPA requires that dissolved metals, nitrate+nitrite, and orthophosphate be filtered within 15 minutes of the collection of the last aliquot. Meeting this holding time goal would be exceedingly difficult for this project, given that the time of last aliquot collection is unknown when samples are collected on a flow-weighted basis. Consequently, a proxy holding time of 24 hours will be used for this study. Dissolved metals and orthophosphate samples exceeding the 24-hour limit will be flagged with a *J* or *R*, based on the quality assurance officer's judgement. Exceeding the pre-filtration holding time will result in the sample being flagged as an estimate (*J*), unless the pre-filtration holding time is exceeded by more than two times, in which case the result will be rejected (*R*).

12.2.3.2. Post-Filtration or Total Holding Times

- For analytes with holding times greater than 7 days:

  Data from samples that exceed the specified maximum post-filtration holding times by less than 48 hours will be considered estimates (*J*). Data from samples that exceed the maximum post-filtration holding times by more than 48 hours will be rejected values (*R*).

- For analytes with holding equal to or less than 7 days:

  Data from samples that exceed the specified maximum post-filtration holding times by less than 24 hours will be considered estimates (*J*). Data from samples that exceed the maximum post-filtration holding times by more than 24 hours will be rejected values (*R*).

12.2.4. Method Blanks

Method blank values will be compared to the MQOs that have been identified for this project. If an analyte is detected in a method blank at or below the reporting limit, no action will be taken. If blank concentrations are greater than the reporting limit, the associated concentration value will be labeled with a *U* (in essence increasing the reporting limit for the affected samples), and associated project samples within five times the *de facto* reporting limit will be flagged with a *J*. In each of these cases, the *de facto* reporting limit for that analyte will be recorded along with the concentration value, equipment will be decontaminated, and samples will be rerun if possible.

12.2.5. Rinsate and Field Blanks

Rinsate blank values will be compared to the MQOs that have been identified for this project. If analytes are detected in the rinsate blanks at concentrations that exceed two times the reporting limit, then associated sample tubing will be cleaned or replaced and associated samples collected since the previous rinsate blank that are within five times the new reporting limit will be flagged with a *J*. At the monitoring locations where corrective actions (e.g., replacement or cleaning of sample tubing) were taken, a follow-up rinsate blank will be collected and analyzed for any parameters exceeding two times the reporting limit in the midpoint rinsate blank.

12.2.6. Reporting Limits

For each sample, the concentration value result and the reporting limit will be presented in each laboratory report. If the reporting limits are not met by the laboratory, the laboratory will be requested to reanalyze the samples or revise the method, if time permits.

12.2.7. Duplicates

Duplicate samples concentration values will be recorded in the data tables. Results exceeding the MQOs for duplicate samples will be flagged as estimates (*J*) or if severely exceeded (e.g., more than twice the objective), then associated values will be rejected (*R*).

12.2.8. Matrix Spikes

Matrix spike results exceeding the MQOs for this project will be recorded in the data tables and associated values will be flagged as estimates (*J*). However, if the percent recovery exceeds the MQOs and a value is less than the reporting limit, the result will not be flagged as an estimate. If the percent recovery of a matrix spike is less than 30 percent, the associated values that are below the detection limit (undetected) values will be rejected (*R*).

12.2.9. Control Standards

Control standard results exceeding the MQOs for this project will be flagged as estimates (*J*). If the objectives are severely exceeded (e.g., more than twice the objective), then associated values will be rejected (*R*).

12.2.10. Sample Representativeness

In general, samples collected in this study will not be submitted for laboratory analysis unless they have met the storm and sample representativeness MQOs. In all cases, the data collected for this study will be labeled with quality assurance flags for both laboratory and field data quality issues related to representativeness.

12.3. Validation Requirements

Not applicable.

12.4. Model Quality Assessment

There is no modelling associated with this project.

13. Data Quality (Usability) Assessment

13.1. Process for Determining Project Objectives Were Met

The process for determining that project objectives have been met will involve several project staff, the ESdat system, and the outcomes of the QA activities (as described above), including audits, data verification, and data validation.

Documentation by the field team will help confirm the condition and usability of the samples. The ESdat system will help by identifying potential data outliers and changes in patterns, such as variable sample volumes and incompleteness of data records. The project manager will review the overall verified and validated data in context of the project objectives, data quality objectives, and measurement quality objectives. Meeting objectives related to field and laboratory procedures will be assessed via the audit.

In particular, the microplastics and 6PPDQ data are novel and this project team will describe the findings with respect to these data carefully.

If any objectives have not been met (e.g., the percent RPD for sample replicates exceeds the MQO), the project manager—together with the field lead and/or the data validator—will decide how to qualify the data and whether or not it can be used in the technical analysis. Depending on how such data are qualified, they may still be considered to have met objectives and to be useable for the data analysis.

After data verification is complete, the project manager or designee will compare the overall data package to the MQOs as specified in Section 5. Data may be rejected for the following reasons:

- It is determined that significant contamination may be present in a sample.
- A sample was taken from an incorrect location.
- A sample was insufficiently preserved, based on pH, or had an exceedance of temperature upon submittal.
- Incompatible equipment, such as incorrect bottle type, was used.
- A sample's hold time was exceeded and is believed to severely impact the results.
- Field duplicate or lab duplicate samples exceed their RPD specified in Table 6 by more than a factor of two.

The reason for any rejected data will be documented. After any rejected data is removed from the data set, data completeness and representativeness will be evaluated. If data completeness goals have not been met, additional measurements may be taken or the lab may be asked to reanalyze samples as possible and as necessary.

13.2. Treatment of Non-Detects

The analytical laboratory will be required to report estimated values for any detections between the MDL and the MRL. Appropriate data qualifiers will be used by the laboratories to indicate if a test resulted in an estimate (*J*) or not detected (*U*). For general summary statistics, the Kaplan-Meier approach will be used when the dataset contains non-detected values. Kaplan-Meier is a nonparametric method for determining the values of non-detect data based on the structure of the detected values. It is used in the calculation of summary statistics and is not a substitution method.

13.3. Data Analysis and Presentation Methods

Staff will use *R* for data analysis and tabulating, graphing, and calculating basic statistics (e.g., minimum, mean, and maximum concentrations). The data analysis will be supplemented with additional multi-variate statistical analyses to compare concentrations across sites. Statistical analysis will include testing for correlations and trends of the chemistry data associated with land use, road density, traffic, basin size, and other GIS-based variables. Statistical analysis may also involve t-tests, ANOVA, and/or non-parametric tests, such as a Siegel-Tukey test for comparing variance among populations (e.g., different basins). The choice of statistics will partly depend on the completeness and quality of the final data set.

Data from the SSPM traps and from the automated samplers, with the exception of the CEC data, will be compared to the data from the 2007 S8.D study. First, a Mann-Whitney U-test will be used to determine if concentrations of pollutants have significantly decreased or increased since the 2007 S8.D study. Boxplots of pollutant concentrations will also be presented. Changes in imperviousness, level of development, and tree cover will be assessed by comparing aerial images from 2012 and 2024. Specifically, Ecopia AI image recognition software will be used to extract development features to compare over time, generating metrics of basin change that will be assessed in combination with the chemistry data. Difference metrics will be calculated for land cover (e.g., increase or decrease in imperviousness) and for water quality (e.g., increase or decrease in dissolved zinc). The data will then be normalized and stepwise linear regression (or a non-parametric analog) will be used to model changes in water quality based on the changes in land use.

The specifics of the GIS analysis methods employed in this study to delineate basins and classify land cover will be presented in a separate deliverable accompanying the GIS data.

To provide context for the results, the CEC data (6PPDQ, Microplastics/TWP, and PFAS) will be compared with values gathered from a literature review of these CECs in stormwater and receiving water.

The presentation of data will be via a project report prepared at the end of the study, three public presentations, and a publicly available project data dashboard.

13.4. Sampling Design Evaluation

The sampling design will be evaluated by assessing the completeness of the monitored data and its suitability for the desired data analysis as explained above. A limited data analysis will be done after the first year of monitoring to identify any sampling design issues and adaptively adjust if needed for the second year of monitoring.

13.5. Documentation of Assessment

The data quality assessment will be documented in the outcomes of the audit, data verification, and data validation. Data flagging and qualification will be tracked in ESdat, and the usability of qualified data will be documented in the project report.

14. References

Ecology. 2015. Western Washington NPDES Phase I Stormwater Permit. Final S8.D Data Characterization 2009-2013. Publication No. 15-03-001. Washington State Department of Ecology, Olympia, Washington. February.

Ecology. 2024a. Municipal Stormwater Permit Outfall Data. Last update May 13, 2024. Washington State Department of Ecology, Olympia, Washington. <https://data.wa.gov/Natural-Resources-Environment/Municipal-Stormwater-Permit-Outfall-Data/d958-q2ci/about_data>.

Ecology. 2024b. Standard Operating Procedure EAP070, Version 2.4, Minimize the Spread of Invasive Species. Publication No. 24-03-204. Washington State Department of Ecology, Olympia, Washington. September.

Ecology. 2024c. Standard Operating Procedure for Collecting Grab Samples from Stormwater Discharge v 1.1. Publication No. 18-10-023. Washington State Department of Ecology, Olympia, Washington. Originally published September 2009; revised March 2024.

Ecology. 2024d. Standard Operating Procedures for Automatic Sampling for Stormwater Monitoring v 1.2. Publication No. 18-10-024.Washington State Department of Ecology, Olympia, Washington. Originally published September 2009; revised March 2024.

Ecology. 2024e. Standard Operating Procedure for Collection of Stormwater Solids Using In-Line Traps v 2.0. Publication No. 18-10-025.Washington State Department of Ecology, Olympia, Washington. Originally published September 2009; revised March 2024.

King County. 2024. Water Quality Benefits Evaluation (WQBE) Toolkit. King County Wastewater Treatment Division. Accessed December 22, 2024. <https://kingcounty.gov/en/dept/dnrp/about-king-county/about-dnrp/sustainability-commitments/clean-water-healthy-habitat/water-quality-benefits-evaluation>.

More, S. L., Miller, J. V., Thornton, S. A., Chan, K., Barber, T. R., & Unice, K. M. 2023. Refinement of a microfurnace pyrolysis-GC-MS method for quantification of tire and road wear particles (TRWP) in sediment and solid matrices. The Science of the Total Environment, 874, 162305.

Native Land. 2024. Native Land Digital. Accessed October 24, 2024. <https://native-land.ca/>.

Saifur and Gardner. 2021. Loading, transport, and treatment of emerging chemical and biological contaminants of concern in stormwater. Saifur, S., & Gardner, C.M. *Water Science and Technology* 83.12 (2021): 2863-2885.

Shafi, M., Lodh, A., Khajuria, M., Ranjan, V. P., Gani, K. M., Chowdhury, S., & Goel, S. 2024. Are we underestimating stormwater? Stormwater as a significant source of microplastics in surface waters. *Journal of Hazardous Materials*, 133445.

SCCWRP. 2020. SCCWRP Microplastic Measurement Methods Evaluation Study. Version 4.03. Southern California Coastal Water Research Project, Costa Mesa, CA. November 10.

Seeley, M. E., & Lynch, J. M. 2023. Previous successes and untapped potential of pyrolysis-GC/MS for the analysis of plastic pollution. Analytical and Bioanalytical Chemistry, 415(15), 2873–2890.

SPU. 2008. Inline Sediment Traps. Water and Sediment Quality Standard Operating Procedure C3100. Revision R0D2. Seattle Public Utilities, Seattle, Washington.

SPU. 2015. Volume 3: Integrated Plan. Seattle Public Utilities, Seattle, Washington.

Tacoma. 2020. 2020 Thea Foss and Wheeler-Osgood Waterways Stormwater Monitoring Quality Assurance Project Plan. City of Tacoma. August 2020.

Tacoma. 2023. Thea Foss and Wheeler-Osgood Waterways 2022 Source Control and Water Year 2022 Stormwater Monitoring Report. City of Tacoma, Washington. March.

Tian, Z., Zhao, H., Peter, K. T., Gonzalez, M., Wetzel, J., Wu, C., Hu, X., Prat, J., Mudrock, E., Hettinger, R., Cortina, A. E., Biswas, R. G., Kock, F. V. C., Soong, R., Jenne, A., Du, B., Hou, F., He, H., Lundeen, R., Kolodziej, E. P. 2021. A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon. Science, 371(6525), 185–189.

TNC. 2024. Stormwater Heatmap. The Nature Conservancy. Accessed December 22, 2024. <https://www.washingtonnature.org/stormwaterheatmap>.

Werbowski, L.M., Gilbreath, A.N., Munno, K., Zhu, X., Grbic, J., Wu, T., Sutton, R., Sedlak, M.D., Deshpande, A.D., Rochman, C.M.. 2021. Urban stormwater runoff: a major pathway for anthropogenic particles, black rubbery fragments, and other types of microplastics to urban receiving waters. *ACS ES&T Water*, *1*(6), 1420-1428.

Appendix A

Quality Assurance Project Plans for Ancillary Laboratory Analyses

HERRERA
Science + Planning + Design

Appendix A

QAPPs for Ancillary Laboratory Analyses

HERRERA
Science + Planning + Design

Appendix A High-Resolution Mass Spectrometry (HRMS) Analysis

1. Overview

For analysis of suspected, unknown, or unmonitored organic contaminants, and to allow future retrospective analysis of sample extract compositions collected as part of this study, we will utilize HRMS instrumentation based upon liquid chromatography quadrupole time-of-flight (LC-QTOF-MS/MS) mass spectrometry. HRMS analysis is an analytical approach that can chemically characterize SPE and SPMM extracts using non-target and suspect screening approaches. Using standard and general sample processing and chromatography approaches, HRMS techniques essentially try and collect data for "all" ionizable and abundant chemical features (exact mass-retention time pairs) present within sample extracts. This process converts chemical data to electronic data, for subsequent screening, identification, and characterization efforts performed on the electronic data files. Typically, hundreds to over 10,000 chemical features are present within samples, the vast majority representing unknown natural or anthropogenic organic compounds. Immediate analysis of suspected and unknown compounds can be performed soon after sample collection and analysis to help understand chemical compositions and source relationships, but the HRMS data is often very amenable to retrospective analysis even long after initial sample analysis. For example, once we used HRMS techniques to identify 6PPDQ as the primary causal toxicant for coho mortality events in 2020 (Tian et al 2020), we were able to go back into HRMS data files from 2016-2020 to identify and semi-quantify 6PPDQ in prior environmental samples.

The development of HRMS techniques for the analysis of water samples took place over that last 2 decades (Schymanski et al. 2014a, Schymanski et al. 2014b, Gago-Ferrero et al. 2015, Hernández et al. 2015). Many recent studies from our lab group have used HRMS analysis as either a primary or complementary analytical tool to understand flows, sources, and abundance of anthropogenic organic contaminants, especially those present in stormwater and roadway runoff (Du et al. 2017, Peter et al 2018, Peter et al. 2019, Du et al 2020, Tian et al 2020, Kumar et al. 2021, Peter et al 2022a, Peter et al. 2022b, Zhao et al 2023, Hu et al 2023). Notably, our current suspect screening lists for HRMS data analysis include >500 fish toxicants, >300 roadway, tire, or PPD antioxidant related contaminants, plus many other pesticides, surfactants, pharmaceuticals, personal care products, and industrial chemicals. Identifications and molecular networking analysis within these data can be used to identify and semi-quantify detected compounds to define and understand relationships between contaminants as well as fate and transport processes. Aggregation of detected features into source and sample specific chemical fingerprints can help to identify and apportion detected contaminants to potential sources, in some cases.

The above HRMS techniques and analysis will be used for exploratory and research purposes to help define contaminant presence and potential land use relationships within the data. The HRMS data will also be used to help understand reaction and transformation processes that may apply to co-detected monitored analytes such as 6PPDQ and other analytes.

1.1 Parameters of interest and potential sources

The anthropogenic compounds present in suspect screening lists may come from sources such as household chemical use, vehicles, agricultural operation, and roadways.

1.2 Regulatory criteria or standards

There are currently no regulatory criteria or standards for the suspect list that will be the focus of this work.

1.3 Water quality impairment studies

There are no water quality impairment studies for the suspect list that are the focus of this study.

1.4 Special training and certifications

No special training is needed. Project staff have experience analyzing environmental samples for various pollutants, including contaminants of emerging concern. The research scientists have the necessary skills in environmental analytical chemistry, especially non-target screening for pollutants and HRMS applications. All laboratory personnel have completed required laboratory training courses as administered by the University of Washington Environmental Health and Safety http://www.ehs.washington.edu/training/.

2 Quality Objectives

2.1 Data quality objectives

The main DQOs for this project are to analyze sample extracts from all locations, totaling 288 samples from 16 locations, excepting any samples that are lost or unsampled. These samples will be analyzed using existing HRMS methods to obtain contaminant compositions and identifications that meet the Measurement Quality Objectives (MQOs) described below.

2.2 Measurement quality objectives

The measurement quality objectives (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 quality of the laboratory analyses are replicates, blanks, and spikes. While consensus protocols for assessment of non-target HRMS QTOF data have not yet been formally established, the 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 CUW Laboratory Standard Operating Procedures (SOPs) for sample collection, sample processing, sample analysis, data handling,

data analysis, and data management. A list of these SOPs are provided in section 5.1; copies are available upon request.

Data collected will be analyzed relative to the following indicators, many of which are defined per the US EPA Environmental Sampling and Analytical Methods (ESAM) Program Glossary (https://www.epa.gov/esam/glossary). Relevant numerical criteria are provided in Table 1.

2.3 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 1.

Table 1. Measurement quality objectives for high resolution mass spectrometry analysis

MQO	Precision Target	Bias Target	Sensitivity Target
Instrument Tune Agilent 6546 QTOF	< 2 ppm mass accuracy variation	--	Resolution for tune solution ions 118 m/z > 35,000 322 m/z > 50,000 622 m/z > 60,000 922 m/z > 60,000 1221 m/z > 60,000 1520 m/z > 60,000 Tune solution ion abundance 500k-1M (118 m/z > 500k)
Internal Reference Solution (Continuous Injection)	--	Detection throughout analytical run	--
Internal Standard Mix	Retention time variation<0.1 min from the calibration standard at the beginning of the run; mass accuracy variation <5 ppm	--	Area response of each analyte within ~20% of initial response
Replicates	--	Features present in ≥ 3 sample replicates	--
Blanks	--	Abundance of features present in each sample ≥ 5 times their abundance in corresponding field and lab blanks	

2.3.1 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. Four key aspects are considered to evaluate precision of non-target data: instrument tuning, background signals, repeated injections of reference standards, and field replicates (Table 1).

- 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.
- 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.

2.3.2 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. Features in sample extracts that have abundances ≤ 5 times those of the same feature in the corresponding field or lab blanks (whichever is higher in the same analytical batch) will be filtered out.

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 (version 10.0.2) using the recursive feature extraction algorithm. Peaks are defined

as having peak 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. The results of the feature alignment allow the identification of common features across samples and blanks.

Feature filtering is refined with an additional level of screening, retaining features which are consistently present across sample replicates with a peak height greater than 5000 (S/N ~17). In each set of sample replicates (replicate field collections or replicate lab extractions), features will be filtered out if the feature is not present in ≥ 3 replicates. 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.

2.3.3 Sensitivity

Sensitivity is a measure of the capability of an instrument 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 resolution: The resolution of the QTOF detector is typically 35,000-60,000 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 resolution are set for individual ions in the tune solution (see Table 1). Additionally, peak abundance of the tuning ions is expected to be 500,000-1,000,000 (with the exception of 118 m/z, which is expected to be > 500,000).
- Instrument response: After alignment of features in samples and blanks, only compounds with peak height above 5000 (S/N ~17) in the sample are considered for further analysis.

2.3.4 Targets for comparability, representativeness, and completeness

2.3.4.1 Comparability

We will use consistent Standard Operating Procedures (SOPs) for all of the methods and processes that are going to be used in this study. The use of standard procedures in this way will ensure that all sampling is replicable and comparable. The SOPs include:

- Extraction and Analysis of 6PPD-Quinone in Water and;
- LC-QTOF-MS/MS Setup, Operation, and Data Analysis

Copies of the SOPs are available from the University of Washington Tacoma Laboratories at the Center for Urban Waters.

The processes described in the SOPs and herein will allow comparability of results across studies. Deviations from these SOPs should be accompanied by appropriate comparability studies to understand how they might impact the recovery and identification of analytes of interest.

2.3.4.2 Representativeness

Representativeness of environmental samples is the degree to which the data accurately reflect the population from which they were taken. Data representativeness will be ensured by selection of sites across a range of conditions that reflect the range of influences encountered throughout the study areas, sampling of multiple events at a given site, and the use of standardized sample processing and analytical procedures.

2.3.4.3 Completeness

Completeness is the proportion of samples collected relative to the total number planned to be collected, and depends on both field sampling and the completion of analytical work by the laboratory. The goal of the study is to collect samples and analyze data for at least 90% of the planned sampling events.

2.4 Acceptance criteria for quality of existing data

Existing water quality monitoring data may be used to inform on the finalization of sampling sites. Existing data were collected according to an Ecology-approved QAPP and/or utilizing methods with clear SOPs. All existing data will be reviewed by project personnel prior to use.

2.5 Model quality objectives

NA.

3 Laboratory Procedures

3.1 Lab procedures table

Table 2. Measurement methods (laboratory).

Analyte	Non-target compounds
Sample Matrix	Water
Sample Preparation Method	Solid phase extraction (water)
Analytical (Instrumental) Method	QTOF MS-only and MS/MS ESI+ and ESI-
Expected Range of Results	200-5000+ unique features; peak area 5000 to >10 million
Feature Detection or Reporting Limit	Peak area >5000, 5-fold higher than controls

3.2 Sample preparation method(s)

Water samples will be prepared and solid phase extracted by methods outlined in the UWT at CUW SOP, "Extraction and Analysis of 6PPD-Quinone in Water".

3.3 Special method requirements

Samples will be analyzed by high resolution mass spectrometry, using a method developed at the UWT at CUW. Details of the method are provided in the UWT at CUW SOP "LC-QTOF-MS/MS Setup, Operation, and Data Analysis".

3.4 Laboratories accredited for methods

Analyses will be performed at the UWT at CUW laboratory. The acquisition and analysis of HRMS data described in the QAPP is not subject to the laboratory accreditation system (a "Request to Waive Required Use of Accredited Lab" form (Ecology 070-152) will be submitted). This non-targeted LC-HRMS methodology is used for the separation and untargeted detection of thousands of organic compounds in a sample. This method is currently non-quantitative and used only to screen compounds within environmental samples to understand sources and contaminant relationships and fate. Compound discovery using LC-HRMS can uncover novel, priority list chemicals that may then be selected to be analyzed semi-quantitatively. This data will be used for research purposes only, and will be flagged as semi-quantitative in reports and data repositories.

4 Quality Control Procedures

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 Sections 8.0 and 9.0, comparing preliminary results to the quality indicators outlined in Section 6.0, and bi-weekly staff meetings to review data outputs and analyses.

4.1 Table of field and laboratory quality control

Table 10. Quality control samples, types, and frequency for non-target analysis. Field duplicates are defined in section 7.2.2.1.

Field Blanks	Minimum 1 per sampling batch
Field Duplicates	Minimum 9% of sampling sites
Instrument blanks	Every 20 samples through an analytical run (minimum)
Method blanks	1 per sample event

Field blanks

Field blanks account for potential exposure to anthropogenic compounds during the sample handling processes. A field blank should be collected with each sampling event along with the field samples. Procedures for field blanks are described elsewhere in this QAPP.

Method blank

A laboratory method blank can account for potential contamination because of the processing of the samples. A minimum of one method blank will be processed with each extraction batch for each matrix.

Spike and Recovery (matrix spike)

Matrix spikes are water samples prepared with a known quantity of targeted chemicals. This type of spike is carried throughout the whole processing scheme to determine the identification and recovery of the targeted chemicals at the laboratory during analysis and to establish control limits for the analytical process. A matrix spike can help determine if ion suppression of detected chemicals will occur because of sample matrix, as well as incidences of false positive/negative within the HRMS workflow. Recoveries of target analytes in our targeted analyte standard mix (see Table 1, Appendix 2) will be tracked using our targeted LC-QQQ analysis (see Appendix 2). These data are useful for determining matrix impacts on sample analyses, but are not ultimately used in a quantitative correction of HRMS data due to the qualitative nature of the HRMS analysis described here.

4.2 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 the stated holding times.

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

4.3 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 collected was 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 (>90%) 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.

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

Samples with rejected data files may be re-analyzed, as appropriate, e.g., in cases where sample collection and processing procedures were followed and verified, but when errors may have occurred during instrument analysis.

4.4 Treatment of non-detects

In the context of non-target data, non-detects (reflecting false negatives for compounds known to be present) 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. Features that fit these criteria in only some sample replicates, but not all sample replicates, will be reported as non-detects. Non-detects will be reported as ND in processed data outputs.

4.5 Data analysis and presentation methods

A complete description of data acquisition and data reduction procedures are described in detail in the SOP titled, "LC-QTOF-MS/MS Setup, Operation, and Data Analysis."

Briefly, the data acquisition is performed in Agilent MassHunter Workstation LC/MS Data Acquisition Version 11.0, Build 11.0.203.0. Data analysis, alignment, and reduction are performed in Profinder 10.0.2 Build 10.0.2.162, Qualitative Analysis Version 10.0 Build 10.0.10305.0, and in Mass Profiler Professional (MPP) 15.1 Build 15.1.20045.0. Recursive extraction and alignment of non-target features is performed in Profinder. MPP is a statistical package to align, filter, and understand relationships across conditions by matching retention time (RT) (±0.01 min) and mass accuracy (±0.01 m/z) of any features extracted by Profinder. Analyses performed within MPP include selection of non-target features present with peak height above 5000 (S/N ~17), and across field or instrument replicates, and at an abundance 5 times greater than feature abundance in blanks. MPP is also used to perform hierarchical cluster analyses and principal component analysis to determine and evaluate relationships between samples. Lists of features can be exported from MPP to Agilent ID Browser Version 10.0 Build 10.0.10543.0 to screen against suspect screening databases, using accurate mass, isotope abundance, and isotope spacing information. Agilent PCDL Manager B.08.00 Build 8209.0 is used to compile suspect screening databases, containing formula, structure, exact mass, and MS/MS spectra information.

Data presentation formats will include cluster analysis diagrams, principal component analysis plots, and lists of identified features with accompanying supporting graphics (e.g., extracted ion chromatograms, MS/MS fragmentation patterns). Data from non-accredited HRMS methods will not be uploaded to archival databases other than those at CUW because the data is exploratory. In needed reports to ECY, HRMS data that has been generated within this project will contain a disclaimer statement indicating that these semiquantitative data were generated using non-accredited methods given the research focus of these studies and the exploratory monitoring outcomes from collected data.

5 Additional Documentation

5.1 Documentation

Below is a list of documents incorporated by reference. Copies available by request from the University of Washington Tacoma Laboratories at Center for Urban Waters

1. Extraction and Analysis of 6PPDQ in Water SOP
2. LC-QTOF-MS/MS Setup, Operation, and Data Analysis SOP

5.2 Appendix B. Glossaries, Acronyms, and Abbreviations

5.2.1 Glossary of General Terms

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

Anthropogenic: Human-caused.

Baseflow: The component of total streamflow that originates from direct groundwater discharges to a stream.

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.

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

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

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

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

Diurnal: Of, or pertaining to, a day or each day; daily. (1) Occurring during the daytime only, as different from nocturnal or crepuscular, or (2) Daily; related to actions which are completed in the course of a calendar day, and which typically recur every calendar day (e.g., diurnal temperature rises during the day, and falls during the night).

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.

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

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

Hyporheic: The area beneath and adjacent to a stream where surface water and groundwater intermix.

Load allocation: The portion of a receiving water's loading capacity attributed to one or more of its existing or future sources of nonpoint pollution or to natural background sources.

Loading capacity: The greatest amount of a substance that a water body can receive and still meet water quality standards.

Municipal separate storm sewer systems (MS4): A conveyance or system of conveyances (including roads with drainage systems, municipal streets, catch basins, curbs, gutters, ditches, manmade channels, or storm drains): (1) owned or operated by a state, city, town, borough, county, parish, district, association, or other public body having jurisdiction over disposal of wastes, stormwater, or other wastes and (2) designed or used for collecting or conveying stormwater; (3) which is not a combined sewer; and (4) which is not part of a Publicly Owned Treatment Works (POTW) as defined in the Code of Federal Regulations at 40 CFR 122.2.

Nonpoint source: Pollution that enters any waters of the state from any dispersed land-based or water-based activities, including but not limited to atmospheric deposition, surface-water runoff from agricultural lands, urban areas, or forest lands, subsurface or underground sources, or discharges from boats or marine vessels not otherwise regulated under the NPDES program. Generally, any unconfined and diffuse source of contamination. Legally, any source of water pollution that does not meet the legal definition of "point source" in section 502(14) of the Clean Water Act.

pH: A measure of the acidity or alkalinity of water. A low pH value (0 to 7) indicates that an acidic condition is present, while a high pH (7 to 14) indicates a basic or alkaline condition. A pH of 7 is considered to be neutral. Since the pH scale is logarithmic, a water sample with a pH of 8 is ten times more basic than one with a pH of 7.

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

Pollution: Contamination or other alteration of the physical, chemical, or biological properties of any waters of the state. This includes change in temperature, taste, color, turbidity, or odor of the waters. It also includes discharge of any liquid, gaseous, solid, radioactive, or other substance into any waters of the state. This definition assumes that these changes will,
or are likely to, create a nuisance or render such waters harmful, detrimental, or injurious to

(1) public health, safety, or welfare, or (2) domestic, commercial, industrial, agricultural, recreational, or other legitimate beneficial uses, or (3) livestock, wild animals, birds, fish, or other aquatic life.

Reach: A specific portion or segment of a stream.

Riparian: Relating to the banks along a natural course of water.

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

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

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

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

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

Synoptic survey: Data collected simultaneously or over a short period of time.

Thalweg: The deepest and fastest moving portion of a stream.

Total suspended solids (TSS): Portion of solids retained by a filter.

Turbidity: A measure of water clarity. High levels of turbidity can have a negative impact on aquatic life.

Watershed: A drainage area or basin in which all land and water areas drain or flow toward a central collector such as a stream, river, or lake at a lower elevation.

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

7Q2 flow: A typical low-flow condition. The 7Q2 is a statistical estimate of the lowest 7-day average flow that can be expected to occur once every other year on average. The 7Q2 flow is commonly used to represent the average low-flow condition in a water body and is typically calculated from long-term flow data collected in each basin. For temperature TMDL work, the 7Q2 is usually calculated for the months of July and August as these typically represent the critical months for temperature in our state.

7Q10 flow: A critical low-flow condition. The 7Q10 is a statistical estimate of the lowest 7-day average flow that can be expected to occur once every ten years on average. The 7Q10 flow is commonly used to represent the critical flow condition in a water body and is typically calculated from long-term flow data

collected in each basin. For temperature TMDL work, the 7Q10 is usually calculated for the months of July and August as these typically represent the critical months for temperature in our state.

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

5.2.2 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. (EPA, 1997)

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

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

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

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

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

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

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

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

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

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

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

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

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

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)

References for QA Glossary

Ecology, 2004. Guidance for the Preparation of Quality Assurance Project Plans for Environmental Studies. https://fortress.wa.gov/ecy/publications/SummaryPages/0403030.html

Kammin, B., 2010. Definition developed or extensively edited by William Kammin, 2010. Washington State Department of Ecology, Olympia, WA.

USEPA, 1997. Glossary of Quality Assurance Terms and Related Acronyms. U.S. Environmental Protection Agency. http://www.ecy.wa.gov/programs/eap/quality.html

USEPA, 2006. Guidance on Systematic Planning Using the Data Quality Objectives Process EPA QA/G-4. U.S. Environmental Protection Agency. http://www.epa.gov/quality/qs-docs/g4-final.pdf

USGS, 1998. Principles and Practices for Quality Assurance and Quality Control. Open-File Report 98-636. U.S. Geological Survey. http://ma.water.usgs.gov/fhwa/products/ofr98-636.pdf

5.2.3 Acronyms and Abbreviations

BMP	Best management practice
CUW	Center for Urban Waters
DO	(see Glossary above)
DOC	Dissolved organic carbon
e.g.	For example
Ecology	Washington State Department of Ecology
EIM	Environmental Information Management database
EPA	U.S. Environmental Protection Agency
et al.	And others
FC	(see Glossary above)
GIS	Geographic Information System software
GPS	Global Positioning System
i.e.	In other words
LC	Liquid chromatography
MEL	Manchester Environmental Laboratory
MQO	Measurement quality objective
MS	Mass spectrometry
NAF	New Approximation Flow
NOAA	National Oceanic and Atmospheric Administration
NPDES	(See Glossary above)
NSDZ	Near-stream disturbance zones
NTR	National Toxics Rule
PBDE	polybrominated diphenyl ethers
PBT	persistent, bioaccumulative, and toxic substance
PCB	polychlorinated biphenyls
PSM	Pre-spawn mortality
QA	Quality assurance
QC	Quality control
QTOF	Quadrupole time-of-flight
RM	River mile
RPD	Relative percent difference
RSD	Relative standard deviation
SOP	Standard operating procedures
SRM	Standard reference materials
TIR	Thermal infrared radiation

TMDL	(See Glossary above)
TOC	Total organic carbon
TSS	(See Glossary above)
USFS	United States Forest Service
USGS	United States Geological Survey
UWT	University of Washington Tacoma
WAC	Washington Administrative Code
WDFW	Washington Department of Fish and Wildlife
WQA	Water Quality Assessment
WRIA	Water Resource Inventory Area
WSTMP	Washington State Toxics Monitoring Program
WSU-P	Washington State University-Puyallup
WWTP	Wastewater treatment plant

Units of Measurement

°C	degrees centigrade
cfs	cubic feet per second
cfu	colony forming units
cms	cubic meters per second, a unit of flow
dw	dry weight
ft	feet
g	gram, a unit of mass
kcfs	1000 cubic feet per second
kg	kilograms, a unit of mass equal to 1,000 grams
kg/d	kilograms per day
km	kilometer, a unit of length equal to 1,000 meters
l/s	liters per second (0.03531 cubic foot per second)
L	Liter
m	meter
mm	millimeter
mg	milligram
mgd	million gallons per day
mg/d	milligrams per day
mg/Kg	milligrams per kilogram (parts per million)
mg/L	milligrams per liter (parts per million)
mg/L/hr	milligrams per liter per hour

mL	milliliter
mmol	millimole or one-thousandth of a mole
mole	an International System of Units (IS) unit of matter
ng/g	nanograms per gram (parts per billion)
ng/Kg	nanograms per kilogram (parts per trillion)
ng/L	nanograms per liter (parts per trillion)
NTU	nephelometric turbidity units
pg/g	picograms per gram (parts per trillion)
pg/L	picograms per liter (parts per quadrillion)
psu	practical salinity units
s.u.	standard units
ug/g	micrograms per gram (parts per million)
ug/Kg	micrograms per kilogram (parts per billion)
ug/L	micrograms per liter (parts per billion)
um	micrometer
uM	micromolar (a chemistry unit)
umhos/cm	micromhos per centimeter
uS/cm	microsiemens per centimeter, a unit of conductivity
ww	wet weight

6 References

Castro, V., J. B. Quintana, I. Carpinteiro, J. Cobas, N. Carro, R. Cela, and R. Rodil. 2021. Combination of different chromatographic and sampling modes for high-resolution mass spectrometric screening of organic microcontaminants in water. Analytical and Bioanalytical Chemistry **413**:5607-5618.

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.

Du, B., Tian, Z., Peter, K.T., Kolodziej, E.P., Wong, C. 2020. "Developing Unique Non-Target High Resolution Mass Spectrometry Signatures to Track Contaminant Sources in Urban Waters." *Environ. Sci. Technol. Letters* **7**(12) 923-930. DOI: 10.1021/acs.estlett.0c00749

Ecology. 2019. Quality Assurance Project Plan. Assessing Sources of Toxic Chemicals Impacting Juvenile Chinook Salmon., Washington State Department of Ecology, Olympia, WA.

Faber, M., R. Sofield, C. A. James, and L. Harding. 2023. Advancing the Evaluation of Estrogenic Mixtures Using High Throughput Screening and Fish Bile Data Integration: A Case Study in Puget Sound. in preparation.

Gago-Ferrero, P., E. L. Schymanski, A. A. Bletsou, R. Aalizadeh, J. Hollender, and N. S. Thomaidis. 2015. Extended Suspect and Non-Target Strategies to Characterize Emerging Polar Organic Contaminants in Raw Wastewater with LC-HRMS/MS. Environmental Science & Technology **49**:12333-12341.

Gipe, A. 2023. Addendum to Quality Assurance Project Plan. Assessing Sources of Toxic Chemicals Impacting Juvenile Chinook Salmon., Washington State Department of Ecology, Olympia, WA.

Hernández, F., M. Ibáñez, T. Portolés, M. I. Cervera, J. V. Sancho, and F. J. López. 2015. Advancing towards universal screening for organic pollutants in waters. Journal of Hazardous Materials **282**:86-95.

Hohrenk-Danzouma, L. L., M. Vosough, V. I. Merkus, F. Drees, and T. C. Schmidt. 2022. Non-target Analysis and Chemometric Evaluation of a Passive Sampler Monitoring of Small Streams. Environmental Science & Technology **56**:5466-5477.

Hu, X., Mar, D., Suzuki, N., Zhang, B., Peter, K.T., Beck, D.A.C., Kolodziej, E.P. 2023. "Mass-Suite: A Novel Open-source Python Package for High Resolution Mass Spectrometry Data Analysis." *J. ChemoInfomatics*. **15**:87. DOI:10.1186/s13321-023-00741-9

James, C. A. 2018. Quality Assurance Project Plan - Characterization of Contaminants of Emerging Concern in Regional Waters. Univertisy of Washington Tacoma laboratories at the Center for Urban Waters, Tacoma, WA.

James, C. A., J. Lanksbury, T. Khangaonkar, and J. West. 2020. Evaluating exposures of bay mussels (Mytilus trossulus) to contaminants of emerging concern through environmental sampling and hydrodynamic modeling. Science of The Total Environment:136098.

James, C. A., and H. Mathews. 2020. Quality Assurance Project Plan. Effects-Based Characterization of Chemicals in Puget Sound Biota. University of Washington Tacoma, Tacoma, WA.

James, C. A., J. P. Miller-Schulze, S. Ultican, A. D. Gipe, and J. E. Baker. 2016. Evaluating Contaminants of Emerging Concern as tracers of wastewater from septic systems. Water Research **101**:241-251.

James, C. A., R. Sofield, M. Faber, D. Wark, A. Simmons, L. Harding, and S. O'Neill. 2023. The screening and prioritization of contaminants of emerging concern in the marine environment based on multiple biological response measures. Sci Total Environ **886**:163712.

Keil, R., K. Salemme, B. Forrest, J. Neibauer, and M. Logsdon. 2011. Differential presence of anthropogenic compounds dissolved in the marine waters of Puget Sound, WA and Barkley Sound, BC. Marine Pollution Bulletin **62**:2404-2411.

Kumar, N., Zhao, H., Awoyemi, A., Kolodziej, E.P., Crago, J. 2021. "Toxicity Testing of Effluent Dominated Stream using Predictive Molecular Level Toxicity Signatures Based on High Resolution Mass Spectrometry: A Case Study of the Lubbock Canyon Lake System". *Environ. Sci. Technol.* **55**(5) 3070-3080. DOI: 10.1021/acs.est.0c05546

Lubliner, B., M. Redding, and D. Ragsdale. 2010. Pharmaceuticals and Personal Care Products in Municipal Wastewater and Their Removal by Nutrient Treatment Technologies. Publication Number 10-03-004., Washington State Department of Ecology, Olympia, WA.

Meador, J. P. 2014. Do chemically contaminated river estuaries in Puget Sound (Washington, USA) affect the survival rate of hatchery-reared Chinook salmon? Canadian Journal of Fisheries and Aquatic Sciences **71**:162-180.

Meador, J. P., A. Yeh, G. Young, and E. P. Gallagher. 2016. Contaminants of emerging concern in a large temperate estuary. Environmental Pollution **213**:254-267.

Nováková, P., H. Švecová, A. Bořík, and R. Grabic. 2023. Novel nontarget LC-HRMS-based approaches for evaluation of drinking water treatment. Environmental Monitoring and Assessment **195**:739.

Overdahl, K. E., R. Sutton, J. Sun, N. J. DeStefano, G. J. Getzinger, and P. L. Ferguson. 2021. Assessment of emerging polar organic pollutants linked to contaminant pathways within an urban estuary using non-targeted analysis. Environmental Science: Processes & Impacts **23**:429-445.

Peter, K.T., Tian, Z., Wu, C., Lin, P., White, S., Du, B., McIntyre, J.K., Scholz, N.L., Kolodziej E.P. 2018. "Using High-Resolution Mass Spectrometry to Identify Organic Contaminants Linked to Urban Stormwater Mortality Syndrome in Coho Salmon." *Environ. Sci. Technol.*, **52**(18) 10317-10327. DOI: 10.1021/acs.est.8b03287

Peter, K.T., Tian, Z., Wu, C., Kolodziej, E.P. 2019. "Application of Non-Target High Resolution Mass Spectrometry Data to Quantitative Source Apportionment." *Environ. Sci. Technol.* **53**(21) 12257-12268. DOI: 10.1021/acs.est.9b04481

Peter, K.T., Lundeen, J.I., Wu, C., Feist, B., [3]Tian, Z., Cameron, J., Scholz, N.L.. Kolodziej, E.P. 2022. "Measuring The Chemical Profile of Biological Decline in Stormwater-Impacted Watersheds." *Environ. Sci. Technol.* **56**(5) 3159-3169. DOI: 10.1021/acs.est.1c08274.

Peter, K.T., Kolodziej E.P., Kucklik, J. 2022. “Assessing Reliability of Non-Target High Resolution Mass Spectrometry Fingerprints for Quantitative Source Apportionment in Complex Matrices.” *Anal. Chem*. **94** (6) 2723-2731. DOI: 10.1021/acs.analchem.1c03202.

Renaud, J. B., L. Sabourin, S. Hoogstra, P. Helm, D. R. Lapen, and M. W. Sumarah. 2022. Monitoring of Environmental Contaminants in Mixed-Use Watersheds Combining Targeted and Nontargeted Analysis with Passive Sampling. Environ Toxicol Chem **41**:1131-1143.

Richardson, S. D. 2012. Environmental Mass Spectrometry: Emerging Contaminants and Current Issues. Analytical Chemistry **84**:747-778.

Schymanski, E. L., J. Jeon, R. Gulde, K. Fenner, M. Ruff, H. P. Singer, and J. Hollender. 2014a. Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. Environmental Science & Technology **48**:2097-2098.

Schymanski, E. L., H. P. Singer, P. Longrée, M. Loos, M. Ruff, M. A. Stravs, C. Ripollés Vidal, and J. Hollender. 2014b. Strategies to Characterize Polar Organic Contamination in Wastewater: Exploring the Capability of High Resolution Mass Spectrometry. Environmental Science & Technology **48**:1811-1818.

Tian, Z., K. T. Peter, A. D. Gipe, H. Zhao, F. Hou, D. A. Wark, T. Khangaonkar, E. P. Kolodziej, and C. A. James. 2020. Suspect and nontarget screening for contaminants of emerging concern in an urban estuary. Environmental Science & Technology:889-901.

Tian, Z., Zhao, H.Q., [3]Peter, K.T., [1]Gonzalez, M., Wetzel, J., [1]Wu, C., [2]Hu, X., Prat, J., Mudrock, E., [1]Hettinger, R., [1]Cortina, A.E., Biswas, R.G., Kock, F.V.C., Soong, R., Jenne, A., [3]Du, B., [2]Hou, F., He, H., [3]Lundeen, R., Gilbreath, A., Sutton, R., Scholz, N.L. Davis, J.W., Dodd, M.C., Simpson, A., McIntyre, J.K., Kolodziej, E.P. 2021. “Ubiquitous Tire Rubber-Derived Chemical Induces Acute Mortality in Coho Salmon.” *Science*. Published online 12/3/20, in print 1/8/21. **371** (6525) 185-189. DOI 10.1126/science.abd6951

Tian, Z., D. A. Wark, K. Bogue, and C. A. James. 2021. Suspect and non-target screening of contaminants of emerging concern in streams in agricultural watersheds. Science of The Total Environment **795**:148826.

US Environmental Protection Agency. 1997. Glossary of Quality Assurance Terms and Related Acronyms. Washington D.C.,.

Wu, J., L. Zhang, and Z. Yang. 2010. A Review on the Analysis of Emerging Contaminants in Aquatic Environment. Critical Reviews in Analytical Chemistry **40**:234-245.

Zhao, H., [2]Hu, X., [3]Tian, Z., Gonzalez, M., Rideout C.A., Peter K.T., Dodd, M.D., Kolodziej, E.P. 2023. “Transformation Products of Tire Rubber Antioxidant 6PPD in Heterogeneous Gas-Phase Ozonation: Identification and Environmental Occurrence.” *Environ. Sci. Technol*. **57**(14) 5621-5632. DOI: 10.1021/acs.est.2c08690

Appendix B

Equipment Cutsheets

6527

STARFLOW QSD ULTRASONIC DOPPLER VELOCITY AND DEPTH INSTRUMENT

MODEL B

The Unidata 6527 Starflow QSD SDI-12 and Modbus Instrument is used to measure water velocity, depth and temperature of water flowing in rivers, streams, open channels and large pipes. When used with a companion Unidata IP data logger, flow rate and total flow can also be calculated.

The 6527 Instrument is robust, reliable and easy to use. It is completely sealed against water ingress, low maintenance, low power, no calibration and no fussy power arrangements required.

Ultrasonic Doppler Principle in Quadrature Sampling Mode is utilised to measure water velocity. The 6527 Instrument transmits ultrasonic energy through its epoxy casing into the water. Suspended sediment particles, or small gas bubbles in the water reflect some of the transmitted ultrasonic energy back to the 6527 Instrument's ultrasonic receiver instrument that processes this received signal and calculates the water velocity.

The 6527 Starflow QSD incorporates ultrasonic depth sensor and an absolute pressure depth sensor. An ultrasonic depth sensor measures water depth using the ultrasonic principle and has a range of up to 5m. An absolute pressure sensor measures pressure forces applied to the strain gauge. Absolute, non-vented, sensor reports a value equal to the sum of the water pressure and the atmospheric pressure above the water. In order to compensate for the atmospheric (barometric) pressure fluctuation, the 6527 Starflow QSD should be connected to the 6515 Starflow QSD barometric reference. The absolute pressure sensor has a range of up to 10m. Having sensors using different depth measurement methods provides flexibility in depth measurement.

The 6527 instrument's low profile form factor minimises disturbance to the flow it's measuring. Furthermore, 6527 instrument measures velocity in both directions and is suitable for use in wide range of water qualities, from sewage to potable water, sea water too.

With a companion Unidata data logger or a telemetered Neon Remote Logger the instrument can be programmed to compute flow rate and total flow in pipes and open channels of known dimensions.

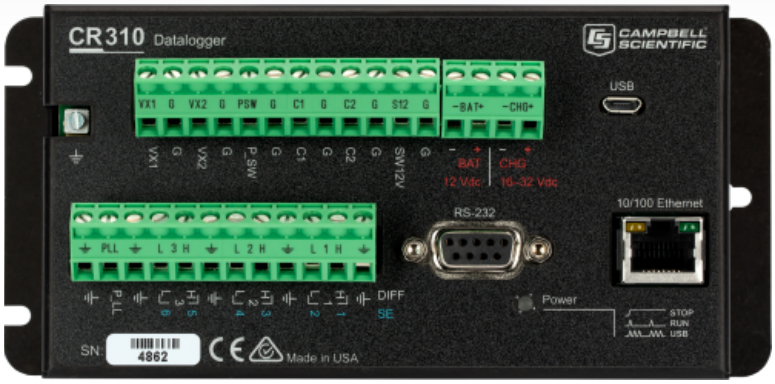
SPECIFICATIONS

PHYSICAL SPECIFICATIONS	
MATERIAL:	Epoxy-sealed body, Marine Grade 316 Stainless Steel Mounting Bracket
SIZE:	135mm x 55mm x 22mm (LxWxH)
WEIGHT:	1kg with 15m of Cable
OPERATING TEMPERATURE:	0°C to 60°C water temperature
VELOCITY RANGE:	20mm/sec to 0.8 m/sec 20mm/sec to 1.6 m/sec (default) 20mm/sec to 3.2 m/sec 20mm/sec to 13.2 m/sec Bidirectional velocity capability, set using configuration tools
VELOCITY ACCURACY:	±1% typical
DEPTH RANGE: Ultrasonic Sensor:	20mm up to 5m above top surface of the instrument 40mm up to 5m from base of the instrument
DEPTH ACCURACY:	Typical ±1%
DEPTH RANGE: Absolute Pressure sensor:	0 to 10m
DEPTH ACCURACY:	Typical ±0.19% for 0m to 5m range Typical ±0.38% for 0m to 10m range
TEMPERATURE:	0°C to 60°C
TEMPERATURE RESOLUTION:	0.1°C
FLOW COMPUTATION:	Flow rate, totalised flow with companion NRT/NRL
CHANNEL TYPE:	Pipe, open channel, natural stream
CABLE:	15 metre, 6 way
CABLE OPTIONS:	User specified up to 50 metres
ELECTRICAL SPECIFICATIONS	
POWER SOURCE:	External Battery 12V – 24V DC
POWER USAGE:	10V to 24V DC, 50µA standby, 100mA active for 1 sec
SDI-12:	SDI-12V 1.3
RS 485:	Modbus RTU

CAMPBELL SCIENTIFIC
WHEN MEASUREMENTS MATTER

CR310

Measurement and Control Datalogger



Compact Data Logger with Ethernet

Ideal for small applications

Overview

The CR310 is a multi-purpose, compact, low-cost measurement and control data logger that includes an integrated 10/100 Ethernet port and removable terminal connectors. This entry-level data logger, with its rich instruction set, can measure most hydrological, meteorological, environmental, and industrial sensors. It will concentrate data, making it available over varied networks and deliver it using your preferred protocol. The CR310 also performs automated on-site or remote decision making for control and M2M communications. The CR310 is ideal for small applications requiring long-term, remote monitoring and control.

The primary differences between the CR300 and CR310 are that the CR310 offers removable connectors and a 10/100 Ethernet connection.

The CR310 has multiple radio options that are suitable for different regions:

- CR310-RF407: US and Canada
- CR310-RF412: Australia and New Zealand
- CR310-RF422: Europe
- CR310-RF427: Brazil

Note: Campbell Scientific does not recommend the CR310 for use as a PakBus router in networks with more than 50 devices. Large arrays or string variables may also reach memory limits. For such applications, a CR1000X Measurement and Control Datalogger is recommended.

Benefits and Features

- Set up easily with PC software and USB connectivity
- Measure with confidence analog and digital sensors
- Internet ready–email, FTP, HTTP/web, TCP–with required add-ons
- Trust in the Campbell Scientific quality, including integral surge and ESD protection
- Save money and space using the integrated Ethernet port
- Network wirelessly to another node or Internet gateway with integrated radio option
- CR310-WIFI ideal for short-range, wireless IP communication
- Wiring made easy through removable terminal block
- Communicate from anywhere when using cellular or satellite peripheral
- Charge batteries using the integrated 12 V battery solar charge regulator
- Measure smart sensors using RS-232 or SDI-12
- Connect with PakBus, Modbus, DNP3, GOES, and other standard communication protocols

- Analyze and control with programmability and multiple general purpose I/O
- Notify with event-driven communications and physical outputs

Detailed Description

Terminal Descriptions

- One switched 12 V terminal (SW12V) for powering sensors or communication devices, 1100 mA @ 20°C
- Two sensor excitation or continuous 0.15 to 5 V terminals (VX1, VX2) for sensor excitation or output control
- Six multipurpose analog input terminals (SE1 - SE6)
  - Analog functions (SE1 - SE6)
    - Analog inputs: 6 single-ended or 3 differential inputs with -100 to +2500 mV and ±34 mV ranges 24 bit ADC
    - 4 to 20 mA or 0 to 20 mA inputs (SE1, SE2 only)
  - Digital I/O functions (SE1 - SE4) consist of 3.3 V logic levels for:
    - High frequency counter (35 kHz)
    - Pulse width modulation
    - Interrupts and timer input
    - Period average (200 kHz, amplitude dependent)
- Two Pulse Counting Terminals (P_SW, P_LL)
  - P_SW
    - Switch closure (150 Hz)
    - High frequency counter (35 kHz)
  - P_LL
    - Low level ac (20 kHz)
    - High frequency counter (20 kHz)
- Two Control Terminals (C1, C2): C terminals are software configurable for digital functions
  - Digital I/O functions consist of 5 V output and 3.3 V input logic levels for:
    - SDI-12
    - High frequency counter (3 kHz)
    - Switch closure (150 Hz)
    - General status/control
    - Voltage source 5 V: 10 mA @ 3.5 V
    - Interrupts
    - Serial asynchronous communication Tx/Rx pair

Specifications

-NOTE-	*Additional specifications are listed in the CR300-Series Specifications Sheet.*
Operating Temperature Range	*Non-condensing environment* -40° to +70°C (standard)
Maximum Scan Rate	10 Hz
Case Material	Powder-coated aluminum
Analog Inputs	6 single-ended or 3 differential (individually configured)
Pulse Counters	8 (P_SW, P_LL, C1, C2, and SE1 to SE4)
Voltage Excitation Terminals	2 (VX1, VX2)
Communications Ports	RS-232 10/100 Ethernet RJ45 USB Micro B
Switched 12 Volt	1 terminal
Digital I/O	7 terminals (C1, C2, P_SW, and SE1 to SE4) configurable for digital input and output. Includes status high/low, pulse width modulation, external interrupt, and communication functions. Exception: The SE4 terminal doesn't do external interrupt.
Input Limits	-100 to +2500 mV
Analog Voltage Accuracy	±(0.04% of measurement + offset) at 0° to 40°C ±(0.1% of measurement + offset) at -40° to +70°C Accuracy specifications do not include sensor or measurement noise.
ADC	24-bit
Power Requirements	16 to 32 Vdc for charger input (CHG) (Current limited to 0.9 A maximum for power converter or solar panel input.)
Power Requirements	10 to 18 Vdc for external batteries (BAT)
Real-Time Clock Accuracy	±1 min. per month
Internet Protocols	Ethernet, PPP, RNDIS, ICMP/Ping, Auto-IP(APIPA), IPv4, IPv6, UDP, TCP, TLS (v1.2), DNS, DHCP, SLAAC,

	NTP, Telnet, HTTP(S), FTP(S), SMTP/TLS, POP3/TLS
Communication Protocols	PakBus, Modbus, DNP3, SDI-12, TCP, UDP, and others
CPU Drive/Programs	80 MB serial flash
Data Storage	30 MB serial flash
Idle Current Drain, Average	10 mA (@ 12 Vdc with Ethernet link idle)
Active Current Drain, Average	56 mA (@ 12 Vdc with Ethernet link active, processor always on)
Dimensions	16.26 x 7.62 x 5.68 cm (6.4 x 3.0 x 2.2 in.)
Weight	288 to 306 g (0.64 to 0.68 lb) depending on communication option selected

CR310-RF407 Option

Radio Type	Frequency Hopping Spread Spectrum (FHSS)
Output Power	5 to 250 mW (user-selectable)
Frequency	902 to 928 MHz (US, Canada)
RF Data Rate	200 kbps
Receive Sensitivity	-101 dBm
Antenna Connector	RPSMA (external antenna required; see www.campbellsci.com/order/rf407 for Campbell Scientific antennas)
Idle Current Drain, Average	12 mA (@ 12 Vdc)
Active Current Drain, Average	< 80 mA (@ 12 Vdc)

CR310-RF412 Option

Radio Type	Frequency Hopping Spread Spectrum (FHSS)
Output Power	5 to 250 mW (user-selectable)
Frequency	915 to 928 MHz (Australia, New Zealand)
RF Data Rate	200 kbps
Receive Sensitivity	-101 dBm
Antenna Connector	RPSMA (external antenna required; see www.campbellsci.com/order/rf412 for Campbell Scientific antennas)
Idle Current Drain, Average	12 mA (@ 12 Vdc)
Active Current Drain, Average	< 80 mA (@ 12 Vdc)

CR310-RF422 Option

Radio Type	868 MHz SRD 860 with Listen Before Talk (LBT) and Automatic Frequency Agility (AFA)
Output Power	2 to 25 mW (user-selectable)
Frequency	863 to 870 MHz (European Union)
RF Data Rate	10 kbps
Receive Sensitivity	-106 dBm
Antenna Connector	(External antenna required; see www.campbellsci.com/order/rf422 for Campbell Scientific antennas.)
Idle Current Drain, Average	9.5 mA
Active Current Drain, Average	20 mA

CR310-RF427 Option

Radio Type	Frequency Hopping Spread Spectrum (FHSS)
Output Power	5 to 250 mW (user-selectable)
Frequency	902 to 907.5 MHz/915 to 928 MHz (Brazil)
RF Data Rate	200 kbps
Receive Sensitivity	-101 dBm
Antenna Connector	RPSMA (External antenna required.)
Idle Current Drain, Average	12 mA (@ 12 Vdc)
Active Current Drain, Average	< 80 mA (@ 12 Vdc)

CR310-WIFI Option

Operational Modes	Client or Access Point
Operating Frequency	2.4 GHz, 20 MHz bandwidth
Antenna Connector	Reverse Polarity SMA (RPSMA)
Antenna	pn 16005 unity gain (0 dBd), 1/2 wave whip, omnidirectional with articulating knuckle joint for vertical or horizontal orientation
Transmit Power	7 to 18 dBm (5 to 63 mW)

CR310-CELL205 Option

-NOTE-	*The CR310-CELL205 option is not compatible with a Verizon cellular network.*
Cell Technologies	4G (LTE CAT-1) 3G (UMTS/HSPA+)
3G Frequency Bands	850, 1700/2100 (AWS), and 1900
4G Frequency Bands	700, 850, 1700/2100 (AWS-1), 1900

Antenna Connector	SMA (External antenna required; see www.campbellsci.com/order/cr310 for Campbell Scientific antennas.)
SIM Interface	3FF (6 position/contacts) Supports SIMs that require 1.8 or 3 V.

CR310-CELL210 Option

-NOTE-	*The CR310-CELL210 option is only compatible with a Verizon cellular network.* CR310-CELL205 Option No
Cell Technologies	4G (LTE CAT-1)
4G Frequency Bands	700, 850, 1700, 1900, 2100
Antenna Connector	SMA (External antenna required; see www.campbellsci.com/order/cr310 for Campbell Scientific antennas.)
SIM Interface	3FF (6 position/contacts) Supports SIMs that require 1.8 or 3 V.

CR310-CELL215 Option

-NOTE-	*The CR310-CELL215 option is intended for use in EMEA countries.*
Cell Technologies	❱ 2G (GSM/GPRS/EDGE) ❱ 3G (UMTS/HSPA+) ❱ 4G (LTE CAT-1)
2G Frequency Bands	900 and 1800 MHz
3G Frequency Bands	850, 900, and 2100 MHz
4G Frequency Bands	800, 850, 900, 1800, 2100, and 2600 MHz
Antenna Connector	SMA (External antenna required; see www.campbellsci.com/order/cr310 for Campbell Scientific antennas.)
SIM Interface	3FF (6 position/contacts) Supports SIMs that require 1.8 or 3 V.

CR310-CELL220 Option

-NOTE-	*The CR310-CELL220 option is intended for use in Australia and New Zealand.*
Cell Technologies	❱ 3G (UMTS/HSPA+) ❱ 4G (LTE CAT-1)
3G Frequency Bands	❱ 850, 900, 1900, and 2100 MHz (EC-21AU) ❱ 850 and 2100 MHz (EC-21AUT)
4G Frequency Bands	❱ 700, 850, 1800, 2100, and 2600 MHz (EC-21AUT) ❱ 700, 900, 1700, 1800, 1900, 2100, and 2600 MHz (EC-21AU)
Antenna Connector	SMA (External antenna required; see www.campbellsci.com/order/cr310 for Campbell Scientific antennas.)
SIM Interface	3FF (6 position/contacts) Supports SIMs that require 1.8 or 3 V.

CR310-CELL225 Option

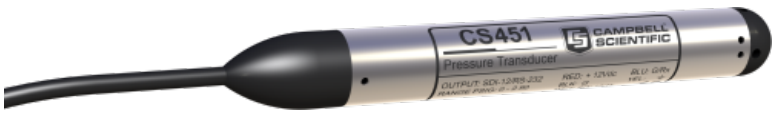
-NOTE-	*The CR310-CELL225 option is intended for use in Japan.*
Cell Technologies	4G (LTE CAT-1)
4G Frequency Bands	800 (lower), 800 (upper), 850+, 900, 1800, and 2100 MHz
Antenna Connector	SMA (External antenna required; see www.campbellsci.com/order/cr310 for Campbell Scientific antennas.)
SIM Interface	3FF (6 position/contacts) Supports SIMs that require 1.8 or 3 V.

For comprehensive details, visit: www.campbellsci.com/cr310

CAMPBELL SCIENTIFIC
WHEN MEASUREMENTS MATTER

CS451

Stainless-Steel Pressure Transducer



Stainless Steel

Ideal for long-term deployment in harsh conditions

Overview

The CS451 is a pressure transducer with a a stainless-steel case. It is used for water-level measurements and can be submerged in most canals, wells, ponds, lakes, and streams. The CS451 outputs either a digital SDI-12 or RS-232 signal to indicate observed pressure and temperature. This output can be read by many of our data loggers.

The CS451 replaces the CS450 transducer. The new transducers have a smaller gap between the water ports and the diaphragm so that less air is trapped that the user must remove during deployment. Trapped air causes the transducer's readings to drift as the air slowly dissolves into the water.

Benefits and Features

- Quality construction to ensure product reliability
- Rugged stainless-steel case protecting the piezoresistive sensor
- Compatible with most Campbell Scientific data loggers
- Fully temperature-compensated
- Low-power sleep state between measurements to reduce power consumption
- Optional weighted nose cone to facilitate submersion
- Optional NPT nose cone to enable usage in closed-pipe applications
- Quick shipment after receipt of order (ARO)

Detailed Description

The CS451 consists of a piezoresistive sensor and a temperature sensor housed in a 316L stainless-steel case. It has a rugged Hytrel cable that remains flexible, even under harsh environmental conditions. The cable incorporates a vent tube to compensate for atmospheric pressure fluctuations. The vent tube terminates inside a desiccant tube, which prevents water vapor from entering the inner cavity of the transducer. The sensor ships with a desiccant tube that can be replaced in the field.

The CS451 has several pressure range options and two accuracy options (see Ordering Info). The standard accuracy option provides ±0.1% FS TEB over the 0° to 60°C temperature range. The high accuracy option provides ±0.05% FS TEB over the 0° to 60°C temperature range and includes a calibration certificate. TEB is the combined errors due to nonlinearity, hysteresis, non-repeatability, and thermal effects over the compensated temperature range, per ISA S51.1. Please note

that the high accuracy option is not available for the 0 to 2.9 psig range option.

Campbell Scientific offers the A150 Desiccated Case that allows the CS451 to be connected to a prewired enclosure (see Ordering Information).

Specifications

Measurement Time	< 1.5 s
Output Options	SDI-12 (version 1.3) 1200 bps; RS-232 9600 bps
Water-Level Resolution	0.0035% FS
Worst-Case Temperature Resolution	0.006°C
Dry Storage Temperature Range	-40° to +100°C WARNING: Sensor could be damaged if encased in frozen ice.
Operating Temperature Range	0° to 60°C WARNING: Sensor could be damaged if encased in frozen ice.
Temperature Accuracy	±0.2°C
Overpressure	2 x pressure range
Power Requirements	6 to 18 Vdc
Cable Type	Hytrel Jacket, five conductor, 26 AWG
NPT Fitting	1/4-in. NPS
Top Cone Material	Delrin
Body Material	316L stainless steel
Element Material	316L stainless steel
Distance	❱ 2.3 cm (0.9 in.) Distance from black line etched on housing to end of standard nose cone ❱ 2.54 cm (1 in.) Distance from black line etched on housing to end of NPT fitting ❱ 9.9 cm (3.9 in.) Distance from black line etched on housing to end of weighted nose cone
Ingress Protection	Exceeds IP68
Diameter	21.34 mm (0.84 in.)
Cable Outer Diameter	❱ 0.589 cm (0.232 in.) nominal ❱ 0.599 cm (0.236 in.) maximum
Length	213.36 mm (6.875 in.)
Cable Weight	0.0421 kg/m (0.0283 lb/ft)
Weight	0.17 kg (0.37 lb) without cable

Air Gap

Standard & Weighted Nose Cone	0.653 cm (0.257 in.)
NPT Fitting	2.72 cm (1.07 in.)

Power Consumption

Quiescent	< 50 µA
Measurement/ Communication	8 mA (1 s measurement)
Maximum	40 mA

Measurement Ranges at Fresh Water Depths

0 to 2.0 m (6.7 ft)	❱ 0 to 2.9 psig The high accuracy (±0.05% FS) option is not available for the 0 to 2.9 psig range option. ❱ 0 to 20 kPa The high accuracy (±0.05% FS) option is not available for the 0 to 2.9 psig range option.
0 to 5.1 m (16.7 ft)	❱ 0 to 7.25 psig ❱ 0 to 50 kPa
0 to 10.2 m (33.4 ft)	❱ 0 to 14.5 psig ❱ 0 to 100 kPa
0 to 20.4 m (67 ft)	❱ 0 to 200 kPa ❱ 0 to 29 psig
0 to 50.9 m (167 ft)	❱ 0 to 500 kPa ❱ 0 to 72.5 psig
0 to 102 m (334.5 ft)	❱ 0 to 1000 kPa ❱ 0 to 145 psig

Accuracy

Standard Accuracy Option	±0.1% full-scale-range TEB Total Error Band (TEB) includes the combined errors due to nonlinearity, hysteresis, nonrepeatability, and thermal effects over the compensated temperature range, per ISA S51.1.
High Accuracy Option	±0.05% full-scale-range TEB The high accuracy (±0.05% full-scale range) option is not available for the 0 to 2.9 psig range option. Total Error Band (TEB) includes the

combined errors due to nonlinearity, hysteresis, nonrepeatability, and thermal effects over the compensated temperature range, per ISA S51.1.

Maximum Cable Length

SDI-12	❱ 60 m (200 ft) 10 sensors connected to a single port ❱ ~457 m (1500 ft) 1 sensor connected to a single port
RS-232	60 m (200 ft)

TRAPEZOIDAL FLUME SPECIFICATION

SECTION 11208
TRAPEZOIDAL FLUMES

PART 1 GENERAL

1.1 SECTION INCLUDES

A. Trapezoidal flumes.

1.2 RELATED SECTIONS

1.3 REFERENCES

A. ASTM D 638 – Standard Test Method for Tensile Properties of Plastics.
B. ASTM D 790 – Standard Test Methods for Flexural Properties of Unreinforced and Reinforced Plastics and Electrical Insulating Materials.
C. ASTM D 2583 – Test Method for Indentation Hardness of Rigid Plastics by Means of a Barcol Impressor.
D. "Trapezoidal Flumes for Open-Channel Flow Measurement." Transactions of The American Society of Agricultural Engineers.

1.4 SUBMITTALS

A. Submit under provisions of Section 01300.
B. Product Data: Test results of representative fiberglass reinforced plastic laminate.
C. Shop Drawings: Show:
   1. Critical dimensions, jointing and connections, fasteners and anchors.
   2. Materials of construction.
   3. Sizes, spacing, and location of structural members, connections, attachments, openings, and fasteners.
D. Samples: 8-inch square sample of representative fiberglass reinforced plastic laminate.
E. Manufacturer's installation instructions.

1.5 DELIVERY, STORAGE, AND HANDLING

A. Store products indoors or in weather protected area until installation. Protect from construction traffic and damage.

PART 2 PRODUCTS

2.1 MANUFACTURER

A. The product shall be manufactured by TRACOM, Inc.; 6575-A Industrial Way, Alpharetta, Georgia 30004; Toll-Free Voice (877) 435-8637, Toll-Free Fax (866) 435-8637, www.tracomfrp.com.
B. Requests for substitution must be made in writing and received by the engineer's office a minimum of ten (10) business days before bid opening. Substitutions shall be made in accordance with the provisions of Section 01600.
C. Substitutions: Manufacturers not pre-approved shall not be allowed.

D. Warranty: Flumes shall be warranted to be free of defects in workmanship and materials for a period of two years from shipment.

2.2 TRAPEZOIDAL FLUMES

A. Flume Type: Provide flumes of the following types:
   1. Style: _____, Size: _____.

B. Construction:
   1. One-piece construction.
   2. Two-piece construction for field assembly (by others), includes T-304 stainless steel connection hardware ***(OPTIONAL)***.

C. Materials:
   1. Fiberglass reinforced plastic.
   2. Gloss inside surfaces, free of irregularities.
   3. Minimum 3/16 inch wall thickness.
   4. Minimum 30% glass by weight.
   5. Isophthalic polyester resin.
   6. Removable pultruded fiberglass bracing at top of flume with T-304 stainless steel hardware.
   7. 2 inch (minimum) top and end stiffening flanges.
   8. Molded-in stiffening ribs, maximum 12 inch center to center spacing.
   9. 15 mil Isophthalic U.V. resistant gel coat on all surfaces, white interior, grey exterior.
   10. Anchor clips drilled for 3/4 inch, pultruded fiberglass construction.
   11. Tensile strength (ASTM D 638): 14,000 PSI.
   12. Flexural strength (ASTM D 790): 27,000 PSI.
   13. Flexural modulus (ASTM D 790): 1,000,000 PSI.
   1. Barcol hardness (ASTM D 2583): 50.

2.3 OPTIONS ***(select all that apply)***

A. Stilling well:
   1. 2 inch coupling, for user-supplied stilling well.
   2. 8 inch diameter attached, with 2 inch opening.
   3. 8 inch diameter detached, with 2 inch coupling, interconnection tubing by others.
   4. 12 inch diameter attached, with 2 inch opening.
   5. 12 inch diameter detached, with 2 inch coupling, interconnection tubing by others.

B. Laminated, high visibility staff gauge:
   1. Graduated in 1/10 foot and 1/100 foot increments, direct read.
   2. Graduated in 1/10 foot, 1/100 foot, and GPM increments, direct read ***(Small-Extra Large 60 sizes only)***.
   3. Graduated in 2mm increments.

C. Ultrasonic mounting bracket:
   1. Fixed position stainless steel.
   2. Horizontally and vertically adjustable stainless steel.

D. Removable T-316 stainless steel bubble tube, for 1/8 inch O.D. bubble line.

E. Removable T-316 stainless steel sample tube, for 3/8 inch O.D. sample line.

F. End Connections:
   1. Inlet and / or outlet ***(specify standard or low-profile) (low profile available for Small – Extra Large 60 sizes only)*** end adapters with:
      i. _____ inch inlet and / or outlet pipe stubs with flexible PVC boot(s) and stainless steel bands to connect to _____ inch, _____ style piping.
      ii. _____ inch, _____ style inlet and / or outlet ANSI 150 lb. flat-face fiberglass flanges.
   2. Inlet / or outlet 45 degree flat wingwalls.

G. Removable stainless steel probe carrier ***(specify length and O.D. of probe)***.

H. Submerged probe / area velocity probe cavity ***(specify length and O.D. of probe)***.
I. Removable open cell fiberglass grating over the flume.
J. Removable flat fiberglass cover with T-304 stainless steel bolt hardware over the flume.
K. Chemical or temperature resistant service ***(the standard flexible boots are PVC, ensure that either the chemical or temperature is compatible with PVC or that alternate end connects are provided)***:
    1. Gel coat only.
    2. Gel coat and resin (required for temperature service).
    3. Maximum temperature: _____ ° F.
    4. Chemical(s) and concentration(s): _____.

PART 3 EXECUTION

3.1 EXAMINATION

A. Verify that the flume dimensions are correct and project conditions are suitable for installation. Do not proceed with installation until condition deficiencies have been corrected.

3.2 INSTALLATION

A. Install products in accordance with engineer's instructions, plans, blueprints, etc.
B. Ensure that the product is installed plumb and that the upstream floor is level.
C. Set the flume at the elevation indicated on the engineer's drawings.
D. Embed the flume in concrete; pour concrete in maximum 6 inch lifts; internally line and brace the flume as necessary to ensure bowing or distortion does not occur.
E. For additional installation instructions refer to latest revision of document T-I.

3.3 ADJUST AND CLEAN

A. Clean surfaces in accordance with the manufacturer's instructions.
B. Remove trash and debris, and leave the site in a clean condition.

END OF SECTION

2150
Area Velocity Flow Module

The 2150 Flow Module uses continuous wave Doppler technology to measure mean velocity. The sensor transmits a continuous ultrasonic wave, then measures the frequency shift of returned echoes reflected by air bubbles or particles in the flow.

Continuous wave Doppler flow meter is ideal for portable flow surveys and permanent installations.

The 2150's "smart" area velocity probe is built on digital electronics, so the analog level is digitized in the sensor itself to overcome electromagnetic interference. The probe is also factory-calibrated for 10-foot (3 meter) span at different temperatures. This built-in calibration eliminates drift in the level signal, providing long-term level stability that reduces recalibration frequency and completely eliminates span recalibration.

In field use, the 2150 is typically powered either by two alkaline, or Teledyne ISCO Rechargeable Lead-acid batteries, within a 2191 Battery Module. Highly efficient power management extends battery life up to 15 months at 15-minute data storage intervals. Other power options (including solar) are available.

Modules may be stacked to customize a system for any site.

Applications:

- Portable and permanent-site AV flow monitoring for inflow and infiltration, capacity assessment, sewer overflow, and other sewer studies
- Measuring shallow flows in small pipes. Our low-profile area velocity sensor minimizes flow stream obstruction and senses velocity in flows down to 1 inch (25 mm) in depth

Standard Features

- Rugged, submersible enclosure meets NEMA 4X, 6P (IP68) environmental specs
- Chemically resistant epoxy-encapsulated sensor withstands abuse, resists oil and grease fouling, and eliminates the need for frequent cleaning
- Replaceable high-capacity internal desiccant cartridge and hydrophobic filter protect sensor reference from water entry and internal moisture
- Pressure transducer vent system automatically compensates for atmospheric pressure changes to maintain accuracy
- The quick-connect sensor can be easily removed and interchanged in the field without requiring recalibration
- Up to four 2100 Series flow modules can be networked by stacking and/or extension cables

2150 Flow Module

Size (HxWxD):	2.9 x 11.3 x 7.5 in (74 x 287 x 191 mm)
Weight:	2.0 lb (0.9 kg)
Materials of Construction:	High-impact polystyrene, stainless steel
Enclosure:	NEMA 4X, 6P (IP68) (self-certified)
Temperature Range:	-40 to 140 °F (-40 to 60 °C) operating & storage
Power Required:	12 VDC nominal (7.0 to 16.6 VDC), 100 mA typical, 1 mA standby
Power Source:	Typically, an 2191 Battery Module, containing 2 alkaline or 2 rechargeable lead-acid batteries. (Other power options are available; ask for details.)
Typical Battery Life:	Using 15-minute data storage interval Energizer® Model 529 alkaline–15 months Rechargeable lead-acid–2.5 months
Program Memory:	Non-volatile programmable flash; can be updated using PC without opening enclosure; retains user program after updating

Built-in Conversions

Flow Rate Conversions:	Up to 2 independent level-to-area conversions and/or level-to-flow rate conversions
Level-to-Area Conversions:	Channel Shapes–round, U-shaped, rectangular, rapezoidal, elliptical, with silt correction; Data Points–Up to 50 level-area points
Level-to-Flow Conversions:	Most common weirs and flumes; Manning Formula; Data Points (up to 50 level-flow points); 2-term polynomial equation
Total Flow Calculations:	Up to 2 independent, net, positive or negative, based on either flow rate conversion

Data Handling and Communications

Data Storage:	Non-volatile flash; retains stored data during program updates. Capacity 395,000 bytes (up to 79,000 readings, equal to over 270 days of level and velocity readings at 15-minute intervals, plus total flow and input voltage readings at 24-hour intervals)
Data Types:	Level, velocity, flow rate 1, flow rate 2, total flow 1, total flow 2, input voltage, temperature
Storage Mode:	Rollover; 5 bytes per reading
Storage Interval:	15 or 30 seconds; 1, 2, 5, 15, or 30 minutes; or 1, 2, 4, 12, or 24 hours. Storage rate variable based on level, velocity, flow rate, total flow, or input voltage
Data Retrieval:	Serial connection to PC or optional 2101 Field Wizard module; optional modules for spread spectrum radio; land-line or cellular modem; 1xRTT. Modbus and 4-20 mA analog available
Software:	Flowlink for setup, data retrieval, editing, analysis, and reporting
Multi-module Networking:	Up to four 2100 Series Flow Modules, stacked and/or remotely connected. Max distance between modules 3300 ft (1000 m)
Serial Communication Speed:	38,400 bps

2150 Area Velocity Sensor

Size (HxWxD):	0.75 x 1.3 x 6.0 in (19 x 33 x 152 mm)
Cable (L x Dia):	33 ft x 0.37 in (10 m x 9 mm) standard. Custom lengths available on request
Weight:	2.2 lbs (1 kg) (including cable)
Materials of Construction:	Sensor–Epoxy, chlorinated polyvinyl chloride (CPVC), stainless steel Cable–Polyvinyl chloride (PVC), chlorinated polyvinyl chloride (CPVC)
Operating Temperature:	32 ° to 140 °F (0 ° to 60 °C)
Level Measurement:	Method– Submerged pressure transducer mounted in the flow stream Transducer Type– Differential linear integrated circuit pressure transducer Range (standard)– 0.033 to 10 ft (0.010 to 3.05 m); (optional) up to 30 ft (9.15 m) Maximum Allowable Level– 34 ft (10.5 m) Accuracy– ±0.01 ft from 0.033 to 10 ft, (±0.003 m from 0.01 to 3.05 m,) Long-Term Stability– ±0.023 ft/yr (±0.007 m/yr) Compensated Range– -32 ° to 122 °F (0 ° to 50 °C)
Velocity Measurement:	Method– Doppler ultrasonic, frequency 500 kHz Typical Minimum Depth– 0.08 ft (25 mm) Range– -5 to +20 ft/s (-1.5 to +6.1 m/s) Accuracy (in water with uniform velocity profile, speed of sound = 4850 ft/s, for indicated velocity range)– ±0.1 ft/s from -5 to 5 ft/s (±0.03 m/s from -1.5 to +1.5 m/s) ±2% of reading from 5 to 20 ft/s (1.5 to 6.1 m/s)
Temperature Measurement:	Accuracy ±3.6 °F (±2 °C)

2150 Ordering Information

Contact your Teledyne ISCO representative for complete ordering details and information on other 2100 Series Modules.

2150 with AV sensor, 2191 Battery Module, and Handle 68-2050-002
2150 Module with AV sensor (only) 68-2050-001
Flowlink® 5 Software 68-2540-200
Energizer® Model 529 Alkaline Lantern Battery (2 required)..... 340-2006-02
Rechargeable Lead-acid Battery (2 required) 60-2004-041
Charger for Lead-acid Batteries (holds 2 batteries) 60-2004-040

Teledyne ISCO
P.O. Box 82531, Lincoln, Nebraska, 68501 USA
Toll-free: (800) 228-4373 • Phone: (402) 464-0231 • Fax: (402) 465-3091

teledyneisco.com

TELEDYNE ISCO
Everywhereyoulook™

L-2115 Rev 2.0
9/18

Rugged TROLL® 100 and 200 Data Loggers

RUGGED TROLL 100 AND 200 DATA LOGGERS ARE DESIGNED FOR LONG- AND SHORT-TERM GROUNDWATER AND SURFACE WATER MONITORING. THESE NON-VENTED (ABSOLUTE) WATER LEVEL DATA LOGGERS MEASURE AND RECORD CHANGES IN WATER LEVEL, PRESSURE, AND TEMPERATURE. ENSURE ACCURATE RESULTS BY USING A RUGGED BAROTROLL® DATA LOGGER. ALL LOGGERS ARE COMPATIBLE WITH THE USER-FRIENDLY VUSITU® MOBILE APP.

AFFORDABLE TITANIUM DATA LOGGERS

- Get reliable data at a budget-friendly price.
- Use in harsh environments. Solid titanium construction offers chemical- and corrosion-resistance and outlasts specially-coated data loggers.
- Select the appropriate logging mode for your project: Linear, Fast Linear, or Event.

FLEXIBLE DEPLOYMENT OPTIONS

- Deploy zero-maintenance loggers in flood-prone areas, high-humidity environments, and remote locations.
- Choose the cable length and termination type that works best for your project.
- Use suspension wire and backshell hanger for applications requiring minimal instrument access.

TOTAL FIELD SUPPORT

- Receive quick-response technical support and online resources.
- Order data loggers and accessories directly from our website.
- Get guaranteed 7-day service for maintenance.

SIMPLIFIED SETUP AND DATA RETRIEVAL

- The VuSitu® mobile app guides you through instrument setup with user-friendly features like Calibration Assistant and Log Setup Assistant.
- VuSitu instantly uploads data files to your HydroVu® account for secure data access, storage and management, all in one place.
- Connect a cabled logger to a telemetry system, radio, controller, or a SCADA/PLC system via Modbus/RS485 or SDI-12 (with the Rugged TROLL 200 and Rugged BaroTROLL).
- Integrate with VuLink® Telemetry for real-time feedback on your remote, continuous monitoring sites in HydroVu.

EXTENDED SHELF LIFE

- The battery indicator on Rugged TROLL 100 and 200 instruments is controlled by an algorithm so that it will not count down until your unit is deployed and has taken at least 1,000 readings or is more than 9 months past its manufacturing date.

Applications:

- **COASTAL WETLAND AND ESTUARY RESEARCH**
- **CREST STAGE GAGING AND STREAM GAGING**
- **DRILLING AND WELL DEVELOPMENT**
- **FLOOD AND STORM SURGE MONITORING**
- **LANDFILL LEACHATE MONITORING**

in-situ.com

Rugged TROLL® 100 and 200 Data Loggers

GENERAL	RUGGED TROLL 100 & 200	RUGGED BAROTROLL
TEMPERATURE RANGES[1]	Operational: 0-50° C (32-122° F) Storage: -40-80° C (-40-176° F) Calibrated: 0-50° C (32-122° F)	Operational: 0-50° C (32-122° F) Storage: -40-80° C (-40-176° F) Calibrated: 0-50° C (32-122° F)
DIAMETER	2.62 cm (1.03 in.)	2.62 cm (1.03 in.)
LENGTH	14.43 cm (5.68 in.)	14.43 cm (5.68 in.)
WEIGHT	137 g (0.30 lb)	137 g (0.30 lb)
MATERIALS	Titanium, Acetal, FKM Fluoroelastomer, Ceramic	Titanium, Acetal, FKM Fluoroelastomer, Ceramic
OUTPUT OPTIONS	Rugged TROLL 100: USB via docking station; Wireless Rugged TROLL Com Rugged TROLL 200: USB via docking station; Wireless Rugged TROLL Com; Modbus/RS485 or SDI-12 via Rugged TROLL 200 Cable	USB or RS232 via docking station; Modbus/RS485 or SDI-12 via Rugged TROLL 200 Cable; Wireless Rugged TROLL Com Device
BATTERY TYPE & LIFE[2]	3.6V lithium; 10 years or 2M readings	3.6V lithium; 10 years or 2M readings
EXTERNAL POWER	Rugged TROLL 100: NA Rugged TROLL 200: 8-36 VDC	8-36 VDC
MEMORY Data records[3] Data logs	2.0 MB 120,000 Rugged TROLL 100: 1 log Rugged TROLL 200: 2 logs	2.0 MB 120,000 1 log
FASTEST LOGGING RATE	1 per second	1 per minute
FASTEST OUTPUT RATE	Rugged TROLL 200 only Modbus & SDI-12: 1 per second	Modbus & SDI-12: 1 per second
LOG TYPES	Linear, Fast Linear, and Event	Linear
SENSOR TYPE/ MATERIAL	**PIEZORESISTIVE; CERAMIC**	**PIEZORESISTIVE; CERAMIC**
RANGE	9 m (30 ft) (Burst: 18 m; 60 ft) 30 m (100 ft) (Burst: 40 m; 134 ft) 76 m (250 ft) (Burst: 112 m; 368 ft)	7 to 30 psi; 0.5 to 2 bar
ACCURACY	±0.05% FS from 0 to 50 °C	±0.05% FS from 0 to 50 °C
RESOLUTION	±0.01% FS or better	±0.01% FS or better
UNITS OF MEASURE	Pressure: psi, kPa, bar, mbar, mmHg Level: in., ft, mm, cm, m	Pressure: psi, kPa, bar, mbar, mmHg, inHg
TEMPERATURE SENSOR	**SILICON**	**SILICON**
ACCURACY	±0.3° C	±0.3° C
RESOLUTION	0.01° C or better	0.01° C or better
UNITS OF MEASURE	Celsius or Fahrenheit	Celsius or Fahrenheit
WARRANTY	**2 YEARS**	**2 YEARS**

NOTES: 1 Temperature range for non-freezing liquids. 2 Typical battery life when used within the factory-calibrated temperature range. 3 1 data record = date/time plus 2 parameters logged for a total of 360,000 data points, no wrapping. Delrin is a registered trademark of E.I. du Pont de Nemours & Co. Specifications are subject to change without notice. Android is a trademark of Google Inc.

VUSITU MOBILE APP FOR WIRELESS CONNECTION

Use the VuSitu Mobile App to view results instantly from your Android™ or iOS™ smartphone or tablet when connected to the Wireless Rugged TROLL Com or the Wireless Rugged TROLL Com. Consolidate all site information and tag data with site photos and GPS coordinates. VuSitu instantly uploads all logs, live recordings, calibration reports and other files to your HydroVu account for secure data storage and convenient access and sharing from any browser or mobile device.

RUGGED TROLL® 200 CABLE

Use the Rugged TROLL Direct-Read Cable to connect your Rugged TROLL 200 Data Logger or Rugged BaroTROLL Data Logger directly to a controller or logger. The cable comes in 2 communication modes:

- Direct Read Cable for SDI-12 (always Stripped and Tinned)
- Direct Read Cable for RS485
  - Twist-lock connector for connection to VuLink or Wireless TROLL Com
  - Top of Well connector for connection to Wireless Rugged TROLL Com or Rugged TROLL Com
  - Stripped and Tinned for connection to third-party telemetry/ data loggers

JACKET OPTIONS	TPU (thermoplastic polyurethane)
CONDUCTORS	4 conductors, 24 AWG, polypropylene insulation
DIAMETER	Cable: 5.1 mm (0.200 in.) Connector: 26.1 mm (1.03 in.)
CABLE LENGTHS	Modbus/RS485: Customizable up to 300 m (1,000 ft) SDI-12: Standard lengths up to 60 m (200 ft)
MINIMUM BEND RADIUS	5X cable diameter
BREAK STRENGTH	68 kg (150 lbs)

WIRELESS RUGGED TROLL® COM COMMUNICATION DEVICE

Use the Wireless Rugged TROLL Com Device for communication between a cabled Rugged TROLL 100/200 or a cabled Rugged BaroTROLL and a VuSitu Mobile App or a laptop/PC.

OPERATING TEMP. RANGE	-5-50° C (23-122° F), 95% relative humidity, non-condensing
STORAGE TEMP. RANGE	-20-50° C (-4-122° F), 95% relative humidity, non-condensing*
MATERIALS	PC/ABS blend, Silicon, Urethane, Stainless Steel, Brass, Santoprene, Poron, Polyethylene, Versapor, Titanium, PEEK, Viton
ENVIRONMENTAL RATING	IP67
DIMENSIONS (LXWXH) WEIGHT (WITH BATTERIES)	6.3 x 1.710 x 1.210 in. 165 g
COMMUNICATION PROTOCOL	Android: SPP; Windows: SPP or USB
OUTPUT CONNECTION	Bluetooth and USB communication
BATTERY TYPE	1 3.7V 8600mWHr Lithium Rechargable cell (UBBL19-FL)
CHARGING REQUIREMENTS	5VDC USB charger (1A or 500 mA)
CERTIFICATIONS	CE, FCC (SSSBC127-X), WEEE
WARRANTY	1 year

www.in-situ.com

6712
Full-size Portable Sampler

The 6700 Series Portable Samplers have set the industry standard, providing the most comprehensive and durable performance available. With the introduction of our 6712, Teledyne ISCO takes another step toward the ultimate by including SDI-12 interface capabilities.

Wide range of bottle configurations, plug-in flow and parameter monitoring

This full-size portable lets you take full advantage of the advanced 6712 Controller, with its powerful pump, versatile programming, and optional plug-in modules for integrated flow measurement. Setup is fast and simple, with online help just a key stroke away.

The environmentally-sealed 6712 controller delivers maximum accuracy and easily handles all of your sampling applications.

In the Standard Programming Mode, the controller walks you through the sampling sequence step-by-step, allowing you to choose all parameters specific to your application. Selecting the Extended Programming Mode lets you enter more complex programs.

Optional land-line and GSM and CDMA cellular telephone modems allow programming changes and data collection to be performed remotely, from a touch-tone phone. They also provide dial-out alarm.

With eleven bottle choices, the 6712 Sampler lets you quickly adapt for simple or intricate sampling routines. Up to 30 pounds (13.5 kg) of ice fits in the insulated base, preserving samples for extended periods, even in extreme conditions. The 6712 with the "Jumbo Base" option holds bottles up to 5.5 gallon (21 liter).

The 6712 Portable Sampler features a vacuum formed ABS plastic shell to withstand exposure and abuse. Its tapered design and trim 20-inch (50.8 cm) diameter result in easy manhole installation and removal. Large, comfortable handles make transporting safe and convenient—even when wearing gloves.

Teledyne ISCO's 6712 Portable Sampler carries a NEMA 4X, 6 (IP67) enclosure rating. Superior capability, rugged construction, and unmatched reliability make the 6712 the ideal choice for portable sampling in just about any application.

Bottle options are available for practically any sequential or composite application.

Applications:

- Wastewater effluent
- Stormwater monitoring
- CSO monitoring
- Permit compliance
- Pretreatment compliance

Standard Features:

- SDI-12 interface provides "plug and play" connection with multi-parameter water-quality sondes and other compatible devices
- Choice of 11 different glass and plastic bottle configurations ranging from 24 x 1liter to 1 x 5.5 gallon
- NEMA 4X, 6 (IP67) controller enclosure
- Rugged ABS plastic shell
- Foam-insulated base holds up to 30 pounds (13.5 kg) of ice to preserve samples even in extreme conditions
- Sample delivery at the EPA-recommended velocity of 2 ft/sec., even at head heights of 26 feet
- Pump revolution counter and patented liquid detection sensor ensure accurate sample volumes—and tells you when tubing should be replaced

6712 Full-size Portable Sampler

Size (H x Dia):	27 x 20 in (68.6 x 50.7 cm)
Weight:	Dry, less battery–32 lbs (15 kg)
Bottle Configurations:	24 – 1 Liter PP or 350 ml Glass 24 – 1 Liter ProPak Disposable Sample Bags 12 – 1 Liter PE or 950 ml Glass 8 – 2 Liter PE or 1.8 Liter Glass 4 – 3,8 Liter PE or Glass 1 – 9,5 Liter PE or Glass 1 – 5.5 gallon (21 Liter)PE or 5 gallon (19 Liter Glass, (with optional Jumbo Base)
Power Requirements:	12 VDC (Supplied by battery or AC power converter.)

Pump

Suction Tubing:	
-Length:	3 to 99 ft (1 to 30 m)
-Material:	Vinyl or Teflon
-Inside Dimension:	3/8 in (1.0 cm)
Pump Tubing Life:	Typically 1,000,000 pump counts
Maximum Lift:	28 ft (8.5 m)
Typical Repeatability:	± 5 ml or ± 5 of the average volume in a set
Typical Line velocity at Head height of:	@ 3 ft (0.9 m) head height: 3.0 ft/s (0.91m/s) @10 ft (3.1 m) head height: 2.9 ft/s (0.87 m/s) @15 ft (4.6 m) head height: 2.7 ft/s (0.83 m/s)
Liquid Presence Detector:	Non-wetted, nonconductive sensor detects when liquid sample reaches the pump to automatically compensate for changes in head heights.

Controller

Dimensions (HxWxD):	10.3 x 12.5 x 10.0 in (26.1 x 31.7 x 25.4 cm)
Weight (dry):	13 lbs (5.9 kg)
Operating Temperature:	32 to 120 °F (0 to 49 °C)
Enclosure Rating:	NEMA 4X, 6 (IP67)
Program Memory:	Non-volatile ROM
Flow Meter Signal Input:	5 to 15 volt DC pulse or 25 millisecond isolated contact closure
Number of Composite Samples:	Programmable from 1 to 999 samples
Real Time Clock Accuracy:	1 minute per month, typical

Software

Sample Frequency:	1 minute to 99 hours 59 minutes, in 1 minute increments. Non-uniform times in minutes or clock times 1 to 9,999 flow pulses
Sampling Modes:	Uniform time, non-uniform time, flow, event. (Flow mode is controlled by external flow meter pulses.)
Programmable Sample Volumes:	10 to 9,999 ml, in 1 ml increments
Sample Retries:	If no sample is detected, up to 3 attempts; user selectable
Rinse Cycles:	Automatic rinsing of suction line up to 3 rinses for each sample collection
Program Storage:	5 sampling programs
Sampling Stop/Resume:	Up to 24 real time/date sample stop/resume commands
Controller Diagnostics:	Tests for RAM, ROM, pump, display, and distributor

Ordering Information

6712 Portable Sampler, Full-size
Includes controller with 512kB RAM, top cover, center section, base, distributor arm, instruction manual, pocket guide 68-6710-070

6712 Portable Sampler, with Jumbo Base
As described above 68-6710-082

Note: Power source, bottle configuration, suction line, and strainer must be ordered separately. Many options and accessories are available for 6712 Samplers; see separate literature for 700 Series Modules and other components to expand your monitoring capabilities. Contact Teledyne ISCO, or your local representative for pricing and additional information.

The 6712 Controller is also an SDI-12 data logger, and has many optional capabilities. Please contact your Teledyne ISCO distributor for more information.

Teledyne ISCO
P.O. Box 82531, Lincoln, Nebraska, 68501 USA
Toll-free: (800) 228-4373 • Phone: (402) 464-0231 • Fax: (402) 465-3091

teledyneisco.com

TELEDYNE ISCO
Everywhereyoulook™

L-1107 Rev 2.0
9/18

Appendix C

Standard Operating Procedures

HERRERA
Science + Planning + Design

Standard Operating Procedures

Per- and Polyfluoroalkyl Substances (PFAS) Stormwater Grab and In-Line Sediment Sampling

Prepared by
Herrera Environmental Consultants, Inc.
2200 Sixth Avenue, Suite 1100
Seattle, Washington 98121
Telephone: 206-441-9080

May 14, 2025

HERRERA
Science + Planning + Design

Contents

Tables

1. Introduction, Scope, and Applicability

This standard operating procedure (SOP) is applicable to the collection of PFAS stormwater and sediment grab samples. These are standard (i.e., typically applicable) operating procedures that may be varied or changed, as required, dependent upon site conditions, equipment limitations, limitations imposed by the procedure, or other procedure limitations. In all instances, the procedures that are ultimately employed should be documented and associated with the final report.

2. Training

The procedures in this SOP are for use only by authorized personnel who have received specific training. Personnel conducting the sampling described in this SOP should have general training in field safety, familiarity with associated water quality parameters and procedures, and knowledge of all relevant components of the associated Quality Assurance Project Plan (QAPP).

3. Method Summary

Sampling situations vary widely; therefore, no universal sampling procedure can be recommended. However, sampling water and sediment for PFAS analysis is generally accomplished such that sampling starts in areas where it is expected or known to be least contaminated and then progresses to areas anticipated or identified to be most contaminated. For all environmental media, hands should be well washed before sampling. Clean, powderless nitrile gloves must be put on before sample collection, handling of sample containers, and handling sampling equipment. The sample container must be kept sealed at all times and only open during the sample collection. The sampling container cap or lid should never be placed on any surface unless it is PFAS-free. The sampling container cap or lid must never be placed directly on the ground. Collection and analysis of QC samples, such as field reagent blanks, equipment rinse blanks, and field duplicates, are important for PFAS analyses because of very low detection limits and widespread commercial use (historical and current) of PFAS-containing products.

Samples must be chilled during storage and shipment and must not exceed 6°C (42.8°F) during the first 48 hours after collection.

4. Sample Preservation, Containers, Handling, and Storage

Once samples have been collected, the following procedure should be followed:

1. Use procedures listed in Section 8.0 for one or two technicians (see Sampling Procedures section).
2. Transfer the sample(s) into suitable sample containers.
3. Do not overfill bottles.
4. Cap the container; place in a double-bagged, clean plastic bag and cool to <6°C.
5. Label inner plastic bag (which can be performed prior to sampling) with sample ID information.
6. Record all pertinent data in the site logbook and on field data sheets.
7. Complete the Chain of Custody record.
8. Decontaminate all sampling equipment (see Table 1,Table 2, andTable 3) prior to the collection of additional samples.

5. Interferences and Potential Problems

Potential sources of PFAS cross-contamination in the typical sampling environment include materials used within the sampling environment; sampling equipment; field clothing and personal protective equipment (PPE); sun and biological protection products; personal hygiene and personal care products (PCPs); food packaging; and the environment itself.

It is recommended, when possible, to exclude materials known to contain PFAS (see tables below), such as those containing polytetrafluoroethylene (PTFE), perfluorinated ethylene-propylene (FEP), ethylene fluoroethylene (ETFE), low-density polyethylene (LDPE), polyvinylidene fluoride (PVDF), pipe thread compound and tape, and waterproof coatings. The Safety Data Sheets (SDSs) of materials should be reviewed before considering materials for use. If PFAS are not listed on the SDS, PFAS may still be present—PFAS may have been used not as a component of the material, but in the manufacturing process itself.

All equipment and materials used in the vicinity of the sample collection should be screened as sources of PFAS contamination prior to sample collection. PFAS screening will be approached in two stages: (1) sampling equipment that will come in direct contact with the sample and (2) materials and equipment that will be in the vicinity of the samples, including sampler clothing, sample coolers, and labels. Stage 1 materials should be thoroughly reviewed and free of known sources of PFAS, whereas Stage 2 materials should avoid known or suspected sources of PFAS unless it impacts field safety. Typical materials that are used in sampling equipment and may contain PFAS are included in Table 1:

Table 1. Sampling Equipment.		
Classification	**Name**	**Commonly Found**
Prohibited	PTFE (e.g., Teflon, Hostaflon)	Hose lining, wiring, gears, sliding action parts
	PVDF (e.g., Kynar)	Tubing, films/coating on aluminum, galvanized or aluminized stee, wire insulators, lithium-ion batteries
	PCTFE (e.g., Neoflon)	Valves, seals, gaskets, food packaging
	ETFE (e.g., Tefzel)	Wire and cable insulation and covers, pipe liner, cable tie wraps
	FEP (e.g., Teflon, Hostaflon, Neoflon)	Wire and cable insulation and covers, pipe linings, labware
	LDPE	Containers, bottles, plastic bags, tubing
Needs Screening	Other	Chemical ice packs, felt tip pens, sticky notes, and plastic binders or clipboards
	Aluminum foil	
Allowable	LDPE bags not in contact with sample	
	HDPE, polypropylene, silicone, acetate	
	Glass bottles or containers known to be PFAS-free	
	Powderless nitrile gloves	

Table 2. Clothing, PPE, Personal Care Products.	
Classification	**Description**
Prohibited	Fabric softener
	New or unwashed clothing
	Clothing made or washed with water, dirt, and/or stain resistant chemicals (e.g., Goretex)
	Clothing treated with insect resistant or UV protection
	Sunscreen applied near sampling area[a]
	Personal care products (cosmetics, dental floss, etc.)
	Food in the sampling or staging area
Needs Screening	Latex gloves
	Water resistant or stain-resistant clothing and PPE
	Tyvek suits or Tyvek coated clothing
Allowable	Powderless nitrile gloves
	PVC or wax-coated fabrics
	Synthetic and natural fibers well laundered with no fabric softeners

[a] Refer to Michigan PFAS Sampling Methodology EGLE 2024) for allowable sunscreens and insect repellants.

Table 3. Decontamination.	
Classification	**Description**
Prohibited	Decon 90
	PFAS treated paper towels
Needs Screening	Municipal Water
	Recycled paper towels or chemically treated paper towels
Allowable	Alconox®, Liquinox®, or Citranox®
	Triple rinse with PFAS-free deionized water
	Cotton cloth or untreated paper towel

Refer to the Michigan Department of Environment, Great Lakes, and Energy's General PFAS Sampling Guidance (EGLE 2024) for a more comprehensive list of known PFAS containing materials and allowable PFAS free alternatives.

In general, field staff will wear well-laundered (washed at least six times without fabric softener) clothing made from synthetic and natural fibers. In general, staff will also wear boots that do not contain GoreTex or Tyvek and have not been recently treated with waterproofing chemicals. On the day of sampling, field staff will avoid use of certain personal care products, including deodorant, floss, moisturizer, and makeup. Sunscreen and insect repellants that are prescreened to be PFAS free may be used if necessary. Immediately prior to sample collection, field staff will avoid handling food packaged in containers or wrappers and ensure the sampling area is clear of potential contaminant sources including chemical ice packs, felt tip pens, sticky notes, and plastic binders or clipboards. Only powder-free nitrile gloves will be worn by the field staff during sampling area preparation and sample collection.

6. Equipment/Apparatus

At minimum, equipment needed for collection of PFAS samples includes the following:

- High flow rate peristaltic pump with 3/8-inch ID silicone tubing (for stormwater grab sampling)"Norton" style sediment trap (for sediment sampling)
- 2x 250-mL and 1x 125-mL (HDPE) sample bottles
- Powder-free nitrile gloves
- Ice
- Coolers
- Chain of Custody records
- Field data sheets or other recordkeeping equipment
- Sample bottle labels
- Safety equipment, including personal protective equipment designated in the Health and Safety Plan
- Decontamination equipment

7. Decontamination Procedures

When possible, Herrera recommends using dedicated or single-use field sampling equipment. When non-dedicated equipment is used at multiple sampling locations, thorough cleaning between uses is required.

With sampling, it is customary that equipment is decontaminated at the conclusion of the sampling event. If the previous user of the equipment is not known and it is unclear how the equipment was handled—especially when it is rental equipment—the equipment should be decontaminated.

Field decontamination procedures for the stormwater sampling equipment and sediment sampling equipment will generally follow the procedures outlined in the QAPP, with the following considerations:

7.1.1. Decontamination Method 1 (EGLE 2024):

- Do not use Decon 90®.
- Do not put equipment away without decontaminating it.
- Laboratory supplied PFAS-free deionized water is preferred for decontamination.
- Alconox®, Liquinox®, and Citranox® can be used for equipment decontamination.
- Sampling equipment can be scrubbed, using a polyethylene or polyvinylchloride (PVC) brush to remove particulates.
- Decontamination procedures should include triple rinsing with PFAS-free water (preferably laboratory-provided, PFAS-free deionized water).
- Municipal drinking water may be used for decontamination purposes if it is known to be PFAS-free.
- Decontaminate sampling equipment after sampling at each location, or at the end of the workday.
- Commercially available deionized water in an HDPE or stainless steel container may be used for decontamination if the water is verified to be PFAS-free, as defined in EGLE (2024).

7.1.2. Decontamination Method 2 (EGLE 2024):

1. In a PFAS-free bucket, wash the equipment with a mixture of PFAS-free water and PFAS-free soap (bucket #1).
2. In a second PFAS-free bucket (bucket #2), rinse the equipment with PFAS-free water.
3. A second rinse should be done with PFAS-free water, using either a third bucket (bucket #3) or, if washed and rinsed, the second bucket (bucket #2).
4. For decontamination of additional equipment, change the decontamination water between cleanings.

8. Sampling Procedures

Upon arrival at the project site, field staff will establish a sample staging area and a decontamination area. The staging area will be free from potential or known sources of PFAS contamination. The decontamination area may include potential sources of PFAS contamination—potential sources would only include sampling equipment or rinse water. Field sampling equipment will be decontaminated, as described below, and moved to the staging area.

Field staff will change to fresh powder-free nitrile gloves after decontaminating equipment, when re-entering the staging area, and as needed to prevent contamination.

8.1. Preparation

1. Determine the extent of the sampling effort, the sampling methods to be employed, and the types and amounts of equipment and supplies needed.
2. Obtain the necessary sampling and monitoring equipment.
3. Clean all sampling equipment using approved decontamination methods.
4. All sampling equipment and sample containers should be free of PFAS.
5. Determine the appropriate number and type of blanks (i.e., field blanks, filter blanks, equipment blanks, etc.).
6. Prepare scheduling and coordinate with staff, clients, and regulatory agency, if appropriate.
7. Perform a general site survey prior to site entry, in accordance with the site-specific Health and Safety Plan.
8. Stakes, flagging, or buoys may be used to mark sampling locations. Care should be taken not to disturb sediment at the sample location. If required, the proposed locations may be adjusted based on site access, property boundaries, and surface obstructions.

8.2. Grab Stormwater Sample Collection

Procedures for collecting grab stormwater samples for PFAS analysis are generally consistent with the typical stormwater grab sampling procedures described in Ecology's Standard Operating Procedures for Collecting Grab Samples from Stormwater Discharges (Ecology 2024), with additional considerations to avoid cross contamination due to the widespread use of PFAS in manufacturing and low water quality criteria. These additional procedures and considerations are presented in the subsections below.

1. When a field blank is required it will be collected in the sample staging area, prior to field sample collection, by pouring laboratory-provided, PFAS-free water into the laboratory-provided sample container. This will occur for one event during the project.

2. If a peristaltic pump is being used to collect the sample, an equipment blank will be collected, using the high flow rate peristaltic pump, by collecting laboratory-provided, PFAS-free water into the laboratory-provided sample container after all field samples have been collected during the monitoring day.
3. If possible, field staff will collect samples starting at the station with the lowest expected PFAS concentrations and ending at the station with the highest expected PFAS concentrations. The high flow rate peristaltic pump with 3/8-inch ID silicone tubing will be decontaminated prior to collection of each field sample.
4. Fill the bottles (2x 250-mL and 1x 125-mL HDPE) at least one-half full from the source. Cap the bottle and invert to rinse the inside of the bottle. Discard the rinse water downstream of the sample collection site. Repeat for a total of three rinses before collecting the sample.
5. Samples will be stored together in a double-bagged plastic bag inside of a sample cooler. Wet ice will be bagged and used to keep the sample cool until delivery to the analytical laboratory. PFAS grab samples will not be stored in a cooler with other sampled parameters but may be stored with other PFAS samples collected across sample locations.

8.3. In-Line Sediment Trap Sample Collection

In-line sediment traps will be installed as described in the associated QAPP. Field staff shall wear new, powder-free nitrile gloves prior to handling the Norton sediment trap, sample bottles, or sample transfer materials.

1. Cap the bottles in-situ and then remove the bottles from the sediment traps.
2. Inspect sediment trap bottles for sediment accumulation. Estimate the volume of sediment by estimating the depth of accumulated sediment in the bottles and the inside diameter of the bottle and multiplying by the area equation for a cylinder (πr^2). The associated QAPP shall specify volume requirements for laboratory analysis. Each monitoring location will have three 1-liter glass sample bottles.
3. Dry the outside of bottles and apply sample labels.
4. Store the bottles in a cooler with ice for transport to the laboratory.
5. At the laboratory, composite the contents of the three sample bottles for each location. Specifically, transfer the sample volume from the sample bottles to a >6-liter, decontaminated stainless-steel bowl, using clean, decontaminated stainless steel equipment. Thoroughly mix the contents of the bowl and remove organic pieces larger than approximately 1 cm (i.e., leaves and twigs). Cover the bowl and let stand for 1 hour (see Sample Processing SOP).
6. Decant the excess water from the bowl and filter for Microplastics/TWP per the Sample Processing SOP.
7. Using a decontaminated stainless-steel spoon, transfer a 5- to 10-gram subsample of the solids slurry into a 250-mL widemouth HDPE sample bottle before being submitted to the laboratory for analysis.

9. Calculations

This section is not applicable to this SOP.

10. Quality Assurance/Quality Control

There are no specific quality assurance (QA) activities that apply to the implementation of these procedures. However, the following general quality control (QC) procedures apply:

1. All field conditions must be documented on field data sheets or within site logbooks.
2. The appropriate number and type of field QA samples need to be included in the sampling plan, in order to confirm that the sampling procedures employed were adequate.

11. Data Validation

This section is not applicable to this SOP.

12. Health and Safety

When working with potentially hazardous materials, follow EPA, OSHA, and corporate health and safety procedures. Before conducting sampling, a health and safety assessment should be conducted to identify site- and job-specific hazards. Health and safety considerations for PFAS stormwater grab sampling may include the following:

- The sampling team member collecting the sample should not get too close to the edge of impoundments where bank failure may cause them to lose their balance or open stormwater sewer structures.
- The person performing the sampling may need to be on a lifeline and wear adequate protective equipment.
- When conducting sampling in confined spaces, appropriate health and safety procedures for confined space entry must be followed.
- Necessity for traffic control and general awareness of traffic hazards should be assessed when conducting sampling near roadways.

13. References

Ecology. 2024. Collecting Grab Samples from Stormwater Discharges, Standard Operating Procedure, Version 1.2. Washington State Department of Ecology, Water Quality Program Olympia, Washington. (Publication 18-10-023).

Interstate Technology Regulatory Council (ITRC), 2023. Sampling Precautions and Laboratory Analytical Methods for Per- and Polyfluoroalkyl Substances (PFAS).

Michigan Department of Environment, Great Lakes, and Energy (EGLE). 2024. General PFAS Sampling Guidance. January. <https://www.michigan.gov/pfasresponse/-/media/Project/Websites/PFAS-Response/Sampling-Guidance/General.pdf?rev=6217442052bf4fedb89bbf786560d645>.

Standard Operating Procedures

6PPD-Quinone Stormwater Grab and Inline Sediment Sampling

Prepared by
Herrera Environmental Consultants, Inc.
2200 Sixth Avenue, Suite 1100
Seattle, Washington 98121
Telephone: 206-441-9080

May 14, 2025

HERRERA
Science + Planning + Design

Contents

1. Introduction, Scope, and Applicability

This standard operating procedure (SOP) is applicable to the collection of 6PPD-quinone (6PPDQ) stormwater grab and in-line sediment samples. These are standard (i.e., typically applicable) operating procedures that may be varied or changed, as required, dependent upon site conditions, equipment limitations, limitations imposed by the procedure, or other procedure limitations. In all instances, the procedures that are ultimately employed should be documented and associated with the final report.

The purpose of this SOP is to establish a uniform procedure for collecting 6PPDQ stormwater grab and in-line sediment samples in a variety of structures, including ditches, open channels, outfalls, pipes, catch basins, and sediment traps. This SOP does not describe selection, installation, and maintenance of sediment traps—these topics must be addressed on a site-by-site basis in the associated Quality Assurance Project Plans (QAPP).

2. Training

The procedures in this SOP are for use only by authorized personnel who have received specific training. Personnel conducting the sampling described in this SOP should have general training in field safety, familiarity with associated water quality parameters and procedures, and knowledge of all relevant components of the associated Quality Assurance Project Plan (QAPP).

3. Method Summary

6PPDQ stormwater grab samples are collected using procedures generally consistent with standard stormwater grab and in-line sediment trap sampling SOPs, including Washington State Department of Ecology (Ecology) SOPs *Collecting Grab Samples from Stormwater Discharges* and *Collection of Stormwater Solids Using In-Line Traps* (Ecology 2024a and 2024b). Special considerations for 6PPDQ sampling are outlined in Ecology's SOP *Sampling 6PPD-Quinone in Receiving Waters* (Ecology 2024c). Certain precautions must be taken to ensure that the sample is handled properly and that the correct sampling materials are used.

These precautions include using only laboratory-provided amber glass sample containers for stormwater grab samples, minimizing headspace in the sample container, minimizing exposure to light, maintaining low temperatures (on ice) following collection, and selecting appropriate materials (such as stainless steel) if intermediate sampling equipment is necessary. General sampling procedures include the following:

- Wear a fresh pair of powder-free nitrile gloves prior to sample collection.
- Label the sample bottle prior to sample collection.

- Remove the lid from the sample container immediately prior to sample collection, with care not to contaminate the sample container or lid.
- If standing in stormwater flow, face upstream and submerge the bottle to approximately half depth (if feasible for stormwater grab samples). Collect the sample volume until as little headspace as possible is visible. Replace the lid.
- Store the sample container, according to method requirements, out of direct sunlight and on ice (kept below 6^0 Celsius (C)).
- The use of a peristaltic pump to collect the sample is permitted. Pump tubing must be backflushed with lab-grade, deionized water prior to sample collection. To rinse the tubing prior to sample collection, 2 liters of pumped sample water shall be discharged onto the ground prior to collecting to sample.

4. Sample Preservation, Containers, Handling, and Storage

Once samples have been collected, the following procedure should be followed:

1. Place the sealed containers in coolers with ice and cool to $<6^0$ C. Ensure samples are not continuously exposed to direct sunlight, in order to maintain sample temperature and prevent photodegradation of 6PPD and 6PPDQ.
2. Record all pertinent data in the site logbook and on field data sheets.
3. Complete the Chain of Custody record.
4. Samples that are shipped to the project laboratory should be shipped overnight to ensure temperatures are met upon receipt.
5. Decontaminate all intermediate sampling equipment, if used, prior to the collection of additional samples.

5. Interferences and Potential Problems

6PPDQ concentrations can change over time due to processes including degradation of 6PPD or sorption to other materials. Because 6PPDQ at low levels is toxic to certain aquatic species, it is important to avoid materials or conditions that can cause small changes in 6PPDQ concentrations. Conditions to avoid include storing samples outside of the $<6^0$C range, exposing samples to extended periods of sunlight, or leaving excessive headspace in the sample bottle. Materials to which 6PPDQ has been shown to sorb should be avoided where possible. These materials include rubber, silicone, and, to a lesser extent, HDPE. However, the short exposure time to the silicone in a peristaltic pump is permissible because it has been shown to not adversely impact 6PPDQ concentrations (Herrera 2024).

6. Equipment/Apparatus

At minimum, equipment needed for collection of 6PPDQ stormwater grab and in-line sediment trap samples includes the following:

- Amber glass sample bottles or jars
  - Grab samples of water should be collected in 2x 250-mL amber glass bottles.
- Powder-free nitrile gloves
- Ice
- Coolers
- Chain of Custody records
- Field data sheets or other recordkeeping equipment
- Sample bottle labels
- Safety equipment including personal protective equipment designated in the Health and Safety Plan

Additional equipment that may be necessary for collection of 6PPDQ stormwater grab and in-line sediment trap samples may include the following:

- Decontamination equipment
- Peristaltic pump with associated tubing
- Stainless-steel sample dipper pole
- Stainless-steel spoon or other sediment transfer tool
- Confined space entry equipment including tripod, winch, harness, 4-gas meter
- Maps/plot plan
- Ziploc bags
- Tape measure
- Camera
- Hand tools for brush removal or monitoring station access
- Tools and equipment for accessing in-line sediment traps

7. Reagents

Reagents may be used for decontamination of sampling equipment, but reagents are not required to preserve sample volumes. The sampling plan or QAPP should specify reagents required for decontamination and handling and disposal procedures.

8. Procedures

8.1. Preparation

1. Determine the extent of the sampling effort and the types and amounts of equipment and supplies needed.
2. Obtain the necessary sampling and monitoring equipment.
3. Clean all intermediate sampling equipment, if needed.
4. Prepare scheduling and coordinate with staff, clients, and regulatory agency, if appropriate.
5. Perform a general site survey prior to site entry, in accordance with the site-specific Health and Safety Plan. For in-line sediment trap retrieval, confined space entry may be required.
6. Stakes or flagging may be used to mark sampling locations.
7. Care should be taken not to disturb sediment at the sample location, if present.

8.2. Grab Stormwater Sample Collection

Prior to sample collection, the field technician will put on a new set of powder-free, nitrile gloves.

1. Stage equipment at the sampling location and prepare to collect samples. Ensure field staff are safely accessing the structure and have solid footing.
2. Remove the lid from the sample container, ensuring not to contaminate the bottle or lid.
3. If sampling from a stormwater outfall, place the sample bottle directly in the center of the discharge, if feasible, and completely fill the container. If sampling directly from discharge is not feasible, attach the sample bottle to an extension pole or use a peristaltic pump to transfer the volume to the sample bottle.
4. Fill the bottle at least one-half full from the source. Cap the bottle and invert to rinse the inside of the bottle. Discard the rinse water downstream of the sample collection site. Repeat for a total of three rinses before collecting the sample.
5. Fill the sample bottle to overfilling.
6. Seal the container with the lid. Ensure there is not significant dirt or debris on the outside of the sample container by quickly rinsing the container in sample volume, if feasible.
7. Store samples per procedures outlined in Section 4.0.

8.3. Inline Sediment Trap Sample Collection

In-line sediment traps will be installed as described in the associated QAPP. Field staff shall wear new, powder-free nitrile gloves prior to handling the Norton sediment trap, sample bottles, or sample transfer materials.

1. Cap the bottles in-situ and then remove the bottles from the sediment traps.
2. Inspect sediment trap bottles for sediment accumulation. Estimate the volume of sediment by estimating the depth of accumulated sediment in the bottles and the inside diameter of the bottle and multiplying by the area equation for a cylinder (πr^2). The associated QAPP shall specify volume requirements for laboratory analysis. Each monitoring location will have three 1-liter glass sample bottles.
3. Dry the outside of bottles and apply sample labels.
4. Store the bottles in a cooler with ice for transport to the laboratory.
5. At the laboratory, composite the contents of the three sample bottles for each location. Specifically, transfer the sample volume from the sample bottles to a >6-liter, decontaminated stainless-steel bowl, using clean, decontaminated stainless steel equipment. Thoroughly mix the contents of the bowl and remove organic pieces larger than approximately 1 cm (i.e., leaves and twigs). Cover the bowl and let stand for 1 hour (see Sample Processing SOP).
6. Decant the excess water from the bowl and filter for Microplastics/TWP per the Sample Processing SOP.
7. Using a decontaminated stainless-steel spoon, transfer a 5- to 10-gram subsample of the solids slurry into an 8-oz amber glass jar before being submitted to the laboratory for analysis.

9. Calculations

This section is not applicable to this SOP.

10. Quality Assurance/Quality Control

There are no specific quality assurance (QA) activities that apply to the implementation of these procedures. However, the following general quality control (QC) procedures apply:

1. All field conditions must be documented on field data sheets or within site logbooks.
2. The appropriate number and type of field QA samples need to be included in the sampling plan, in order to confirm that the sampling procedures employed were adequate.

11. Data Validation

This section is not applicable to this SOP.

12. Health and Safety

When working with potentially hazardous materials, follow EPA, OSHA, and corporate health and safety procedures. Before conducting sampling, a health and safety assessment should be conducted to identify site- and job-specific hazards. Health and safety considerations for PFAS stormwater grab sampling may include the following:

- The sampling team member collecting the sample should not get too close to the edge of impoundments where bank failure may cause them to lose their balance or open stormwater sewer structures.
- The person performing the sampling may need to be on a lifeline and wear adequate protective equipment.
- When conducting sampling in confined spaces, appropriate health and safety procedures for confined space entry must be followed.
- Necessity for traffic control and general awareness of traffic hazards should be assessed when conducting sampling near roadways.

13. References

Ecology. 2024a. Collecting Grab Samples from Stormwater Discharges, Standard Operating Procedure, Version 1.2. Publication #18-10-023. Washington State Department of Ecology, Water Quality Program Olympia, Washington.

Ecology. 2024b. Collection of Stormwater Solids Using In-Line Traps, Standard Operating Procedure, Version 2.1. Publication #18-10-025. Washington State Department of Ecology, Water Quality Program Olympia, Washington.

Ecology. 2024c. Standard Operating Procedure EAP093, Version 1.0: Sampling 6PPD-Quinone in Receiving Waters. Publication #24-03-205. Washington State Department of Ecology, Environmental Assessment Program, Olympia, Washington.

Herrera. 2024. Technical Memorandum: 6PPDQ Roadway Runoff Stormwater Composite Sampling Protocol Recommendation. Prepared for Washington State Department of Ecology, by Herrera Environmental Consultants, Inc., Seattle, Washington.

Standard Operating Procedure

6PPD-Quinone by Triple Quadrupole LC-MS/MS, EPA Draft Method 1634
January 2024

City of Tacoma
Environmental Services Laboratory

DocuSigned by:
Tom Chontofalsky
9B21F7BAB0E54D5...

09/26/2024

Tom Chontofalsky
Environmental Lab Scientist II – Author

Date

Signed by:
Monica Herbert
9E03038E4A0C412...

09/25/2024

Monica Herbert
Environmental Lab Scientist III – Organics Lead

Date

DocuSigned by:
Terri Torres
EBC808004E434A0...

09/24/2024

Terri Torres
Environmental Lab Scientist III – QA Officer

Date

DocuSigned by:
Tiffany Ryan
9D4B4D82A1DE4B7...

09/24/2024

Tiffany Ryan
Assistant Division Manager

Date

Disclaimer:
Please note that the City of Tacoma's Environmental Services Laboratory Standard Operating Procedures (SOPs) are adapted from published methods. They are intended for internal use only and are specific to the equipment, personnel, and samples analyzed at the Environmental Services Laboratory. This SOP is not intended for use by other laboratories, nor does it supplant official published methods. Distribution of this SOP does not constitute an endorsement of a particular procedure or method.

This document is uncontrolled after printing. The official approved version is accessed through the laboratory's document management system.

Any reference to specific equipment, manufacturer, or supplies is for descriptive purposes only and does not constitute an endorsement of a particular product or service by the author or by the City of Tacoma.

Although the lab follows the SOP in most instances, there may be instances in which the lab uses an alternative methodology or procedure with quality assurance and management approval. Deviation will include documentation through the use of non-conforming work documents.

SOP Revision History

Revision Date	Rev Number	Summary of Changes	Sections	Reviser(s)
7/3/2023	1	New SOP	All	Tom, Mark, Terri, Jeff, and Michelle
2/7/2024	2	Updated to include draft EPA method 1634 reference and criteria	1,2,4,6,7,8,9,10,13	Terri, Monica
6/6/2024	3	Column update.	5	Monica
9/12/2024	4	Added modification table, clarified spikes		Tom, Terri

Related Documents

SOPs, Methods, or Manuals that support this procedure
1008_Standard and Reagent Preparation and Documentation
EPA Functional Guidelines
Variance Memo
Chemical Hygiene Health and Safety Plan
SOP 1005 Corrective Action
SOP 1017 Control Charts
1026_Manual Integration
EPA Draft Method 1634, Determination of 6-PPD-Quinone in Aqueous Matrices Using Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS)
EPA Definition and Procedure for Determination of the Method Detection Limit, Revision 2

Related Records

logs, labels, bench sheets, or report templates associated with this procedure
SDS Repository
Element\Print\bch_COT_OrgPrep_v1.rpt
Element\Laboratory\Standards
Element\Print\lex_COT_default.rpt
Element\Print\cub_default

1.0 Scope and Application

1.1 Method 1634 is for the determination of 6PPD-Quinone (6PPD-Q) in aqueous matrices, predominantly stormwater and surface water by liquid chromatography/mass spectrometry (LC-MS/MS). 6PPD-Q is formed in the environment from the conversion of the tire additive N-(1,3-dimethylbutyl)-N'-phenyl-*p*-phenylenediamine (PPD) in the presence of ozone.

1.2 The method calibrates and quantifies 6PPD-Quinone using retention time, a precursor ion, a product quantifier ion, at least 1 product qualifier ion, and the ratio between these two product ions.

1.3 The analysis portion of this method is to be used by, or under the direct supervision of analysts experienced in the use of Agilent chromatography (LC-MS/MS) systems, and Mass Hunter software.

2.0 Summary of Method

2.1 Aqueous samples are prepared and extracted using 250 mL of sample spiked with isotopically labeled 6PPD-Quinone (extracted internal standard, EIS) D5-6PPD-Quinone. Samples are extracted using solid phase Extraction (SPE).

2.2 The extract (SPE eluent) is spiked with the non-extracted internal standard (NIS or IIS) $13C_6$-6PPD-Quinone solution and injected on the high-performance liquid chromatograph (HPLC) equipped with a C18 column interfaced to a tandem mass spectrometer (LC/MS/MS) in the multiple reaction monitoring (MRM) mode. The 6PPD-Q is identified by comparing the acquisition of the mass transitions and retention time to reference spectra and retention time for the calibration standards acquired under identical LC/MS/MS conditions.

2.2.1 Quantitative determination of 6PPD-Q concentration is made using the primary quantitation ion (Q1) with respect to the EIS, and the results for 6PPD-Q are recovery corrected by the isotope dilution method.

2.2.2 The EIS recoveries are determined similarly against the NIS and are used as general indicators of overall analytical quality.

3.0 Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in liquid chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory method blanks.

3.2 Data from all blanks, samples, and spikes must be evaluated for interferences. Determine if the source of interference is in the preparation and/or cleanup of the samples and take corrective action to eliminate the problem.

3.3 Cross contamination may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes. After analysis of a sample containing high concentrations of analytes, one or more laboratory method blanks should be analyzed.

3.4 Matrix interference may be caused by contaminants that are present in the sample. The extent of matrix interference will vary considerably from sample to sample, depending on the source sampled. Positive identifications must be confirmed by retention times, precursor ions, product ions, and product ion ratios. Samples can exhibit matrix suppression so extracting a subsample or dilution of the extract may be necessary to minimize the matrix interference.

4.0 Safety

4.1 Refer to City of Tacoma, Environmental Services Laboratory's "Chemical Hygiene Health and Safety Plan", current revision, for standard lab safety practices.

4.2 Appropriate Personal Protective Equipment (PPE) must be worn at all times. When in the laboratory, safety goggles must be worn at a minimum. When handling chemicals, reagents, or samples, a laboratory coat and gloves must be worn. Oven-safe gloves are located near the glassware oven and must be worn when handling hot glassware.

4.3 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level.

4.3.1 Pure standards should be handled by trained personnel, with suitable protection to skin and eyes, and care should be taken not to breathe the vapors or ingest the materials.

4.3.2 Solutions must be prepared in a hood, following universal safety measures. Make certain the vacuum exhaust hose used during the filtering is securely anchored inside of a fume hood to prevent vapors from being released into the working environment.

4.3.3 Acetonitrile – Flammable, Poison, Irritant.

4.3.4 Formic Acid – Flammable, Corrosive, Toxic, Irritant.

4.3.5 Methanol – Flammable, Poison, Irritant.

4.3.6 Hexane – Flammable, Irritant.

4.4 Read all applicable Safety Data Sheets (SDS) before using this procedure. SDS are found in paper format in the red binders across from room 220, or in electronic format at \\fspwes01\GROUP\Lab ES\Documentation\MSDS\SDSList.accdb

5.0 Equipment and Supplies

5.1 Vacuum manifold for SPE Cartridges

5.2 SPE extraction disks – Agilent Bond Elut HLB, 200 mg, 6 mL tube – (Agilent Cat # 561-2146) or Bakerbond Speedisk H2O-Philic DVB (8072-07) or equivalent

5.3 15 mL Polypropylene Centrifuge Tubes with caps (eluent collection), Agilent Cat #5610-2039 or equivalent.

5.4 Disposable polypropylene collection tubes (13 x 100 mm, 8 mL)

5.5 Syringes and Pipettes – assorted sizes.

5.6 Class A volumetric flasks of various sizes.

5.7 12 mL amber screw top vials with Teflon™ lined screw tops.

5.8 2 mL autosampler vials with crimp-top caps or screwcaps.

5.9 Glass wool, silane-treated (Agilent Cat # 8500-1572 or equivalent)

5.10 Disposable glass pipets

5.11 Bottles, HDPE or glass, with HDPE or polypropylene caps. Various sizes. To store prepared reagents.

5.12 Liquid chromatography, triple quadrupole mass spectrometer system (LC-MS/MS). Thermo Scientific Quantiva, Agilent 6470, or equivalent.

5.13 Analytical column – A reverse phase column: Agilent InfinityLab Poroshell 120 EC-C18, 2.1 x 50 mm, 1.9 um (p/n 699675-902) Agilent Masshunter data acquisition and processing system - capable of controlling the LC-MS/MS and the continuous acquisition of all mass spectra and ions obtained throughout the duration of the chromatographic program.

6.0 Reagents and Standards

Note: Reagent and Standard preparations are recorded in Element® following SOP 1008 Standard and Reagent Preparation and Documentation.

6.1 Deionized water (DI) – LCMS grade.

6.2 Methanol – HPLC grade or equivalent.

6.3 Acetonitrile – HPLC grade or equivalent.

6.4 Hexane – Pesticide grade or equivalent.

6.5 Formic acid - (greater than 96% purity or equivalent), verified by lot number before use, store at room temperature.

6.6 Aqueous reagent A: Acetonitrile with 0.1% Formic Acid – Add 1mL Formic Acid to a final volume of 1L of Acetonitrile. Reagent can be purchased premade. Store at room temperature, shelf life 2 months.

6.7 Aqueous reagent B: DI Water with 0.1% Formic Acid – Add 1mL Formic Acid to a final volume of 1L of DI water (section 6.1). Reagent can be purchased premade. Store at room temperature, shelf life 2 months.

6.8 Mass labeled surrogate/extractable internal standard (EIS) Stock. D5-6PPD-Quinone: HPC Standards 688151 or equivalent. Store according to vendor specifications.

6.8.1 EIS/SS Intermediate (2000 ng/mL): Dilute 40 uL EIS Stock (6.1) to 2 mL with Acetonitrile.

6.8.2 EIS/SS Spike (200 ng/mL): Dilute 200 uL of EIS Intermediate (6.1.1) to 2 mL with Acetonitrile.

6.9 Stock labeled injection internal standard solution (IIS) Stock. 13C6-6PPD-Quinone: Cambridge Isotope laboratories, Inc. CLM-12293-1.2 or equivalent. Store according to

vendor specifications.

6.9.1 IIS Intermediate (2000 ng/mL): Dilute 40 uL of IIS Stock (6.9) to 2 mL with Acetonitrile.

6.9.2 IIS Spike (20 ng/mL): Dilute 10 uL of IIS Intermediate (6.9.1) to 2 mL with Acetonitrile. 10 uL of IIS Spike is added to 1 mL of extract prior to analysis.

6.10 6PPD-Quinone Stock (**100 ug/mL)**: Certified standard stock solutions from certified standard vendors (Cambridge Isotope Laboratories ULM-12288-1.2, or equivalent). Store according to vendor specifications.

6.10.1 6PPD-Quinone Intermediate Stock (1000 ng/mL): Prepare standard by diluting 20 µL of the stock standard solution (6.10) to 2 mL with Acetonitrile.

6.10.2 6PPD-Quinone ICAL Standard (5 ng/mL): Prepare standard by diluting 5 µL of the Intermediate Stock solution (6.10.1) to 1 mL with Acetonitrile.

6.10.3 ICAL Standards: Dilute in acetonitrile the 6PPD-Quinone ICAL Std or Intermediate Stock, to the calibration concentrations and add 10 uL of EIS Spike (6.8.2) to a final concentration of 2 ng/mL. The suggested ICAL concentrations are 0.025, 0.1, 0.5, 1, 2, 5, 10, 25, 50, and 100 ng/mL. Any standard, surrogate, or spike concentrations may differ from those stated in this SOP.

Calibration Level	µL of ICAL Std (6.10.2)	uL of Intermediate Std (6.10.1)	µL of EIS Spike (6.8.2)	uL of Acetonitrile
CAL1 (0.025 ppb iCal)*	5		10	985
CAL2 (0.1 ppb iCal)	20		10	970
CAL3 (0.5 ppb iCal)	100		10	890
CAL4 (1 ppb iCal)	200		10	790
CAL5 (2 ppb iCal)**	400		10	590
CAL6 (5 ppb iCal)		5	10	985
CAL7 (10 ppb iCal)		10	10	980
CAL8 (25 ppb iCal)		25	10	965
CAL9 (50 ppb iCal)		50	10	940
CAL10 (100 ppb iCal)		100	10	890

See Table 2 for more details
*Concentration used for LCV
** Concentration used for CCV

6.11 200 ppb Target Work Spike for BS/LCS/MS/MSD: Dilute 200 uL of 6PPD-Quinone Intermediate Stock (6.10.1) to 1 mL with Acetonitrile.

6.12 25 ppb MRL Check Spike: Dilute 125 uL of Target Work Spike (6.11) to 1 mL with Acetonitrile.

6.13 6PPD-Quinone second calibration verification stock (SCV **100 ug/mL.**): Certified standard stock solution HPC Standards 688152, or equivalent). Store according to vendor specifications. Note this solution is from a second-source different from the calibration standard.

6.13.1 6PPD-Quinone Intermediate Verification Stock (1000 ng/mL): Prepare standard by diluting 20 µL of the stock standard solution (6.13) to 2 mL with Acetonitrile.

6.13.2 6PPD-Quinone ICAL Verification Standard (2 ng/mL): Prepare standard by diluting 2 µL of the Intermediate Verification Stock solution (6.13.1) and 10 uL of EIS (6.8.2) to 1 mL with Acetonitrile.

6.14 6PPD-Quinone calibration verification stock (CCV): Prepare standard by diluting 2 µL of the Intermediate Stock solution (6.10.1) and 10 uL of EIS (6.8.2) to 1 mL with Acetonitrile.

6.15 6PPD-Quinone Instrument Sensitivity Check / Low-Level CCV stock (LCV): Prepare standard by diluting 5 µL of the ICAL Standard (6.10.1) and 10 uL of EIS (6.8.2) to 1 mL with Acetonitrile.

6.16 When not being used, store standard solutions in the dark at less than 6 °C, but not frozen, unless the vendor recommends otherwise, in screw-capped vials with PTFE-lined caps. The laboratory must maintain records of the certificates for all standards, as well as records for the preparation of intermediate and working standards, for traceability purposes.

6.17 Allow all solutions to warm to room temperature prior to use. Mix using a vortex mixer prior to taking aliquots for use. Standards should not be used past the expiration date listed on the standard.

7.0 Sample Collection, Preservation, and Handling

7.1 Samples are collected in 250 mL amber glass with PTFE lined caps free of headspace. The samplers should acquire pre-cleaned bottles from the analytical laboratory for sampling.

7.2 All samples are protected from light, iced, or refrigerated at ≤ 6 °C from the time of collection until receipt by the laboratory. Once received the samples may be stored protected from light at 0 - 6 ºC until sample preparation. After extraction sample extracts should be stored protected from light, in the refrigerator at 0 - 6 ºC while not being analyzed.

7.3 Aqueous samples must be extracted within 14 days from sample collection.

7.4 Extracts must be analyzed within 28 days from extraction.

8.0 Quality Control and Method Performance

8.1 Initial Demonstration of Capability (DOC) is performed by each analyst once prior to reporting sample results and to demonstrate any modifications to the method meet equivalency requirements (see sections 9.1.2.1 and 9.2 of the method). The DOC is repeated if a major change is made to the extraction, analysis method or equipment or for any modifications to the method. DOC consists of the analysis of four replicates of the Laboratory Control Sample. A least one method blank must also be included. All sample processing steps that are to be used for processing samples, including preparation, extraction and concentration, must be included in this test. Follow SOP 1017_Control Charts to generate the DOC recovery chart with average recovery and standard deviation. At least one method blank, matching the matrix being analyzed, must be prepared with the DOC batch. All sample processing steps that are to be used

for processing samples, including preparation and extraction, cleanup and concentration, must be included in this test. The DOC is acceptable if the relative standard deviation (RSD) and average % recoveries are within the limits for initial precision and recovery (70-130% recovery and 20% RSD). If analytes fail to meet these requirements, the test may be repeated only for those analytes that failed. IDOC/DOC data is stored in the analyst's training folder in as a PDF file in Training Records.

8.2 Method Detection Limit (MDL) is defined as the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results. It is determined the first time the method is performed on each instrument and repeated annually, or if there is a major change in the procedure or equipment. For new instrumentation, analyze a minimum of two spiked replicates and two method blank replicates on the new instrument. If both method blank results are below the existing MDL, then the existing MDL is validated. Combine the new spiked sample results to the existing spiked sample results and recalculate the MDL_s. If the recalculated MDL_s is within 0.5 - 2.0 times the existing MDL, then the existing MDL is validated. For ongoing annual verification, perform at least 7 replicates, two per quarter per instrument is suggested, enter these samples as MRL Check Samples in the preparation bench sheet.

8.2.1 The MDL_s is recalculated every 13 months using the previous 2 years of MDL_s data as:

$$\mathrm{MDL_s} = \mathrm{t}_{(\mathrm{n-1,\ 1-\alpha=.99})} S_s$$

where:

$t_{(n-1,\ 1-\alpha\ =\ 0.99)}$ = the Student's *t*-value appropriate for the single-tailed 99th percentile *t* statistic and a standard deviation estimate with n-1 degrees of freedom.

S_s = sample standard deviation of the replicate spiked sample analyses

8.2.2 Additionally, the MDLb is determined every 13 months using Method Blank (MB) data for the prior two years to include all MBs associated with the analysis (but not those associated with rejected or re-analyzed client samples); the laboratory has the option to use only the last 6 months of method blank data, or the 50 most recent method blanks, whichever is greater. If none of the method blanks give numerical results for an individual analyte, the MDLb does not apply. If some (but not all) of the method blanks for an individual analyte give numerical results, set the MDLb equal to the highest method blank result. If more than 100 method blanks are available, set MDLb to the level that is no less than the 99th percentile of the method blank results. If all of the method blanks for an individual analyte give numerical results, then calculate the MDLb as:

$$\mathrm{MDL_b} = \overline{\mathrm{X}} + \mathrm{t}_{(\mathrm{n-1,\ 1-\alpha=.99})} S_b$$

where:

$\overline{X}$= mean of the method blank results (use zero if the mean is negative)

$t_{(n-1,\ 1-\alpha\ =\ 0.99)}$ = the Student's *t*-value appropriate for the single-tailed 99th percentile *t* statistic and a standard deviation estimate with n-1 degrees of freedom.

S_b = sample standard deviation of the replicate method blank sample analyses

8.2.3 The verified MDL is the greater of the MDL_s or MDL_b. If the verified MDL is within 0.5 to 2.0 times the existing MDL, and fewer than 3% of the method blank results (for the individual analyte) have numerical results above the existing MDL, then the existing MDL may be left unchanged. Otherwise, adjust the MDL to the new verification MDL. (The range of 0.5 to 2.0 approximates the 95th percentile confidence interval for the initial MDL determination with six degrees of freedom.)

8.2.4 Add spike standard at a concentration of at or below current MRL level but above the MDL to each batch. Analyze this MDL Check (Batch-MRL#) in the same manner as samples. MDL results are imported to Element/DataTool for the MDL calculation. MDL data is stored on \\fspwes01\Transfer\Organic MDLs as a PDF file. For additional details, refer to Definition and Procedure for Determination of the Method Detection Limit, Revision 2 and the laboratory SOP 1016 Determining and Applying Method Detection Limits.

8.3 Blind to the Analyst (BTTA) Study is performed annually at a minimum.

8.3.1 A 250 mL sample made from DI water is spiked by a chemist who will not perform the extraction or analysis at a concentration known only by that chemist and the QA Manager.

8.3.1.1 Measure 250 mL of DI water into a 250 mL amber glass with PTFE lined cap.

8.3.1.2 Spike the sample with a known quantity of standard using gas tight syringe.

8.3.1.3 The chemist must document how the samples was made, including volumes, concentrations, standards, IDs, date made, sample concentration and who made the sample.

8.3.2 The sample will be logged into LIMS for 6PPD-Q analysis, labeled and placed in the cooler as any other client sample. The sample is to be extracted and analyzed like any other client sample.

8.3.3 Follow the normal review process for the BTTA sample as would be performed for any other sample. Make sure to include a variance memo for any QC failures, as would be done for any other sample.

8.3.4 Following extraction and analysis the QA manager will review the results and grade the sample reports. The graded report is submitted by the QA manager to Washington Department of Ecology.

8.1 Instrumental Blanks (acetonitrile only): Prior to the start of the analytical sequence, analyze an instrument blank (acetonitrile only) to ensure no instrument contamination has occurred. In addition, an instrument blank should be analyzed any time carryover contamination is suspected (i.e., after a sample with high analyte concentrations is analyzed), and when new lots of solvent are purchased.

8.2 Method Blank (Batch#-BLK#): Analyze at least one method blank (MB/BLK) per sample batch or at least 5% of samples prepared – equivalent to 1 per 20 field samples or less. The MB is made up using DI water and extracted using the same preparation procedure as the associated samples. The blanks must be free from contamination at a concentration at 1/2 the reporting limit (RL). If contamination is shown in the Method Blank, halt sample analysis and correct the issue. Samples affected by the Method Blank should be re-analyzed, or qualified if re-analysis is not possible.

8.2.1 If the blank contains a concentration greater than the MDL and the sample concentration is less than the reporting limit, report the MRL value with a "U".

8.2.2 If the sample concentration is greater than or equal to 5 times the blank value but less than 10 times the blank value, qualify the datum with a “J”.

8.2.3 If the sample concentration is less than or equal to 5 times the blank value, qualify the datum with a “UJ”.

8.2.4 If gross contamination exists in the blank, positive sample results may require rejection and be qualified as unusable "R". Non-detected target compounds do not require qualification unless the contamination is so high that it interferes with the analyses of non-detected compounds. Unusable data may require re-extraction.

8.2.5 Complete a QC Variance form for any result requiring qualification.

8.3 Laboratory Control Sample (LCS) or Blank Spike (Batch#-BS#): A laboratory control sample (LCS) is spiked and analyzed at a frequency of one per batch. The acceptable recoveries are currently 70-130%. Re-analyze samples associated with any LCS failures or qualify the data where reanalysis is not possible. After the analysis of 30 LCS samples, the limits are updated on a regular basis based on 2 standard deviations from the mean.

8.3.1 Reanalyze the LCS if recovery is outside the criteria after evaluating whether system maintenance could improve recovery and taking any actions indicated. Analyze a second LCS if one was extracted with the batch. Evaluate repeat analyses only for the analytes that failed the initial analysis. Consult with Environmental Laboratory Scientist (ELS) III if recovery is still outside the criteria to determine whether re-extraction is possible within sample holding times. Report data associated with the best recovery. Delete results in Element® for non-reported LCS, leaving one internal standard as an indication of the additional analysis.

8.3.2 Qualify results with a “J” for detects and “R” for non-detects if the LCS recovery is less than the lower recovery limit. Complete a QC Variance form.

8.3.3 Results qualified as rejected (“R”) are not usable for regulatory purposes. Notify the Project Client for further action.

8.3.4 Qualify results with a “J” for detects and complete a QC Variance form if the LCS recovery exceeds the upper recovery limit. Do not qualify non-detects.

8.3.5 Complete a QC Variance form for any result requiring qualification.

8.4 Matrix Spike/Matrix Spike Duplicate (Batch#-MS# and Batch#-MSD#): ***A matrix spike is analyzed only if requested by the client.*** The spike recoveries should be within laboratory control limits of 50-150%.

8.4.1 Do not qualify results if the sample concentration exceeds the spike concentration by a factor of four or more. Do not report the MS/MSD if the parent sample concentration exceeds the calibration.

8.4.2 Reanalyze MS or MSD if recovery is outside the criteria after evaluating whether LC/MS-MS system maintenance could improve recovery and taking any actions indicated. Consult with the senior analyst if recovery is still outside the criteria to determine whether re-extraction is possible within sample holding times. Report data associated with the best recovery. Delete results in Element for non-reported MS/MSD leaving an internal standard as an indication of the additional analysis.

8.4.3 If the MS and MSD recoveries are less than the lower recovery limit or ≤10%, whichever is lower, qualify results of the source sample with "J" for detects and "R" for non-detects. Qualify non-detects as "UJ" if recoveries are less than the lower recovery limit but not less than 10%. Complete a QC Variance form.

8.4.4 If the MS and MSD recoveries exceed the upper recovery limit, qualify results of the parent MS/MSD sample with a "J" for detects and complete a QC Variance form. Do not qualify non-detects.

8.4.5 If the RPD exceeds the limit, qualify detected analyte results of the source MS/MSD sample with a "J", and complete a QC Variance form. Do not qualify non-detects.

8.4.6 Complete a QC Variance form for any result outside control limits or any MS/MSD not reported due to concentration of parent sample.

8.5 Sample duplicate (Batch#-DUP#). ***A duplicate is analyzed only if requested by the client.*** The duplicate relative percent difference (RPD) should be less than or equal to 40% for sample duplicates and matrix spike duplicates. If the RPD fails due to inhomogeneity or matrix interference, qualify the failing analytes in the source sample.

8.6 Extracted (EIS)/Surrogate: To assess method performance on the sample matrix, the laboratory must spike all samples with the isotopically labeled compound standard solution (EIS) and all sample extracts with the non-extracted Internal Standards (IIS) spiking solution. The recovery limits for the EIS are 25-200%. After the analysis of 30 samples, the limits are updated on a regular basis based on 2 standard deviations from the mean.

8.6.1 Analyze each sample according to the procedures in Section 10.0. Peak responses of the quantitation and confirmation ions must be at least three times the background noise level (S/N 3:1). The quantitation ion must have a S/N ≥ 10:1 if there is no confirmation ion. Retention times must fall within ± 0.4 minutes of the predicted retention times from the midpoint standard of the ICAL or opening CCV, whichever was used to establish the RT window

position for the analytical batch. Compute the percent recovery of the isotopically labeled compound using the non-extracted internal standard method. The recovery of must be within 25-200% or current limits.

8.6.2 If the recovery of the EIS falls outside of these limits, method performance is unacceptable for 6PPD-Q in that sample. Additional cleanup procedures must then be employed to attempt to bring the recovery within the normal range. If the recovery cannot be brought within the normal range, the extracts may be diluted.

8.6.2.1 Samples may be diluted only aif the EIS meet the S/N and retention time requirements and still recover at greater than 5%. For example, if the EIS recovery of the affected analyte in the undiluted analysis is 50%, then the sample cannot be diluted more than 10:1; if the EIS recovery of the affected analyte in the undiluted analysis is 30%, then the sample cannot be diluted more than 6:1. If sample extract dilution does not correct the recovery, a new extraction should be performed.

8.6.3 For water matrices with a backup sample container that is the same sample volume or higher than the original, a higher amount of EIS spike is recommended. If there is no secondary container available, a new sample may need to be collected. If neither of these approaches solve the recovery issue, consult with the lab project manager and QA manager on how to proceed.

8.6.4 If after dilution and/or re-extraction, any EIS recovery is still outside limits and there is no further sample available for extraction or dilution, qualify the 6PPD-Q in the analysis as indicated below. Use professional judgment if sample dilution is a factor in EIS recovery.

8.6.4.1 For target analyte detections where the recovery exceeds the upper recovery limit qualify results as estimated, “J". Do not qualify non-detects.

8.6.4.2 If the recovery is less than the lower recovery limit but greater than 10% or the lower control limit in Table 8 whichever is less, qualify results as estimated “J".

8.6.4.3 If the recovery is less than 10% or the lower limit in Table 8 whichever is less, qualify results as “J" for detects and “R” for non-detects.

8.7 Non-Extracted Internal Standards (IIS): Retention times must fall within ± 0.4 minutes of the predicted retention times from the midpoint standard of the ICAL or opening CCV, whichever was used to establish the RT window position for the analytical batch. The IIS area in the field samples and QC samples must be within 50-200% of the most recent CCV, the most recent CCV must be within 50-200% of the mid-point o of the calibration.

8.7.1 If sample dilution is required, the NIS response in the diluted extract is no longer required to be within ±50% of the response (peak area) in the most recent CCV.

8.8 Control Charts – Control charts are maintained in Element® according to SOP 1017_Control Charts.

8.9 Corrective Action – QC results outside of limits and deviations from the SOP are documented according to SOP 1005_Corrective Action.

8.10 See a QC guidance summary table in Appendix A.

9.0 Calibration and Standardization

9.1 Calibration is required whenever the laboratory takes an action that changes the chromatographic conditions or might change or affect the initial calibration criteria, or if either the CCV or Instrument Sensitivity Check (LCV) acceptance criteria have not been met.

9.2 The instrument must have a valid mass calibration following the manufacturer specified procedure prior to initial sample analysis and at least annually following this initial calibration. The mass calibration is updated on an as-needed basis (QC failures, ion masses fall outside of the instrument required mass window or major instrument maintenance). Record maintenance in Element® Instrument Maintenance Log under the instruments name.

9.3 Mass calibration must be verified after each mass calibration, prior to any sample analysis. Mass calibration must be performed per the instrument manufacturer's instructions. A mass calibration verification must be performed using standards whose mass range brackets the masses of interest (quantitative and qualitative ions). Record maintenance in Element® Instrument Maintenance Log under the instruments name.

9.4 Instrument Tune

9.4.1 Perform a check tune prior to an initial calibration to monitor the instrument status. The check tune requirements are set by the manufacturer and are noted on the check tune report.

9.4.2 If there are more than 10 parameters out of spec or MS2 abundance for 2122 ion is less than 15000, check the tune solution and spray nozzle and/or adjust the failing tune parameter in manual tune. Perform another check tune. If this fails, then instrument maintenance and/or a full autotune are required.

9.4.3 All check tunes are accessible via the MassHunter acquisition software.

9.1 Perform initial calibration and repeat if any major changes or maintenance (column removal or replacement, etc.) are performed or if continuing calibration fails. Record maintenance in Element® Instrument Maintenance Log under the instruments name.

9.1.1 Prepare calibration standards at a minimum of seven concentration levels with the lowest standard at or below the MRL (this point can be used as the instrument sensitivity check (see section 9.4). The initial calibration solutions contain the entire suite of EIS and NIS compounds, and target analytes. All solvents used in standard preparation need to be LCMS grade or better.

9.1.2 Sufficient instrument sensitivity is established if a signal-to-noise ratio ≥ 3:1 for the quantification ions and the confirmation ions for the target analyte, or ≥ 10:1 for the EIS and NIS, which only have a quantification ion, can be achieved when analyzing the lowest concentration standard within the quantitation range that the laboratory includes in its assessment of calibration linearity. .

9.1.3 Calculations are performed by the instrument's software. Agilent MassHunter Software has many options for calibration curves which may be used. The calibration must meet or exceed one of the following two criteria. *Note that when using a weighted regression or non-linear regression calibration, the regression must be weighted inversely proportional to concentration and must not be forced through zero. Additionally, when using a weighted regression calibration, linearity must be assessed using Option 2*:

Option 1: Calculate the relative standard deviation (RSD) of the RF values of the initial calibration standards. The RSD must be ≤ 20% to establish instrument linearity.

$$mean\ RR\ or\ RF = \frac{\sum_{i=1}^{n}(RR\ or\ RF)i}{n}$$

$$SD = \sqrt{\frac{\sum_{i=1}^{n}(RR\ or\ RFi - mean\ RR\ or\ RF)^2}{\mathrm{n}}}$$

$$RSD - \frac{SD}{mean}x100$$

where:
RR or RFi = RR or RF for calibration standard i
n = Number of calibration standards

Option 2: Calculate the relative standard error (RSE) of the initial calibration standards. The RSE for all method analytes must be ≤ 20% to establish instrument linearity.

$$RSE = 100\ x \sqrt{\sum_{i=1}^{n} \frac{\left[\frac{x'_i - x_i}{x_i}\right]^2}{n-p}}$$

where,
x_i = Nominal concentration (true value) of each calibration standard
x'_i = Measured concentration of each calibration standard
n = Number of standard levels in the curve
p = Type of curve (2 = linear, 3 = quadratic)

9.1.4 Response factor (RF) for the Extracted Internal Standard (EIS) compound:

$$RF_s = \frac{A_{EIS}}{A_{NIS}} \times \frac{C_{NIS}}{C_{EIS}}$$

where: A_{EIS} = Area of EIS

C_{EIS} = Concentration of EIS

A_{NIS} = Area of Non-Extracted Internal Standard (NIS)

C_{NIS}= Concentration of NIS

9.2 Non-extracted Internal Standard Area

9.2.1 Each time an initial calibration is performed, use the data from all the initial calibration standards used to meet the linearity test in Section 9.1. Record the area for the NIS compound of the mid-point of the calibration for use in evaluating opening CCV results for sample analyses.

9.1 Retention Time window position and width (Once per ICAL and at the beginning of the analytical sequence).

9.1.1 Position of method analyte, EIS analyte, and IIS analyte peaks shall be set using the midpoint standard of the ICAL curve when ICAL is performed. On days when ICAL is not performed, the initial CCV retention times or the midpoint standard of the ICAL curve can be used to establish the RT window position.

9.1.2 Method analyte, EIS analyte, and IIS analyte RTs must fall within 0.4 minutes of the predicted retention times from the midpoint standard of the ICAL or initial daily CCV, whichever was used to establish the RT window position for the analytical batch.

9.2 Independent Calibration Verification (ICV/SCV). The initial calibration curve for each target analyte must be checked immediately with a standard from a source different from that used for the initial calibration, preferably an alternate vendor is used (section 6.6.2). If an alternate vendor is not available, a different lot number from the same vendor may be used.

9.2.1 Analyze the ICV standard directly after calibration. The ICV is evaluated with every new calibration curve that is analyzed.

9.2.2 The analyte recovery should be within +/- 30% of the expected concentration. If the ICV does not meet quality criteria, the instrument may be recalibrated. If the ICV failed due to problems other than calibration, remake the standard and reanalyze. If the ICV passes, continue the sequence. If it fails, abort the sequence, determine the problem, and recalibrate the instrument. On a case-by-case basis, per client and supervisor approval, samples associated with an ICV not meeting acceptance limits can be reported so long as they are addressed in the case narrative and qualified as estimates.

9.3 Back Calculation (Residuals). Re-calculate each standard concentration level using the updated, and passing, calibration curve. The percent difference between the calculated concentration and the expected concentration for each analyte at that level should not be more than 30%; except for the lowest standard used in the curve, where 10% of analytes are allowed to be within 50%. If these requirements are not met, the ICAL for those analytes fails and should be reanalyzed.

9.3.1 If the initial calibration does not meet the acceptance criteria, any sample results associated with the out-of-control analyte(s) should be qualified as estimated “J.”

9.4 Instrument Sensitivity Check / Low-Level Continuing Calibration Verification (LCV).

9.4.1 Analyze a Low-Level continuing calibration verification (LCV) standard at a minimum prior to the analysis of samples and blanks. The LCV analyzed is a MRL-level standard and should be prepared from the same source as calibration standards. The acceptance range is +/- 50%, signal-to-noise ≥ 3:1 for quantitation and confirmation ions and an ion ratio of ± 50%. If a LCV does not meet quality criteria, analysis must be halted, and the sensitivity of the LC/MS/MS system adjusted before analysis of field or QC samples

9.5 Continuing calibration Verification (CCV).

9.5.1 Analyze a continuing calibration verification (CCV) standard at a minimum prior to the analysis of samples and blanks, at the end of an analytical run sequence containing samples, and after every 10 field samples. The exception is after an acceptable initial calibration is run, when 10 samples may be analyzed before a CCV is required. The CCV analyzed is a mid-level standard and should be prepared from the same source as calibration standards. The acceptance range is +/- 30%. If a CCV does not meet quality criteria, recalibrate the instrument and reanalyze all samples following the failing CCV. If an analyte in the CCV failed because of high recovery, but that analyte was not detected in a sample extract, then that sample extract need not be reanalyzed. On a case-by-case basis, samples associated with a CCV not meeting acceptance limits can be reported as long as they are qualified as estimates. Note draft EPA method 1634 uses the term calibration verification (VER) instead of CCV.

9.5.2 Complete a QC Variance form for any result requiring qualification.

9.6 The lowest level calibration standard must meet a signal-to-noise ratio of 3:1 for the quantitation ions and confirmation ions, and 10:1 for quantitation ions that have no confirmation ion and be at a concentration less than or equal to the LOQ.

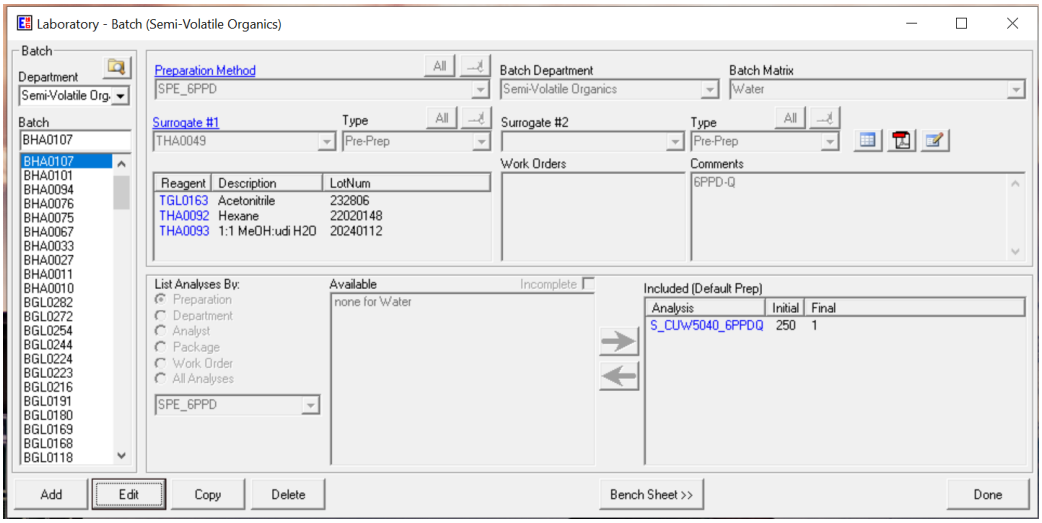
10.0 Procedure

10.1 Preparation of samples:

10.1.1 Cleaning of glassware, tools, and surfaces: Satisfactory cleaning may be accomplished by rinsing with acetonitrile as needed andwashing with DI water.

10.1.2 Review Extraction Bench Sheets **prior** to sample preparation. Environmental Laboratory Scientist (ELS) II or III signs and dates on the Analyst Approval line. Note in the sample comments column on the bench sheet.

10.1.2.1 In Element: Laboratory > Batch > Add



Select the appropriate Preparation Method, Batch Matrix, Surrogate and Analysis. Add reagents by right clicking in the Reagent box.

Save > Bench Sheet>>

The Bench Sheet will be called up. Select Edit > Add to add client samples by container, and any applicable QC samples. Right click highlighted samples to change Initial/Final volumes, Surrogate Amounts, Spike IDs/amounts and select Source Samples for all MS/MSD and DUP (if required).

Select Save, then the print icon.

10.1.3 Allow all solutions to warm to room temperature prior to use. Mix using a vortex mixer prior to taking aliquots for use. Standards may be scaled up or down as needed. Standards should not be used past the expiration date listed on the standard.

10.1.4 Typical sample size is 250 mL. The entire sample must be extracted. Sub-sampling is allowed (with qualification), but only for matrix problems or high 6PPD-Quinone samples and with the approval of the lab project manager. Seal the sample bottles with screw caps and mix the contents well by inverting and shaking before loading onto the SPE cartridge.

10.1.5 Mark the meniscus on the extraction jar for later determination of sample volume extracted. After extraction, fill bottle to the meniscus line with water and measure the volume. Record the initial (mL) volume on the bench sheet and enter into Element™ Prepare a MB, LCS and MRL. These must be of the same volume as a typical sample in the batch (250 mL).

10.1.6 Spike all samples with 100 µL of EIS (D5-6PPD-Quinone surrogate) (section 6.8.2). Spike the LCS (and MS/MSD if requested) with 100 µL of the Target Work Spike (section 6.11). Spike the MRL Check with 100 µL of the MRL Check Spike standard (section 6.12).

10.1.7 The samples are now ready for extraction.

10.1.8 Prepare the SPE Cartridge manifold with solvent collection and sample collection waste bottles.

10.1.9 Place a Bond Elut HLB 6cc (200 mg) extraction cartridge on the SPE Cartridge vacuum extraction manifold for each sample and QC. If the samples contain a high level of suspended solids pack clean salinized glass wool to half the height of the available space in the SPE barrels.

10.1.10 Condition the cartridge with 5 mL of Acetonitrile followed at a flow rate of approximately 2.5-3 mL/min. Follow this with 10 mL of DI water but do not let the cartridge go dry following the DI water conditioning step.

10.1.11 Load the samples into the reservoirs and elute through the cartridges at 2.5-3 mLs/min. Care should be taken not to allow cartridge to go dry.

10.1.12 Rinse the sample bottle with 10 mLs of DI water and transfer the bottle rinse to the SPE reservoir, washing the walls of the reservoir and elute through the cartridges at 2.5-3 mLs/min.

10.1.13 Rinse the SPE cartridge with approx. 5 mL 1:1 MeOH:DI water then 5 mL of Hexane. Increase the vacuum to maximum for at least 5 minutes to dry the SPE cartridge.

10.1.14 Remove from vacuum and place a 15 mL poly centrifuge tube under each SPE cartridge to use as eluent collection tube.

10.1.15 Add 5mL of Acetonitrile to the sample bottle. Cap and shake well to extract any analytes from the inside surface. Add this to the SPE reservoir and elute.

10.1.16 Set sample bottles aside to fully dry once all rinse is poured into the HLB SPE reservoir.

10.1.17 Elute the sample from the HLB SPE cartridge with an additional 5 mL Acetonitrile collecting the eluent.

10.1.18 Bring to a final volume of 10 mL and transfer to a 12 mL screw top vial.

10.1.19 The sample is now ready for analysis.

10.2 Prepare the samples by transferring 1 mL of the sample extract into an autosampler vial. To each 1 mL volume, add 10 µL of 13C6-6PPD-Quinone non-extracted internal standard NIS (IIS) spiking solution (6.9.2). Cap the vial, shake or vortex to mix well, and then transfer to the LC/MS-MS for analysis.

10.3 If the Mass detector system has been turned off: turn on the mass detector.

10.4 If needed, clean source prior to analysis. Be sure to cool source before opening, then rinse and wipe down interior of the spray chamber with isopropyl alcohol. Sonicate source transfer tube in a mixture of 50% DI water and 50% isopropyl alcohol. Dry the transfer tube before installing. Maintenance may vary depending on the level of

cleanliness. See manufacture user guide.

10.5 See Appendix A for maintenance information.

10.6 Turn on autosampler, pump and degas unit. If the HPLC has been idle and the reservoirs are empty or new reagent has been added to the reservoir(s), prime the pumps by opening the pump bypass valve and pressing the purge button. Close valve after pump is primed. Priming procedure may vary between instruments.

10.7 Start Triple Quadrupole (Agilent MassHunter) software. Ensure that all systems are communicating, and status lights are green. Load the correct acquisition software method.

10.8 Instrument Setup. See Appendix B for a printout of the current method.

10.9 Recommended analytical run sequence is:

- Instrument Blank
- Instrument Sensitivity Check/ Low-Level CCV (LCV)
- CCV
- Method Blank
- LCS
- Up to 10 injections of sample extracts, diluted extracts, laboratory duplicate extracts and MS/MSD extracts (if requested)
- CCV
- Instrument Blank
- Up to 10 more injections of sample extracts, diluted extracts, laboratory duplicate extracts, and MS/MSD extracts (if requested)
- CCV

10.10 Qualitative Identification of Target Compounds

10.10.1 Target compound identification is made by precursor and product ions as well as retention time matching. A current laboratory-generated standard must be present and compared.

10.10.2 Using available software, search for the target compound in the established retention time window. Examine chromatograms and determine if a positive identification is present. See references for more information.

10.10.3 Examine baseline and peak integration to insure proper area integration. If the compound is present but not properly integrated, then manually integrate the peak. Retain the original peak integration with the properly integrated peak with the sample data.

10.10.4 Examine transition and all product ions for confirmation ions to further validate the compound identification.

10.10.5 If there is evidence of retention time shift, use relative retention to the surrogate or internal standard along with confirming ions to validate the identification.

10.10.6 Technical Acceptance Criteria is determined by qualitative analysis of ion retention times, transition ions (precursor and product ions),

chromatography, and ion abundance ratios.

10.10.7 Peak responses for 6PPD-Q must be at least three times the background noise level (signal-to-noise ratio [S/N] ≥3:1) and the EIS response must have S/N of at least 10:1. If the S/N ratio is not met due to high background noise, the laboratory must correct the issue (e.g., perform instrument troubleshooting and any necessary maintenance , such as cleaning the ion source, or replacing the LC column) and the instrument must be recalibrated (Section 10). If the S/N ratio is not met, but the background is low, then the analyte is to be considered a non-detect.

10.10.8 6PPD-Q must elute within ± 0.1 minutes of the EIS.

10.10.9 The relative retention times (RRTs) must be within ±0.4 RRT units of the standard RRT. Use professional judgment when there is a question if 0.4 RRT units may be too broad, or too narrow.

10.10.10 Verify the presence of product ions and check their corresponding ratios of the analyte in the sample. The acceptance window for the ion abundance ratio of each target analyte above the MRL is 50% to 150% of the mid-point calibration standard. The acceptance window for the ion abundance ratio of each target analyte below the MRL is 50% to 150% of the daily CCV standard. The relative response ratio is calculated by dividing the qualifier/confirmation ion area by the quantifier ion area.

10.11 Quantitative analysis of target analytes

10.11.1 When a compound has been identified, the quantification of that compound will be calculated using extracted internal standard (isotope dilution) calibration. Isotope dilution calibration requires the determination of relative response (RR) defined by the following equation.

$$RR = \frac{A_t(C_t)}{A_{EIS}(C_{EIS})}$$

Where:

A_t = Target Compound Area

C_t = Target Compound Concentration

A_{EIS} = Extracted Internal Standard Area

C_{EIS} = Extracted Internal Standard Concentration

10.11.2 For the analytes determined by isotope dilution, the relative response (RR) (labeled to native) vs. concentration in the calibration solutions is computed over the calibration range according to the procedures described below. A minimum of six calibration points are employed. The calculation of each analyte concentration, relative to its labeled analog, is determined using the area responses of the primary product ions for each calibration standard. This calculation is usually done by the analytical software.

10.11.3 Keep manual integration of peaks to a minimum following SOP

1026 Manual Integration.

10.11.4 If the response for any compound exceeds the highest calibration standard, dilute the extract (see section 6.19), and reanalyze. Target dilutions to fall between the mid-point and top of the calibration range. Adjust the volume of internal standard added proportionately to the volume of solvent added when diluting an extract already spiked with internal standard.

10.11.5 Calculate the concentration of each identified analyte in the sample as follows:

10.11.6 Liquids

$$C_F = \frac{C_I(V_F)(D)}{V_I}$$

Where: C_F = Final Concentration (ug/L)

C_I = On Column Concentration (ng/mL)

V_F = Final Volume of Extract (mL)

D = Dilution Factor

V_I = Initial Volume of Sample (mL)

10.11.7 Solids

$$C_F = \frac{C_I(V_F)(D)(1000)}{w\left(\frac{S}{100}\right)}$$

Where: C_F = Final Concentration (ug/kg)

C_I = On Column Concentration (ng/mL)

V_F = Final Volume of Extract (mL)

D = Dilution Factor

w = Weight of Sample Extracted (g)

S = Percent Solids

11.0 Pollution Prevention and Waste Management

11.1 Store vials for disposal containing sample extracts or expired standards in the designated waste containers for this satellite area until disposed of by lab pack in accordance with the Dangerous Waste Management Plan. Keep in-use waste disposal container under the hood in Room 230. This is the designated satellite collection area for this waste stream. When waste container is full, notify the Hazardous Waste Manager for removal to Hazardous Waste Storage area. Refer to the waste stream fact sheets for details: CH1130589 (Organic extractions waste & expired standards in vials).

11.2 Collect waste solvents in an appropriate waste container and dispose of in accordance with the Dangerous Waste Management Plan. Refer to the waste stream fact sheet for

details: CH573177 (Waste solvents).

11.3 Post-extraction sample wastewater is disposed of immediately following extraction by filtering through bucket containing charcoal and then drained into the lab sink in accordance with WSFS 0005 (non-hazardous liquid waste, carbon treated).

12.0 References

12.1 EPA Draft Method 1634 January 2024

12.2 US EPA Functional Guidelines: http://www.epa.gov/sites/production/files/2015-03/documents/somnfg.pdf

12.3 City of Tacoma Environmental Services Chemical Hygiene Health and Safety Plan, current revision

12.4 City of Tacoma Environmental Services Quality Assurance Manual, current revision

12.5 City of Tacoma Environmental Services Dangerous Waste Disposal Manual, current revision

13.0 Tables, Diagrams, Flowcharts and Validation Data

Appendix A: Method Modifications

Appendix B: QC guidance table

Appendix C: Routine Maintenance Schedule

Appendix D: Instrument Setup

Appendix A: Method Modifications

1634 Method Section(s)	SOP Modification	ES Lab SOP section(s)
2.1, 7.2.2, 7.2.4, 9.2.1.1, 9.3.3, 11.1.2, 13.3.1, 13.3.2, 13.4.2, 20.2	The EIS used by the Tacoma Environmental Services Laboratory is *D5-6PPD-Quinone* not 13C6-6PPD-Q as listed in the method.	2.1, 6.8, 10.1.6
2.2, 7.2.2, 7.2.5, 9.2.1.1, 11.3.1, 13.3.2, 20.2	The NIS *or IIS* used by the Tacoma Environmental Services Laboratory is *$13C_6$-6PPD-Quinone* not D5-6PPD-Q as listed in the method.	2.2, 6.9, 10.2
7.1.6	The Tacoma Environmental Services Laboratory uses Acetonitrile with *0.1%* Formic Acid instead of the method solution of Acetonitrile with 0.2% Formic Acid	6.6
7.1.7	The Tacoma Environmental Services Laboratory uses DI water with *0.1%* Formic Acid instead of the method solution of reagent water with 0.2% Formic Acid.	6.7
7.1.8 and 7.1.9	The Tacoma Environmental Services Laboratory does not use *Acetic acid* for this method. Acetic Acid is listed in the Reagents and Standards section of the method only.	
7.1.10, 7.1.11 and 6.0	The Tacoma Environmental Services Laboratory uses a rinse of *acetonitrile* as needed and *DI water* to clean extraction equipment. *DI water* is used for QC checks instead of NH4OH/acetonitrile solution used by the method. *Note that Method Blanks are used as QC checks by the Tacoma Environmental Services Laboratory*.	8.2, 10.1.122
10.4.3.4, 13.8, 14.3.2	The method lists both evaluation of the IIS (NIS) against the average of the ICAL and against the most recent CCV (VER). Additionally due to software limitations the Tacoma Environmental Services Laboratory is unable to evaluate to the mean of the ICAL. The Tacoma Environmental Services Laboratory evaluates the opening CCV against the *mid-point* of the ICAL and *samples and QC against the opening CCV.*	8.7, 9.2.1 and Appendix B
11.1.1	*The Tacoma Environmental Services Laboratory marks the meniscus on the extraction jar* for later determination of initial sample volume extracted instead of determining the initial sample volume gravimetrically per the method.	10.1.5
11.2.7, 11.2.8	The Tacoma Environmental Services Laboratory rinses the sample bottle with *10 mLs of reagent water and then 5 mL of Acetonitrile,* instead of the methods guidance of 5 mL of 50:50 methanol:reagent water prior to drying SPE cartridge.	10.1.12
	The Tacoma Environmental Services Laboratory rinses the SPE with 5 mL of MeOH:water followed by 5 mL of Hexane. The method includes the 5 mL of MeOH:water as the bottle rinse but does not use Hexane at all.	10.1.13
11.2.10 and 11.2.11	The Tacoma Environmental Services Laboratory uses an *additional 5 mL Acetonitrile* to elute the SPE column but does not rinse the bottle with this additional Acetonitrile volume per the method. Additionally, the method only calls for an additional 4 mls of Acetonitrile. The Tacoma Environmental Services Laboratory brings the final volume of extract to 10 mL, the method does not adjust the final volume.	10.1.17 and 10.1.18
11.3.1	The Tacoma Environmental Services Laboratory *transfers 1 mL of the sample extract into an autosampler vial and then adds the NIS/IIS*. The method adds the NIS to the entire extract final volume.	10.4.8

Method Terminology	Equivalent SOP Terminology
IPR	IDC/DOC
OPR	LCS
VER	ICV or CCV
NIS	IIS or NIS
EIS	EIS or Surrogate
ICS	LCV

Appendix B: QC guidance table

QC Parameter	Frequency	Criteria	Corrective Action	Qualification
Mass Calibration	The mass calibration must be performed at least annually on an as-needed basis (e.g., QC failures, ion masses fall outside of the instrument required mass window, major instrument maintenance, or if the instrument is move).	Instrument must have a valid mass calibration following the manufacturer specified procedure prior to any sample analysis.	Problem must be corrected. No samples may be analyzed under a failing mass calibration.	NA
Mass Calibration Verification	Mass calibration must be verified after each mass calibration, prior to any sample analysis. Mass calibration must be performed per the instrument manufacturer's instructions.	A mass calibration verification must be performed using standards whose mass range brackets the masses of interest (quantitative and qualitative ions). Check the mass calibration by measuring the amount of peak drift from the expected masses. If the peak apex has shifted more than approximately 0.2 Da, recalibrate the mass axis following the manufacturer's instructions.	Problem must be corrected. No samples may be analyzed under a failing mass calibration verification.	NA
Retention Time (RT) window position establishment	Once per ICAL and at the beginning of the analytical sequence.	Position of method analyte, EIS analyte, and IIS analyte peaks shall be set using the midpoint standard of the ICAL curve when ICAL is performed. On days when ICAL is not performed, the initial CCV retention times or the midpoint standard of the ICAL curve can be used to establish the RT window position.	Correct problem and reanalyze samples.	NA
Retention Time (RT) window width	Once per ICAL and at the beginning of the analytical sequence.	6PPD-Q must fall within ± 0.1 minutes of the NIS/IIS. 66PP-Q, NIS/IIS and EIS also must fall within 0.4 minutes of the predicted retention times from the midpoint standard of the ICAL or initial daily CCV.	Correct problem and reanalyze samples.	NA

QC Parameter	Frequency	Criteria	Corrective Action	Qualification
Initial Calibration (ICAL)	At instrument set-up and after ICV or CCV failure, prior to sample analysis, at a minimum annually.	Initial calibration is performed using a series of at least six solutions, with at least five of the six calibration standards being within the quantification range, and with the lowest standard at or below the LOQ. (If a second-order calibration model is used, then one additional concentration is required, with at least six of the seven calibration standards within the quantitation range.) The initial calibration solutions contain the EIS, IIS and target compound. Sufficient instrument sensitivity is established if a signal-to-noise ratio ≥ 3:1 for the quantitation ions and the confirmation ions, or ≥ 10:1 if the analyte only has a quantitation ion, can be achieved when analyzing the lowest concentration standard within the quantitation range that the laboratory includes in its assessment of calibration linearity ICAL must meet one of the two options below: Option 1: The RSD of the RFs or RRs for all method analytes, isotopically labeled compounds and EIS must be ≤20%. Option 2: The% RSE for all method analytes, isotopically labeled compounds and EIS must be ≤20%.	No samples shall be analyzed until ICAL has passed.	NA
Extracted Internal Standard (EIS)	Must be added to every field sample, standard, blank, and QC sample.	Recovery of the EIS is calculated by internal standard quantification against the IIS using an Response Ratios or Response Factors (See equation section 9.1.4). Recovery criteria for EIS is 25-200%.	Correct problem. If required, re-extract and reanalyze associated field and QC samples. If recoveries are acceptable for QC samples, but not field samples, the field samples must be re-extracted and analyzed (greater dilution may be needed).	Apply J qualifier and discuss in the Case Narrative only if reanalysis confirms failures.
Injection Internal Standards (IIS)	Must be added to every prepared field sample, standard, blank, and QC sample prior to instrumental analysis. IIS analyte recovery is calculated determined against the average IIS analyte area of the ICAL standards.	Retention times must fall within ± 0.4 minutes of the predicted retention times from the midpoint standard of the ICAL or opening CCV, whichever was used to establish the RT window position for the analytical batch. The IIS area in the field samples and QC samples must be within 50-200% of the most recent CCV, the most recent CCV must be within 50-200% of the area of	Correct problem and reanalyze samples.	NA

QC Parameter	Frequency	Criteria	Corrective Action	Qualification
		the mid-point of the calibration.		
Initial Calibration Verification (ICV):	After each Initial Calibration (ICAL), prior to sample analysis; analyze a second source standard.	Calculated concentration must be within ±30% of the true value.	No samples shall be analyzed until calibration has been verified.	NA
Continuing Calibration Verification (CCV)	Prior to sample analysis, after every 10 field samples, and at the end of the analytical sequence.	Calculated concentrations must be within ±30% of their true value.	Correct problem and reanalyze samples. If reanalysis cannot be preformed, data must be qualified and explained in a variance memo.	Apply J qualifier to all results for the specific analyte(s) in all samples since the lab acceptable CCV.
Instrument Sensitivity Check / Low-Level Continuing Calibration Verification (LCV)	Prior to ICAL or sample analysis	The signal-to-noise ratio of ≥ 3:1 for the quantification ions and the confirmation ions for the target analyte and ≥ 10:1 for the EIS and NIS. Calculated concentration of 6PPD-Q must be within ±50% of true value.	Correct problem and reanalyze samples.	NA
Instrumental Blanks (acetonitrile only)	Immediately following the highest standard analyzed, following any sample with a high concentration where carryover is suspected, each new solvent lot and daily prior to sample analysis.	Concentration target analyte detected must be ≤ the LOQ.	No samples shall be analyzed until instrument blank has met acceptance criteria. Note: Successful analysis following the highest standard analyzed determines the highest concentration that carryover does not occur.	NA
Method Blank (MB)	One per preparatory batch.	No method analyte can be detected ½ > MRL or >1/10th the amount measured in field samples in the batch, whichever is greater.	Correct problem, re-extract and reanalyze MB and all QC samples and field samples processed with the contaminated blank. If reanalysis cannot be performed, data must be qualified and explained in a	Apply J qualifier to all results for the specific analyte(s) in all samples in the associated preparatory batch.

QC Parameter	Frequency	Criteria	Corrective Action	Qualification
			variance memo.	
Laboratory Control Sample/Laboratory Control Sample Duplicate (LCS/LCSD)	One per preparatory batch.	Blank spiked with the target analyte. Recovery criteria is 70-130%.	Correct problem, re-extract and reanalyze LCS and all QC samples and field samples for failed analytes if sufficient sample material is available. If reanalysis cannot be performed, data must be qualified and explained in a variance memo.	Apply J qualifier to all results for the specific analyte(s) in all samples in the associated preparatory batch.
MRL Check Standard	One per preparatory batch.	Blank spiked with all analytes at concentrations one to two times the LOQ. Recovery criteria is 40-160%.	Correct problem, re-extract and reanalyze LCS and all QC samples and field samples for failed analytes if sufficient sample material is available. If reanalysis cannot be performed, data must be qualified and explained in a variance memo.	Apply J qualifier to all results for the specific analyte(s) in all samples in the associated preparatory batch.

Appendix C. Routine Maintenance Schedule:

Daily:

- Prime both pump channels prior to running.
- Fill Eluent bottles, needle wash bottle, seal wash bottle.
- Rinse the ionization chamber with wet (isopropyl alcohol) kim wipe. Check that Instrument Tune is not expired.
- Empty eluent collection bottle.

Check:

- Source Pressure (3.5-4.0 torr is normal)
- High Vac number (9.4×10^{-5} torr is normal)
- Collision cell gas on (1.7×10^{-5} torr is normal)

Weekly:

- Check and drain rough pump reservoir.
- Clean and replace ionization transfer capillary.
- Clean ionization chamber cones.

As Required:

- Update Software
- Run instrument tune
- Preventive Maintenance every year – performed by the instrument manufacturer.

Appendix D: Instrument Setup

Acquisition Method Report

Agilent | Trusted Answers

MS QQQ Mass Spectrometer

Ion Source	AJS ESI	**Tune File**	D:\MassHunter\Tune\QQQ \G6470B \Autotune_20231201_073728\atunes_20231201_083439.TUNE.XML
Stop Mode	No Limit/As Pump	**Stop Time (min)**	No limit
Time Filter	On	**Time Filter Width (min)**	0.05
LC->Waste Pre Row	N/A	**LC->Waste Post Row**	N/A

Time Segments

Index	Start Time (min)	Scan Type	Ion Mode	Div Valve	Delta EMV (+)	Store	Cycle Time (ms)	Triggered?	MRM Repeats
1	0.4	DynamicMRM	ESI+Agilent Jet Stream	To MS	400	Yes	500	No	3

Time Segment 1

Scan Segments

Cpd Group	Cpd Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Cell Acc (V)	Ret Time (min)	Ret Window
IIS	13C6-6-PPD-Quinone	Yes	305.1	Unit/Enh (6490)	247.1	Unit/Enh (6490)	110	36	4	4.7	3
IIS	13C6-6-PPD-Quinone	Yes	305.1	Unit/Enh (6490)	221.1	Unit/Enh (6490)	110	20	4	4.7	3
Target	6-PPD-Quinone	No	299.1	Unit/Enh (6490)	256.1	Unit/Enh (6490)	140	20	4	4.7	3
Target	6-PPD-Quinone	No	299.1	Unit/Enh (6490)	241.1	Unit/Enh (6490)	105	32	4	4.7	3
Target	6-PPD-Quinone	No	299.1	Unit/Enh (6490)	187.1	Unit/Enh (6490)	105	32	4	4.7	3
EIS	D5-6-PPD-Quinone	No	304.1	Unit/Enh (6490)	246.1	Unit/Enh (6490)	110	36	4	4.7	3
EIS	D5-6-PPD-Quinone	No	304.1	Unit/Enh (6490)	220.1	Unit/Enh (6490)	110	20	4	4.7	3
EIS	D5-6-PPD-Quinone	No	304.1	Unit/Enh (6490)	192.1	Unit/Enh (6490)	110	20	4	4.7	3

Source Parameters

Parameter	Value (+)	Value (-)
Gas Temp (°C)	300	300
Gas Flow (l/min)	10	10
Nebulizer (psi)	40	40
Sheath Gas Temp (°C)	375	375
Sheath Gas Flow (l/min)	11	11
Capillary (V)	2500	2500
Nozzle Voltage/Charging (V)	0	0

Scan Parameters

Data Stg	Threshold
Centroid	0

Chromatograms

Chrom Type	Label	Offset	Y-Range
TIC	TIC	0	1500000

Instrument Curves

Actual

Acquisition Method Report

Agilent | Trusted Answers

Sampling Speed	
Draw Speed	200.0 µL/min
Eject Speed	100.0 µL/min
Wait Time After Drawing	1.2 s
Injection	
Needle Wash Mode	Standard Wash
Injection Volume	5.00 µL
Standard Needle Wash	
Needle Wash Mode	Flush Port
Duration	3 s
High Throughput	
Injection Valve to Bypass for Delay Volume Reduction	No
Sample Flush-Out Factor	5.0
Overlapped Injection	
Overlap Injection Enabled	No
Needle Height Position	
Draw Position Offset	0.0 mm
Use Vial/Well Bottom Sensing	No
Stoptime	
Stoptime Mode	As Pump/No Limit
Posttime	
Posttime Mode	Off

Name: Binary Pump **Module: G7120A**

Flow	0.400 mL/min
Use Solvent Types	Yes
Stroke Mode	Synchronized
Low Pressure Limit	0.00 bar
High Pressure Limit	590.00 bar
Max. Flow Ramp Up	100.000 mL/min²
Max. Flow Ramp Down	100.000 mL/min²
Expected Mixer	Jet Weaver V35 Mixer
Stroke A	
Automatic Stroke Calculation A	Yes
Stoptime	
Stoptime Mode	Time set
Stoptime	4.40 min
Posttime	
Posttime Mode	Time set
Posttime	0.30 min

Solvent Composition

	Channel	Ch. 1 Solv.	Name 1	Ch. 2 Solv.	Name 2	Selected	Used	Percent (%)
1	A	100.0 % Acetonitrile V.03		100.0 % Acetonitrile V.03	ACN 0.1% Formic	Ch. 2	Yes	30.00 %
2	B	100.0 % Water V.03		100.0 % Water V.03	H2O 0.1% Formic Acid	Ch. 2	Yes	70.00 %

Timetable

	Time (min)	A (%)	B (%)	Flow (mL/min)
1	Start. Cond. min	30.00 %	70.00 %	0.400 mL/min
2	0.50 min	30.00 %	70.00 %	0.400 mL/min
3	4.25 min	70.00 %	30.00 %	0.400 mL/min
4	4.35 min	100.00 %	0.00 %	0.400 mL/min

Name: Column Comp. **Module: G7116B**

Left Temperature Control	
Temperature Control Mode	Not Controlled
Enable Analysis Left Temperature	

Standard Operating Procedure
6PPD-Quinone Soil Extraction

City of Tacoma
Environmental Services Laboratory

DocuSigned by:
Tom Chontofalsky
9B21E7BAB9E54D5...

09/24/2024

Tom Chontofalsky
Environmental Lab Scientist II – Author

Date

Signed by:
Fatima Alsinai
60AE08CEE557463...

09/24/2024

Fatima Alsinai
Environmental Lab Scientist In-training - Author

Date

DocuSigned by:
Monica Herbert
FF0A1B0442CB4DF...

09/24/2024

Monica Herbert
Environmental Lab Scientist III – Organics Lead

Date

DocuSigned by:
Terri Torres
FBC808064E434A6...

09/24/2024

Terri Torres
Environmental Lab Scientist III – QA Manager

Date

Signed by:
Tiffany Ryan
9D4B4D82A1DE4B7...

09/24/2024

Tiffany Ryan
Assistant Division Manager

Date

Disclaimer:
Please note that the City of Tacoma's Environmental Services Laboratory Standard Operating Procedures (SOPs) are adapted from published methods. They are intended for internal use only and are specific to the equipment, personnel, and samples analyzed at the Environmental Services Laboratory. This SOP is not intended for use by other laboratories, nor does it supplant official published methods. Distribution of this SOP does not constitute an endorsement of a particular procedure or method.

This document is uncontrolled after printing. The official approved version is accessed through the laboratory's document management system.

Any reference to specific equipment, manufacturer, or supplies is for descriptive purposes only and does not constitute an endorsement of a particular product or service by the author or by the City of Tacoma.

Although the lab follows the SOP in most instances, there may be instances in which the lab uses an alternative methodology or procedure with quality assurance and management approval. Deviation will include documentation through the use of non-conforming work documents.

SOP Revision History

Revision Date	Rev Number	Summary of Changes	Sections	Reviser(s)
	1	New SOP	All	

Related Documents

SOPs, Methods, or Manuals that support this procedure
1008_Standard and Reagent Preparation and Documentation
EPA Functional Guidelines
Variance Memo
Chemical Hygiene Health and Safety Plan
SOP 1005 Corrective Action
SOP 1017 Control Charts
EPA Draft Method 1634, Determination of 6-PPD-Quinone in Aqueous Matrices Using Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS)
EPA Definition and Procedure for Determination of the Method Detection Limit, Revision 2

Related Records

logs, labels, bench sheets, or report templates associated with this procedure
SDS Repository
Element\Print\bch_COT_OrgPrep_v1.rpt
Element\Laboratory\Standards
Element\Print\lex_COT_default.rpt
Element\Print\cub_default

1.0 Scope and Application

1.1 This SOP covers the extraction of 6PPD-Quinone (6PPD-Q) in solid matrices. 6PPD-Q is formed in the environment from the conversion of the tire additive N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (PPD) in the presence of ozone.

1.2 The analysis portion of this method, which is covered in SOP 5040 6PPD Quinone by Triple Quadrupole LC-MS-MS, is to be used by, or under the direct supervision of analysts experienced in the use of Agilent chromatography (LC-MS/MS) systems, and Mass Hunter software.

2.0 Summary of Method

2.1 Solid samples are prepared and extracted using 0.25 g of sample spiked with isotopically labeled 6PPD-Quinone (EIS) D5-6PPD-Quinone. Samples are extracted using sonication with Acetone.

2.2 The extract is spiked with the non-extracted internal standard (NIS or IIS) $13C_6$-6PPD-Quinone solution and injected on the liquid chromatograph (HPLC) equipped with a C18 column interfaced to a tandem mass spectrometer (LC/MS/MS) in the multiple reaction monitoring (MRM) mode. The 6PPD-Q is identified by comparing the acquisition of the mass transitions and retention time to reference spectra and retention time for the calibration standards acquired under identical LC/MS/MS conditions.

2.2.1 Quantitative determination of 6PPD-Q concentration is made using the primary quantitation ion (Q1) with respect to the EIS, and the results for 6PPD-Q are recovery corrected by the isotope dilution method.

2.2.2 The EIS recoveries are determined similarly against the NIS and are used as general indicators of overall analytical quality.

3.0 Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in liquid chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory method blanks.

3.2 Data from all blanks, samples, and spikes must be evaluated for interferences. Determine if the source of interference is in the preparation and/or cleanup of the samples and take corrective action to eliminate the problem.

3.3 Cross contamination may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes. After analysis of a sample containing high concentrations of analytes, one or more laboratory method blanks should be analyzed.

3.4 Matrix interference may be caused by contaminants that are present in the sample. The extent of matrix interference will vary considerably from sample to sample, depending on the source sampled. Positive identifications must be

confirmed by retention times, precursor ions, product ions, and product ion ratios. Samples can exhibit matrix suppression so extracting a subsample or dilution of the extract may be necessary to minimize the matrix interference.

4.0 Safety

4.1 Refer to City of Tacoma, Environmental Services Laboratory's "Chemical Hygiene Health and Safety Plan", current revision, for standard lab safety practices.

4.2 Appropriate Personal Protective Equipment (PPE) must be worn at all times. When in the laboratory, safety goggles must be worn at a minimum. When handling chemicals, reagents, or samples, a laboratory coat and gloves must be worn. Oven-safe gloves are located near the glassware oven and must be worn when handling hot glassware.

4.3 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level.

4.3.1 Pure standards should be handled by trained personnel, with suitable protection to skin and eyes, and care should be taken not to breathe the vapors or ingest the materials.

4.3.2 Solutions must be prepared in a hood, following universal safety measures. Make certain the vacuum exhaust hose used during the filtering is securely anchored inside of a fume hood to prevent vapors from being released into the working environment.

4.3.3 Acetonitrile – Flammable, Poison, Irritant.

4.3.4 Acetone – Flammable, Irritant

4.4 Read all applicable Safety Data Sheets (SDS) before using this procedure. SDS are found in paper format in the red binders across from room 220, or in electronic format at \\fspwes01\GROUP\Lab ES\Documentation\MSDS\SDSList.accdb

5.0 Equipment and Supplies

5.1 Analytical balance capable of accurately weighing to the nearest 0.1 mg.

5.2 4 mL amber screw top vials with Teflon™ lined screw tops.

5.3 8 mL amber screw top vials with Teflon™ lined screw tops.

5.4 Syringes and Pipettes – assorted sizes for the preparation of standards.

5.5 Nitrogen evaporation device - The N-Evap by Organomation Associates, Inc. may be used if the FMS™ PowerVap Concentrator is not available.

5.5.1 The N-Evap water bath does not need to be used, as all evaporation is done at room temperature.

5.6 4 oz Amber glass jar

5.7 12 mL amber screw top vials with Teflon™ lined screw tops.

5.8 2 mL amber screw top vials with Teflon™ lined screw tops.

5.9 Vortex mixer

5.10 Centrifuge

5.11 Ultrasonic cleaner

6.0 Reagents and Standards

Note: Reagent and Standard preparations are recorded in Element® following SOP 1008 Standard and Reagent Preparation and Documentation.

6.1 Acetonitrile – HPLC grade or equivalent.

6.2 Acetone – HPLC grade or equivalent

6.3 Calcium Bentonite Clay - Pharmaceutical Grade

6.4 Prepare calibration standards as indicated in SOP 5040_6PPD-Quinone Extraction and analysis by Triple Quadrupole LC-MS-MS, section 6.10.3 using the clay diluent in place of Acetonitrile.

6.4.1 Weigh 0.25 g of Bentonite clay into a 12 mL amber glass vial. Add 10 mL of Acetonitrile and vortex for 10-15 secs. Centrifuge for 5 mins at 2000 rpm. Pour solvent layer into a clean 12 mL amber glass vial.

6.5 Mass labeled surrogate/extractable internal standard (EIS). D5-6PPD-Quinone (100 ug/mL): HPC Standards 688151 or equivalent. Store according to vendor specifications.

6.5.1 EIS/SS Intermediate (2000 ng/mL): Dilute 20 uL EIS Stock to 1 mL with Acetonitrile.

6.5.2 EIS/SS Spike (200 ng/mL): Dilute 100 uL of EIS Intermediate to 1 mL with Acetonitrile.

6.6 Mass labeled injection internal standard solution (IIS). 13C6-6PPD-Quinone (100 ug/mL): Cambridge Isotope laboratories, Inc. CLM-12293-1.2 or equivalent. Store according to vendor specifications.

6.6.1 IIS Intermediate (2000 ng/mL): Dilute 20 uL of IIS Stock to 1 mL with Acetonitrile.

6.6.2 IIS Spike (20 ng/mL): Dilute 10 uL of IIS Intermediate to 1 mL with Acetonitrile. 10 uL of IIS Spike is added to 1 mL of extract prior to analysis.

6.7 6PPD-Quinone (100 ug/mL 6PPD-Quinone): Certified standard solutions from certified standard vendors (Cambridge Isotope Laboratories ULM-12288-1.2, or equivalent). Store according to vendor specifications.

6.7.1 6PPD-Quinone Intermediate Stock (1000 ng/mL): Prepare standard by diluting 20 µL of the stock standard solution to 2 mL with Acetonitrile.

6.8 Standard Reference Material (SRM)/Laboratory Control Sample (LCS): To prepare the SRM/LCS with a concentration of 25 ppb, weigh 1 g of clay (section 6.3) into a 4 mL amber glass vial and spike with 25 uL of the 1000ppb

6PPD_Quinone Intermediate Stock (6.7.1). Add 2 mL of Acetone per gram of clay and vortex for 10-15 secs. Completely dry the SRM by placing the vial under nitrogen flow using N-evap (at a rate of 4 LPM) at room temperature (do not submerge in the water bath). This process takes approximately 1 hour. Assign an expiration date matching the stock standard.

6.9 Method Detection Limit Check Sample (MRL): Prepare MRL check with a concentration of 5 ppb: weigh 1 g of clay (section 6.3) into a 4 mL amber glass vial and spike with 5 uL of the 1000ppb 6PPD_Quinone Intermediate Stock (6.7.1). Add 2 mL of Acetone per gram of clay and vortex for 10-15 secs. Completely dry the MRL by placing the vial under nitrogen flow using N-evap (at a rate of 4 LPM) at room temperature (do not submerge in the water bath). This process takes approximately 1 hour. The MRL Check standard should not be used past the expiration date listed on the stock standard.

6.10 When not being used, store standard solutions in the dark at less than 6 °C, but not frozen, unless the vendor recommends otherwise, in screw-capped vials with PTFE-lined caps. The laboratory must maintain records of the certificates for all standards, as well as records for the preparation of intermediate and working standards, for traceability purposes. Scan these as PDFs named with the Standard ID_01 into LIMS at fs005\element\PDF\Standards\.

6.11 Allow all solutions to warm to room temperature prior to use. Mix using a vortex mixer prior to taking aliquots for use. Standards should not be used past the expiration date listed on the standard.

7.0 Sample Collection, Preservation, and Handling

7.1 Collect samples in 4 oz (or larger) pre-cleaned, amber glass jars with Teflon lined lids.

7.2 All samples are protected from light, iced, or refrigerated at ≤ 6 °C from the time of collection until receipt by the laboratory. Once received, the samples may be stored protected from light, at < 6 ºC until sample preparation. After extraction, sample extracts should be stored protected from light, in the refrigerator at 0 – 6 ºC while not being analyzed.

7.3 Samples must be extracted within 14 days from sample collection or 1 year, if frozen -20°C.

7.4 Extracts must be analyzed within 28 days from extraction.

8.0 Quality Control and Method Performance

8.1 Initial Demonstration of Capability (DOC) is performed by each analyst once prior to reporting sample results. The DOC is repeated if a major change is made to the extraction, analysis method or equipment or for any modifications to the method. The DOC consists of the analysis of four replicates of the Laboratory Control Sample. A least one method blank must also be included. All sample processing steps that are used for processing samples, including preparation, extraction and concentration, must be included in this test. Follow SOP 1017_Control Charts to generate the DOC recovery chart with average recovery and standard deviation. The DOC is acceptable if the relative standard deviation (RSD) is less than 20% and the average recoveries are within 70-130%. If any analyte fails to meet these requirements, the test may be repeated only for those analytes that failed. DOC

data are stored in the analyst's training folder as a PDF file in Training Records.

8.2 Method Detection Limit (MDL) is defined as the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results. It is determined the first time the method is performed on each instrument and repeated annually, or if there is a major change in the procedure or equipment. For new instrumentation, analyze a minimum of two spiked replicates and two method blank replicates on the new instrument. If both method blank results are below the existing MDL, then the existing MDL is validated. Combine the new spiked sample results to the existing spiked sample results and recalculate the MDLs. If the recalculated MDLs is within 0.5 - 2.0 times the existing MDL, then the existing MDLs is validated. For ongoing annual verification, perform at least 7 replicates, two per quarter, per instrument is suggested. Enter these samples as MRL Check Samples in the preparation bench sheet. The MDLs is recalculated every 13 months using the previous 2 years of MDLs data as:

$$\mathrm{MDL_s} = \mathrm{t}_{(\mathrm{n-1,\ 1-\alpha=.99})} S_s$$

where:

$\mathrm{t}_{(\mathrm{n-1,\ 1-\alpha = 0.99})}$ = the Student's *t*-value appropriate for the single-tailed 99th percentile *t* statistic and a standard deviation estimate with n-1 degrees of freedom.

S_s = sample standard deviation of the replicate spiked sample analyses

Additionally, the $\mathrm{MDL_b}$ is determined every 13 months using Method Blank data for the prior two years to include all BLKs associated with the analysis (but not those associated with rejected or re-analyzed client samples); the laboratory has the option to use only the last 6 months of method blank data, or the 50 most recent method blanks, whichever is greater. If none of the method blanks give numerical results for an individual analyte, the $\mathrm{MDL_b}$ does not apply. If some (but not all) of the method blanks for an individual analyte give numerical results, set the $\mathrm{MDL_b}$ equal to the highest method blank result. If more than 100 method blanks are available, set $\mathrm{MDL_b}$ to the level that is no less than the 99th percentile of the method blank results. If all of the method blanks for an individual analyte give numerical results, then calculate the $\mathrm{MDL_b}$ as:

$$\mathrm{MDL_b} = \overline{\mathrm{X}} + \mathrm{t}_{(\mathrm{n-1,\ 1-\alpha=.99})} S_b$$

where:

$\overline{\mathrm{X}}$= mean of the method blank results (use zero if the mean is negative)

$\mathrm{t}_{(\mathrm{n-1,\ 1-\alpha = 0.99})}$ = the Student's *t*-value appropriate for the single-tailed 99th percentile *t* statistic and a standard deviation estimate with n-1 degrees of freedom.

S_b = sample standard deviation of the replicate method blank sample analyses

The verified MDL is the greater of the MDLs or MDLb. If the verified MDL is within 0.5 to 2.0 times the existing MDL, and fewer than 3% of the method blank results (for the individual analyte) have numerical results above the existing MDL, then the existing MDL may be left unchanged. Otherwise, adjust the MDL to the new verification MDL. (The range of 0.5 to 2.0 approximates the 95th percentile confidence interval for the initial MDL determination with six degrees of freedom.)

8.3 Create a MRL standard (section 6.9). Extract and analyze the MRL Check in the same manner as samples. MDL results are imported to Element/DataTool for the MDL calculation. MDL data is stored on \\fspwes01\Transfer\Organic MDLs as a PDF file. For additional details, refer to Definition and Procedure for Determination of the Method Detection Limit, Revision 2 and the laboratory SOP 1016 Determining and Applying Method Detection Limits.

8.4 Blind to the Analyst (BTTA) Study is performed annually, at a minimum.

8.4.1 A 1 g sample made using clay (section 6.3) that is spiked by a chemist who will not perform the extraction or analysis at a concentration known only by that chemist and the QA Manager.

8.4.1.1 Measure 1 g clay into a 4 mL amber glass vial. Spike the sample with a known quantity of standard using gas tight syringe. Add 2 mL of acetone and vortex for 10-15 secs. Completely dry the sample by placing vial under nitrogen flow (4 LPM) using N-evap at room temperature (do not submerge in the water bath). This process takes approximately 1 hour.

8.4.1.2 The chemist must document how the sample was made, including volumes, concentrations, standards, IDs, date made, sample concentration and who made the sample.

8.4.2 The sample will be logged in to LIMS for 6PPD-Q analysis, labeled and placed in the cooler, as with any other sample. The sample is to be extracted and analyzed like any other client sample.

8.4.3 Follow the normal review process for the BTTA sample, as would be performed for any other sample, including variance memos for any QC failures or anomalies.

8.4.4 Following extraction and analysis, the QA manager will review the results and grade the sample reports. The graded report is submitted by the QA manager to Washington State Department of Ecology.

8.5 Method Blank (Batch#-BLK#): Analyze at least one method blank (BLK) per sample batch or at least 5% of samples prepared – equivalent to 1 per 20 field samples or less. The BLK is made up using Clay (section 6.3) and extracted using the same preparation procedure as the associated samples. The blanks must be free from contamination at a concentration at 1/2 the reporting limit (RL/MRL). If contamination is shown in the Method Blank, halt sample analysis and correct the issue. Samples affected by the Method Blank should be re-analyzed, or qualified if re-analysis is not possible.

8.5.1 If the blank contains a concentration greater than the MDL and the sample concentration is less than the reporting limit, report the MRL value with a "U".

8.5.2 If the sample concentration is greater than 10 times the blank value, no data qualification is required.

8.5.3 If the sample concentration is greater than or equal to 5 times the blank value but less than 10 times the blank value, qualify the data as estimated

with a “J”.

8.5.4 If the sample concentration is less than or equal to 5 times the blank value qualify the data as not detected at or above the associated estimated concentration with a “UJ”.

8.5.5 If gross contamination exists in the blank, positive sample results may require rejection and be qualified as unusable, "R". Non-detected target compounds do not require qualification unless the contamination is so high that it interferes with the analyses of non-detected compounds. Unusable data may require re-extraction.

8.5.6 Complete a QC Variance form for any result requiring qualification.

8.6 Laboratory Control Sample (LCS) or Standard Reference Material (Batch#-BS#): A laboratory control sample (LCS) is created (section 6.8) and extracted and analyzed at a frequency of one per batch or every 20 samples. The acceptable recoveries are 70-130%. Re-analyze samples associated with any LCS failures or qualify the data where reanalysis is not possible. After the analysis of 30 LCS samples, the lab limits are updated on a regular basis based on 2 standard deviations from the mean.

8.6.1 Reanalyze LCS if recovery is outside the criteria after evaluating whether system maintenance could improve recovery and taking any actions indicated. Analyze a second LCS if one was extracted with the batch. Evaluate repeat analyses only for the analytes that failed the initial analysis. Consult with Environmental Laboratory Scientist (ELS) III if recovery is still outside the criteria to determine whether re-extraction is possible within sample holding times. Report data associated with the best recovery.

8.6.2 Qualify results with a “J” for detects and “R” for non-detects if the LCS recovery is less than the lower recovery limit. Complete a QC Variance form.

8.6.3 Results qualified as rejected (“R”) are not usable for regulatory purposes. Notify the Project Client for further action.

8.6.4 Qualify results with a “J” for detects and complete a QC Variance form if the LCS recovery exceeds the upper recovery limit. Do not qualify non-detects.

8.6.5 Complete a QC Variance form for any result requiring qualification.

8.7 Matrix Spike/Matrix Spike Duplicate (Batch#-MS# and Batch#-MSD#): ***A matrix spike is analyzed only if requested by the client.*** The spike recoveries should be within laboratory control limits of 50-150% with an RPD of 50%.

8.7.1 Do not qualify results if the sample concentration exceeds the spike concentration by a factor of four or more. Do not report the MS/MSD if the parent sample concentration exceeds the upper calibration limit.

8.7.2 Reanalyze the MS or MSD if recovery is outside the criteria after

evaluating whether LC/MS-MS system maintenance could improve recovery and taking any actions indicated. Consult with the senior analyst if recovery is still outside the criteria to determine whether re-extraction is possible within sample holding times. Report data associated with the best recovery.

8.7.3 If the MS and MSD recoveries are less than the lower recovery limit or ≤10%, whichever is lower, qualify results of the source sample with “J” for detects and “R” for non-detects. Qualify non-detects as “UJ” if recoveries are less than the lower recovery limit but not less than 10%. Complete a QC Variance form.

8.7.4 If the MS and MSD recoveries exceed the upper recovery limit, qualify results of the parent MS/MSD sample with a “J” for detects and complete a QC Variance form. Do not qualify non-detects.

8.7.5 If the RPD exceeds the limit, qualify detected analyte results of the source MS/MSD sample with a “J”, and complete a QC Variance form. Do not qualify non-detects.

8.7.6 Complete a QC Variance form for any result outside control limits or any MS/MSD not reported due to concentration of parent sample.

8.8 Sample duplicate (Batch#-DUP#). ***A duplicate is analyzed only if requested by the client.*** The duplicate relative percent difference (RPD) should be less than or equal to 50% for sample duplicates and matrix spike duplicates. If the RPD fails due to inhomogeneity or matrix interference, qualify the failing analytes in the source sample.

8.9 Extracted Internal Standard (EIS)/Surrogate: To assess method performance on the sample matrix, the laboratory must spike all samples with the isotopically labeled compound standard solution (EIS) and all sample extracts with the non-extracted Internal Standards (IIS) spiking solution. The recovery limits for the EIS are 25-200%. After the analysis of at least 30 samples, the limits are updated on a regular basis based on 2 standard deviations from the mean.

8.9.1 Analyze each sample according to the procedures in Section 10.0. Peak responses of the quantitation and confirmation ions must be at least three times the background noise level (S/N 3:1). The quantitation ion must have a S/N ≥ 10:1 if there is no confirmation ion. Retention times must fall within ± 0.4 minutes of the predicted retention times from the midpoint standard of the ICAL or initial CCV, whichever was used to establish the RT window position for the analytical batch. Compute the percent recovery of the isotopically labeled compound using the non-extracted internal standard method. The recovery of must be within 25-200% or current limits.

8.9.2 If the recovery of the EIS falls outside of these limits, method performance is unacceptable for 6PPD-Q in that sample. Additional cleanup procedures must then be employed to attempt to bring the recovery within the normal range. If the recovery cannot be brought within the normal range, the extracts may be diluted.

8.9.2.1 When entering the dilution into the batch sheet correct the EIS

spike concentration for the dilution (i.e. Original spike amount was 20 uL, so on a 10X dilution of the extract the EIS spike amount is entered as 2 uL). Make sure to note that this correction is to correct for dilution in the comments.

8.9.2.2 The sample may only be diluted to the level that the EIS meet the S/N and retention time requirements and are still recovered at greater than 5%. For example, if the EIS recovery of the affected analyte in the undiluted analysis is 50%, then the sample cannot be diluted more than 10:1; if the EIS recovery of the affected analyte in the undiluted analysis is 30%, then the sample cannot be diluted more than 6:1. If sample extract dilution does not correct the recovery, a new extraction should be performed.

8.9.3 If after dilution and/or re-extraction any EIS recovery is outside limits and there is no further sample available for extraction or dilution, qualify the 6PPD-Q in the analysis as indicated below. Use professional judgment if sample dilution is a factor in EIS recovery.

8.9.3.1 For target analyte detections where the recovery exceeds the upper recovery limit qualify results as estimated, “J". Do not qualify non-detects.

8.9.3.2 If the recovery is less than the lower recovery limit but greater than 10%, qualify results as estimated “J".

8.9.3.3 If the recovery is less than 10% or the lower limit in Table 8 whichever is less, qualify results as “J" for detects and “R” for non-detects.

8.10 Non-Extracted Internal Standards (IIS): Retention times must fall within ± 0.4 minutes of the predicted retention times from the midpoint standard of the ICAL or opening CCV, whichever was used to establish the RT window position for the analytical batch. The IIS area in the field samples and QC samples must be within 50-200% of the most recent CCV, the most recent CCV must be within 50-200% of the mean area of the calibration.

8.10.1 If sample dilution is required, the NIS response in the diluted extract is no longer required to be within ±50% of the response (peak area) in the most recent CCV.

8.11 Control Charts – Control charts are maintained in Element® according to SOP 1017 Control Charts.

8.12 Corrective Action – QC results outside of limits and deviations from the SOP are documented according to SOP 1005 Corrective Action.

8.13 See a QC guidance summary table in Appendix A.

9.0 Calibration and Standardization

9.1 Refer to the following SOPs for calibration of balances and thermometers, glassware cleaning procedures, and the preparation of reagents and standards

1006 Reagent Preparation and Documentation

1008 Standards and Reagent Preparation and Documentation

1011 Glassware Cleaning

1013 Thermometer Calibration and Temperature Control

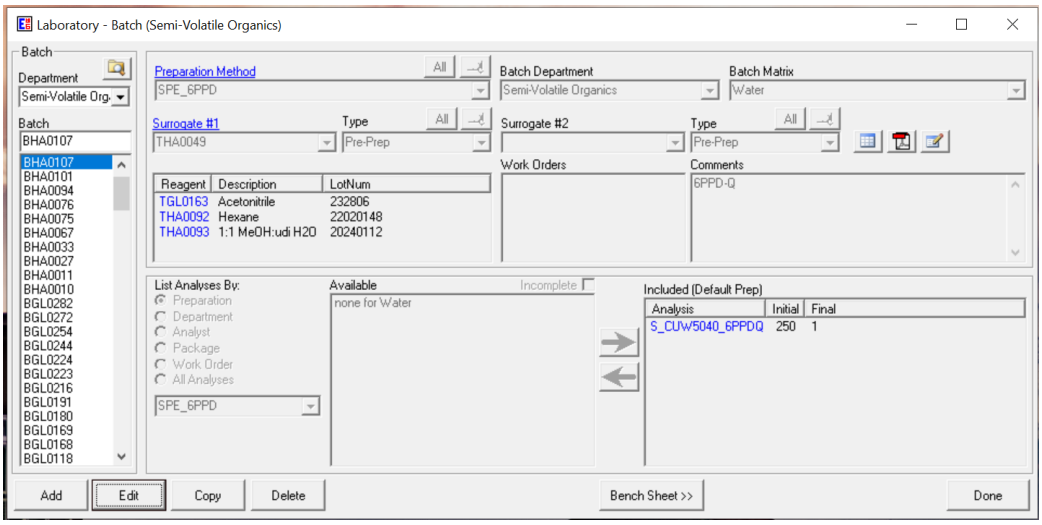
1015 Analytical Balance Calibration and Maintenance

10.0 Procedure

10.1 Cleaning of glassware, tools, and surfaces: Satisfactory cleaning may be accomplished by rinsing with acetonitrile, and then washing with any detergent and water.

10.2 Review Extraction Bench Sheets **prior** to sample preparation. Environmental Laboratory Scientist (ELS) II or III signs and dates on the Analyst Approval line. Note that in the sample comments column on the bench sheet,

10.2.1 In Element: Laboratory > Batch > Add



Select the appropriate Preparation Method, Batch Matrix, Surrogate and Analysis. Add reagents by right clicking in the Reagent box.

Save > Bench Sheet>>

The Bench Sheet will be called up. Select Edit > Add to add client samples by container, and any applicable QC samples. Right click highlighted samples to change Initial/Final volumes, Surrogate Amounts, Spike IDs/amounts and select Source Samples for all MS/MSD and DUP (if required).

Select Save, then the print icon.

10.2.2 All Sediment samples will be cryo-milled. Follow SOP 1022_Cryomill Sample Processing.

10.2.3 Prepare samples including (method blank, LCS (BS), MRLs, and SRMs) by weighing approx. 0.2 g to 0.25 g of sample into an 8 mL amber glass vial.

10.2.4 Spike all samples with 10 µL of 200 EIS (surrogate) (section 6.5.2).

10.2.5 The samples are now ready for extraction.

10.2.5.1 Add exactly 5 mL of Acetone to sample tubes.

10.2.5.2 Vortex for 10-15 seconds.

10.2.5.3 Sonicate for approx. 30 minutes.

10.2.5.4 Centrifuge sample for 5 minutes at 2000 rpm.

10.2.5.5 Manually decant the approx. 5 mL solvent layer into a 12 mL vial. Carefully pour it in to avoid spilling.

10.2.5.6 Place the 12 mL vial under the N-evap (4 LPM) until the Acetone is completely evaporated off.

10.2.5.7 Using a pipette, re-elute extract with 1 mL of Acetonitrile.

10.2.5.8 Vortex for 15-20 seconds and sonicate for 2 mins.

10.2.5.9 Using a pipette, transfer the 1 mL extract into a 2 mL amber glass vial. To each 1 mL volume, add 10 µL of injection IS (IIS) spiking solution. Cap the vial, shake or vortex to mix well, and then transfer to the LC/MS-MS for analysis. Refrigerate extract if not analyzed right away.

10.2.5.10 The sample is now ready for analysis per SOP 5040 6PPD Quinone by Triple Quadripole LC-MS-MS.

11.0 Pollution Prevention and Waste Management

11.1 Store vials for disposal containing sample extracts or expired standards in the designated waste containers for this satellite area until disposed of by lab pack in accordance with the Dangerous Waste Management Plan. Keep in-use waste disposal container under the hood in Room 230. This is the designated satellite collection area for this waste stream. When waste container is full, notify the Hazardous Waste Manager for removal to Hazardous Waste Storage area. Refer to the waste stream fact sheets for details: CH1130589 (Organic extractions waste & expired standards in vials).

11.2 Collect waste solvents in an appropriate waste container and dispose of in accordance with the Dangerous Waste Management Plan. Refer to the waste stream fact sheet for details: CH573177 (Waste solvents).

11.3 Upon extraction, the solid sample waste is collected and disposed following the Dangerous Waste Management Plan. Disposal details are outlined in the waste stream fact sheet: 0006 (non-hazardous solid waste).

12.0 References

12.1 US EPA Functional Guidelines: http://www.epa.gov/sites/production/files/2015-03/documents/somnfg.pdf

12.2 City of Tacoma Environmental Services Chemical Hygiene Health and Safety Plan, current revision

12.3 City of Tacoma Environmental Services Quality Assurance Manual, current revision

12.4 City of Tacoma Environmental Services Dangerous Waste Disposal Manual, current revision

Standard Operating Procedures

Microplastics and Tire Wear Particles
Stormwater Grab and In-Line Sediment Sampling

Prepared by
Herrera Environmental Consultants, Inc.
2200 Sixth Avenue, Suite 1100
Seattle, Washington 98121
Telephone: 206-441-9080

May 14, 2025

HERRERA
Science + Planning + Design

Contents

Tables

1. Introduction, Scope, and Applicability

This standard operating procedure (SOP) is applicable to the collection of microplastics (MP) in stormwater and sediment grab samples, which includes the analysis of tire wear particles (TWP). This standard operating procedure may be varied or changed, as required, dependent upon site conditions, equipment limitations, limitations imposed by the procedure, or other procedure limitations. In all instances, the procedures that are ultimately employed should be documented and associated with the final report.

2. Training

The procedures in this SOP are for use only by authorized personnel who have received specific training. Personnel conducting the sampling described in this SOP should have general training in field safety, familiarity with associated water quality parameters and procedures, and knowledge of all relevant components of the associated Quality Assurance Project Plan (QAPP).

3. Method Summary

The MP sample method for stormwater samples is bulk water collection from the monitoring station. This will be achieved by pumping water from targeted sample depths into a large collection container (20 liters). This method will allow sufficient water to be sampled without in-field filtration or sieving.

The MP sample method for sediment samples (i.e., stormwater solids) is collection by in-line sediment traps as described in the associated QAPP. Any equipment coming into direct contact with the sample should be plastic-free.

Samples must be chilled during storage and shipment and must not exceed 6°C (42.8°F) during the first 48 hours after collection.

4. Sample Preservation, Containers, Handling, and Storage

Once samples have been collected, the following procedure should be followed:

1. Use procedures listed in Section 8.0 (Sampling Procedures) for one or two technicians.
2. Once filled, cap the container and label it with the sample ID.
3. Record all pertinent data in the site logbook and on field data sheets.
4. Complete the Chain of Custody record.
5. Decontaminate all sampling equipment (see Tables 1, 2, and 3) prior to the collection of additional samples.

5. Interferences and Potential Problems

Potential sources of MP cross-contamination in the typical sampling environment include materials used within the sampling equipment; gloves, clothing, and personal protective equipment (PPE) that contain plastics; food packaging; and the environment itself. In addition, because TWP will be analyzed, sources of rubber can potentially cause interference, and rubberized equipment should avoid contact with the MP samples.

It is recommended, when possible, to exclude materials known to contain MP (see tables below), such as those containing polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), poly(methyl methacrylate) (PMMA), polycarbonate (PC), and rubber materials, including tires, rubber bands, pencil erasers, and some packaging materials. The Safety Data Sheets (SDSs) of materials should be reviewed before considering materials for use. If MP is not listed on the SDS, then MP may still be present.

All equipment and materials used in the vicinity of the sample collection should be screened as sources of MP contamination prior to sample collection. Screening for MP will be approached in two stages: (1) sampling equipment that will come in direct contact with the sample and (2) materials and equipment that will be in the vicinity of the samples, including sampler clothing, and labels. Stage 1 materials should be thoroughly reviewed and either free of known sources of MPs or used in such a way as to avoid contact with plastic areas. Stage 2 materials should avoid known or suspected sources of MPs unless it impacts field safety. Typical materials that are used in sampling equipment and may contain MPs are included in Table 1:

Table 1. Sampling Equipment.

Classification	Name	Commonly Found
Prohibited	Plastic or vinyl containers	Containers, bottles, plastic bags, tubing
Needs Screening	Other	Chemical ice packs, aluminum foil, bottle labels
Allowable	Plastic equipment that does not contact sample or sample container	Pens and mechanical pencils, and plastic binders or clipboards, etc. (kept outside sampling area)
	Glass or other materials known to be plastic-free	Containers, bottles, sample processing equipment
	Powderless nitrile gloves	
	Silicone tubing	Peristaltic pump

Table 2. Clothing, PPE, Personal Care Products.	
Classification	**Description**
Prohibited	Clothing made with nylon or polyester
Needs Screening	Clothing made from plastic or synthetic fibers[a]
	Water resistant or stain-resistant clothing and PPE
	Tyvek suits or Tyvek coated clothing
	Cosmetic products (e.g. lipstick, mascara, eye shadow)
Allowable	Powderless nitrile gloves
	Natural fibers well laundered with no fabric softeners

[a] Clothing and PPE with no suitable plastic-free alternative should be in good condition, i.e., not breaking down or shedding material. Field personnel should use caution not to touch such clothing or equipment while wearing nitrile gloves.

Table 3. Decontamination.	
Classification	**Description**
Prohibited	Plastic sponge or brush
Needs Screening	Stormwater
	Wrapping and packaging
Allowable	Alconox®, Liquinox®, or Citranox®
	Triple rinse with deionized water
	Cotton cloth, natural sponge, or untreated paper towel

In general, field staff will wear clothing and shoes/boots without black rubber soles. In particular, shoe wear should be new and not aging or shedding material. Immediately prior to sample collection, field staff will avoid handling plastic or rubber containers or wrappers and ensure the sampling area is clear of potential contaminant sources, pens, and plastic binders or clipboards. Powder-free nitrile gloves will be worn by the field staff during sampling area preparation and sample collection of other parameters; these gloves will either be new or will be washed with MP-free water prior to MP sample collection. Field staff will avoid clothing made with synthetic polymers, particularly nylon and polyester (polyethylene terephthalate). Any potential source of contamination that is necessary for field work but has no suitable plastic-free alternative (e.g., PPE, such as high-visibility clothing, rain gear, or shoes/boots) will be documented in field logs.

6. Equipment/Apparatus

At minimum, equipment needed for collection of MP samples includes the following:

- High flow rate peristaltic pump with 3/8-inch ID silicone tubing (for stormwater grab sampling)
- 22-liter stainless-steel jugs (stormwater)
- "Norton" style sediment trap (for sediment sampling)

- Sample bottles (stainless steel or glass)
- Powder-free nitrile gloves, washed with MP-free DI water
- Chain of Custody records
- Field data sheets or other recordkeeping equipment
- Sample bottle labels
- Safety equipment, including personal protective equipment designated in the Health and Safety Plan
- Decontamination equipment

7. Decontamination Procedures

When possible, use dedicated or single-use field sampling equipment. When non-dedicated equipment is used at multiple sampling locations, thorough cleaning between uses is required.

Field decontamination procedures for the stormwater sampling equipment and sediment sampling equipment will generally follow the procedures outlined in the QAPP. No special decontamination is required for MP samples; rather, standard cleaning of the sample equipment suffices. However, if equipment will be reused for MP sampling the following steps should be taken to avoid plastic in the decontamination process:

- Only use scrub brushes or sponges made with natural materials.
- Use laboratory-provided MP-free deionized water during all decontamination steps or triple rinse with laboratory-provided MP-free if standard deionized water was used during earlier decontamination steps.
- Decontaminated equipment may be stored in aluminum foil that has been screened for MP for a short time prior to use.

8. Sampling Procedures

Upon arrival at the project site, field staff will establish a sample collection staging area for stormwater samples. The staging area will be free from potential or known sources of plastics and rubber that could contact the sample. Field staff will use washed, powder-free nitrile gloves during the sample collection and when handling the sample jug. Details for sampling procedures are described in the sections below.

8.1. Preparation

1. Obtain the necessary sampling and monitoring equipment, including for quality control samples such as blanks and duplicates.

2. Clean all sampling equipment using approved cleaning methods.
3. All sampling equipment and sample containers should be free of plastic and rubber.
4. Muster all sampling equipment at the sampling station.

8.2. Grab Stormwater Sample Collection

Procedures for collecting stormwater grab samples for MP analysis are generally consistent with the typical stormwater grab sampling procedures described in Ecology's Standard Operating Procedures for Collecting Grab Samples from Stormwater Discharges (Ecology 2024). However, additional considerations are needed to avoid cross contamination due to the widespread use of plastics. These additional procedures and considerations are presented in the subsections below.

1. A field rinsate blank will be collected in the sample staging area at one location, prior to field sample collection, by pumping laboratory-provided, MP-free water into the laboratory-provided sample container. The field blank should be open to air for the same amount of time that a sample is processed and open to air, in order to account for particle atmospheric fallout (particularly from field staff clothing).
2. New or station-specific dedicated 3/8-inch inside diameter silicone tubing will be used in the pump at each sample collection at a given location.
3. Field staff will make sure to position the pump intake so deposited sediments are not entrained during pumping.
4. To flush the sample line, the first 2 liters of sample water will be disposed of by discharging the pump to the ground. Pump 20 liters of water into a prelabeled, 22-liter stainless steel jug.
5. After sample collection the jug will be immediately sealed with its associated stainless-steel lid and transported to the laboratory for analysis. MP water samples do not need to be placed on ice during transportation.

8.3. In-Line Sediment Trap Sample Collection

In-line sediment traps will be installed as described in the associated QAPP. Field staff shall wear new, powder-free nitrile gloves prior to handling the Norton sediment trap, sample bottles, or sample transfer materials.

1. Cap the bottles in-situ and then remove the bottles from the sediment traps.
2. Inspect sediment trap bottles for sediment accumulation. Estimate the volume of sediment by estimating the depth of accumulated sediment in the bottles and the inside diameter of the bottle and multiplying by the area equation for a cylinder (πr^2). The associated QAPP shall specify volume requirements for laboratory analysis. Each monitoring location will have three 1-liter glass sample bottles.

3. Dry the outside of bottles and apply sample labels.
4. Store the bottles in a cooler with ice for transport to the laboratory.
5. At the laboratory, composite the contents of the three sample bottles for each location. Specifically, transfer the sample volume from the sample bottles to a >6-liter, decontaminated stainless-steel bowl, using clean, decontaminated stainless steel equipment. Thoroughly mix the contents of the bowl and remove organic pieces larger than approximately 1 cm (i.e., leaves and twigs). Cover the bowl and let stand for 1 hour (see Sample Processing SOP).
6. Decant the excess water from the bowl and filter for Microplastics/TWP per the Sample Processing SOP.
7. Using a microplastics/ TWP-free decontaminated stainless-steel spoon, transfer a 200- to 400-gram subsample of the solids slurry into an 8-oz amber glass jar before being submitted to the laboratory for analysis.

9. Calculations

This section is not applicable to this SOP.

10. Quality Assurance/Quality Control

There are no specific quality assurance (QA) activities that apply to the implementation of these procedures. However, the following general quality control (QC) procedures apply:

1. All field conditions must be documented on field data sheets or within site logbooks.
2. The appropriate number and type of field QA samples need to be included in the sampling plan, in order to confirm that the sampling procedures employed were adequate. Field QA samples include:
   a. Equipment rinsate blanks – collected by pumping laboratory-provided, plastic-free water through the peristaltic pump into the laboratory-provided sample container while in the field.
   b. Duplicates – additional samples to be collected in the same manner as regular field samples, at the same time and location, to be analyzed with the original sample.

11. Data Validation

This section is not applicable to this SOP.

12. Health and Safety

When working with potentially hazardous materials, follow EPA, OSHA, and corporate health and safety procedures. Before conducting sampling, a health and safety assessment should be conducted to identify site- and job-specific hazards. Health and safety considerations for PFAS stormwater grab sampling may include the following:

- The sampling team member collecting the sample should not get too close to the edge of impoundments where bank failure may cause them to lose their balance or open stormwater sewer structures.
- The person performing the sampling may need to be on a lifeline and wear adequate protective equipment.
- When conducting sampling in confined spaces, appropriate health and safety procedures for confined space entry must be followed.
- Necessity for traffic control and general awareness of traffic hazards should be assessed when conducting sampling near roadways.

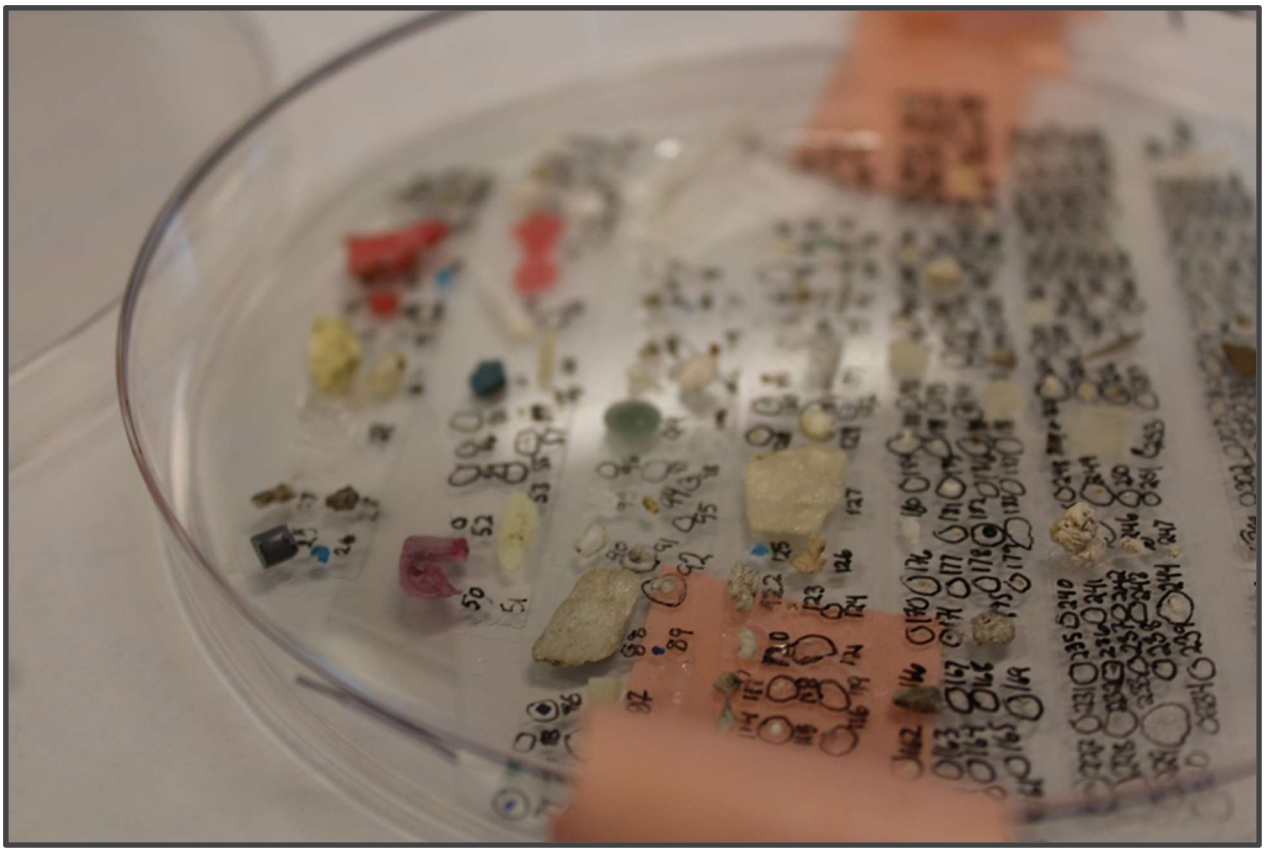
13. References

Ecology. 2024. Collecting Grab Samples from Stormwater Discharges, Standard Operating Procedure, Version 1.2. Washington State Department of Ecology, Water Quality Program Olympia, Washington. (Publication 18-10-023).

Harrold, et al. 2022. A peristaltic pump and filter-based method for aqueous microplastic sampling and analysis. Harrold, Z., Arienzo, M.M., Collins, M., Davidson, J.M., Bai, X., Sukumaran, S., & Umek, J. *ACS ES&T Water* 2022, *2*(2), 268-277.

SCCWRP. 2020. SCCWRP Microplastic Measurement Methods Evaluation Study. Version 4.03. Southern California Coastal Water Research Project, Costa Mesa, CA. November 10.

SCCWRP MICROPLASTIC MEASUREMENT METHODS EVALUATION STUDY



Fourth Version for Participant Use

Version 4.03 November 10, 2020

TABLE OF CONTENTS

Contact Information

If you have any questions, please contact the following individuals:

- Charles Wong (charlesw@sccwrp.org): Overall queries and logistics (e.g., sample shipment, data submission, meetings, instrument use at SCCWRP, etc.)
- Hannah De Frond (hannah.defrond@utoronto.ca): Materials and extraction SOPs
- Suja Sukumaran (suja.sukumaran@thermofisher.com): FTIR
- Eunah Lee (eunah.lee@horiba.com): Raman

Changes in version 4.03 since last version 4.02 (September 14, 2020)

- **IMPORTANT!** There is an updated data submission spreadsheet template, available at http://microplastics.sccwrp.org. Major change is that the RawDataResults tab has an additional column, called InstrumentType (column D on the template), with values in a new lookup table called lu_instrumentype. This fixes a bug, and now allows submission of data from multiple analysis techniques (e.g., FTIR and Raman for labs doing both) at the same time, and in the same submission. If you already have filled-out data, please transfer it to the new template. The old template will no longer work! You should be able to cut-and-paste everything in all tabs except RawDataResults. For that one, you can cut-and-paste twice to account for the new InstrumentType column (i.e., old columns A-C for LabID, SampleID, and SampleType go to new columns A-C on the new template, and all other columns from SizeFraction onwards to the right of InstrumentType on the new template).
- **IMPORTANT!** Appendix J edited to note that the data template spreadsheet has example lines of formatted "data", including SampleID and ParticleID names, that will successfully pass the data checker upon submission. These lines are identified with the fictitious LabID "ACME". The data checker and database will ignore these lines (if unmodified) if you submit your data with them still present.
- **IMPORTANT!** As noted in the SOPs, please heat to 55°C (up from 50°C) during filtering, and add only a small amount of sample at a time so that it doesn't have a chance to cool off. This can help with slow filtering through the smallest filter pore size fractions. If slow filtering continues to be an issue, please contact Charles Wong (see Contact Info page right after Table of Contents) and we will work it out.
- **IMPORTANT!:** Nile Red concentrations should be 10 μg/mL not 10 μg/L!
- **IMPORTANT!**: Appendix J edited to note that in the data template spreadsheet's RawDataResults table, 1 row = 1 particle from an analysis type (InstrumentType). Up to 30 of these per size class, color, and morphology will be "picked" (if applicable) for chemical characterization, and the rest can be noted as unidentified. We'll determine how many particles of each type you found by counting the total number of rows present in this table.
- Revised due data for submission of simulated clean water data now November 23, 2020 to account for temporary closing of data portal on October 30 to accommodate fixes to bugs that became apparent once data started to be submitted.
- Modified instructions in section C of each SOP on particle storage (on double-sided tape for Raman or ATR-FTIR, on a reflective surface for reflectance FTIR) to include: "*This is a suggestion only; please store particles as you see fit. When using double-sided tape, the tape should not be laid directly into the base of a petri dish. Instead, we recommend using projector paper or a glass slide as a base to lay the tape on. The choice between the two may depend on the stage of the instrumentation you are using for chemical analyses. When using ATR-FTIR*

spectroscopy, double sided tape should only be used for larger size fractions to avoid the crystal coming into contact with the tape."

- Added to each SOP regarding picking particles: "*For smaller size fractions, particles do not need to be manually picked as this is not only challenging but provides a high likelihood for particle loss. These particles can instead be analyzed directly from the filter substrate.*"
- Updates to reflect changes in lab and Augmentation status due to continuing COVID issues.
- Appendix J edited for mandatory naming format of image files of particles.
- Updated Appendix J to note that image files for particles must be in JPEG or PNG format.
- Updated Appendix J to note that the maximum length for PhotoID is 75 characters not 25.
- In Appendix J, LaserWavelength in RamanSettings is now a primary field.
- In Appendix J, the MagnificationRange field under Microscopy and Nile Red tabs should be overall magnification, not a range.
- In Appendix J, changed all Time fields in Raman, Microscopy, PyroGCMS, FTIR, and NileRed to decimals (floats) not integers.
- In Appendix J, SpectraRange in Raman and FTIR is a text field, accepting either a number (e.g., 700) or a range (e.g., 300-800).
- In Appendix J, length, width and timeimagemeasurement in RawDataResults have been changed from integers to decimals (floats).
- In Appendix J, B1separationtime, B2separationtime, and KOHDigestionTime in SampleExtraction changed from integers to decimals (floats).
- In Appendix J, added comment in the Accessories field under the FTIR tab to include the type of detector used e.g., "room temperature", "cooled", "imaging". (This information can also be included in the Comments field for the tab)

Changes in version 4.02 since last version 4.01 (March 17, 2020)

- **IMPORTANT!** Updated SOP for fish tissue to rinse with KOH solution, not water, so as not to change the solution density for the separation step!
- **IMPORTANT!** Updated SOP for sediment to soak and rinse shipping container with $CaCl_2$ solution to get off particles stuck on the container inside walls.
- Changed participating lab status, including new labs, and deleted labs that had to drop out due to COVID-19 complications.
- Added new Augmentations that have developed since the previous version.
- Updated other Augmentations (some deleted, others modified) given pandemic issues.
- Fixes to Appendix J (e.g., Instrument Information Table repeated twice, corrections in testing data portal).
- Added URL and details for data submission to Appendix J.
- Updated data submission deadlines and dates/formats of subsequent data analysis workshops to reflect COVID-19 pandemic issues.

Changes in version 4.01 since last version 4.00 (February 24, 2020)

- Changed participating lab status, including new labs (UMD and USGS).
- Added note in main text and in SOP (Appendix C) about storage conditions and about vacuum filtering warm simulated clean water samples to shorten process for intercalibration samples.
- Added note in main text SOP (Appendix F) about storage and filtering of dirty water samples.
- Adjusted timeline to reflect workshop date (October 5-6, 2020).
- Added note regarding effect of COVID-19.
- Fixed some typos.

Changes in version 4.00 since last version (3rd Interim Draft, August 11, 2019)

- Addition of this section to see changes at a glance.
- Addition of Contact Information section.
- More participating laboratories added (Tables 1, and Appendix A tables).
- Additions and changes to several Augmentation studies.
- Addition of Appendix B: One-pager for good laboratory practices for microplastics analysis (from November 2019 instrumental training course at SCCWRP).
- Significant changes to all SOPs for extraction from matrices (Appendices C-F), including addition of suggested vendors for supplies and pictorial flow diagrams of procedures at end of each Appendix. Please READ CAREFULLY.
- Addition of Appendix G: Flow diagram for processing and analysis. Follow this for the steps you will do, and the decision points along the way.
- Addition of Appendices H and I for basic operations of FTIR and Raman, as per SCCWRP November 2019 training course. Uses instruments at SCCWRP as specific examples. Not intended as a training manual.
- Addition of Appendix J on data reporting. This section goes through every data field that will be in the spreadsheet template to be used for data reporting; you will submit this through a web-based portal to upload your results. The template and portal are currently in development and will be provided to study participants upon completion.
- Lots of little edits throughout.

Introduction

Microplastics, a ubiquitous global pollutant, have contaminated marine, freshwater and terrestrial ecosystems around the world. Since the 1970s, when small pieces of plastics were discovered floating in the middle of the North Atlantic Ocean (Carpenter et al., 1972), researchers have been working to understand the many sources from which microplastics originate, the diverse ways they move through ecosystems, and their impacts on wildlife and humans.

Microplastics originate from a variety of industrial and commercial production processes; they are used as exfoliants in personal care products, they shed from woven textiles, and are produced as part of the plastic manufacturing process (i.e., pre-production plastic pellets), among other sources. Moreover, larger plastic particles, once introduced to the environment, break down over time to become increasingly small microplastics. Microplastics can travel from one ecosystem compartment to another via air, water, and biota.

Measuring microplastics is challenging. Standard light microscopy allows for quantification of larger particles, but loses effectiveness as the size range decreases from millimeters to microns. Moreover, measurement of microplastics can be easily confounded by other non-plastic materials, such as paper and natural plant material, that can be present in the same size ranges. Scientists have developed methods using a number of technologies, notably Raman spectroscopy, Fourier Transformed Infrared spectroscopy and Pyrolysis gas chromatography, to not only better quantify the number and shape of microplastic morphologies (e.g., fiber, fragment, sphere), but to also distinguish among polymer types (e.g., polyethylene, polyester, polyurethane). These methods show great promise, but remain largely provisional or investigator-specific. They have not yet been validated and standardized for widespread use.

Application of these methods has enhanced understanding about the pervasive nature of microplastics, which in turn has spurred new legislation and management programs intended to more tightly regulate microplastics emissions and monitor its presence in the environment. The State of California in particular has passed four such bills in the last few years (see breakout box), two of

Recent California Microplastics Legislation

House Resolution 1321 (2015): Bans the manufacturing of rinse-off cosmetics that contain intentionally-added plastic microbeads

Assembly Bill 888 (2015): Prohibits the distribution of a personal care product containing plastic microbeads used to exfoliate or cleanse in a rinse-off product in the State of California.

Senate Bill 1263 (2018): Requires the Ocean Protection Council to adopt and implement a Statewide Microplastics Strategy related to microplastic materials that pose an emerging concern for ocean health.

Senate Bill 1422 (2018): Requires the California State Water Resources Control Board develop standardized methods for analyzing microplastics in drinking water and defining acceptable levels.

which require building the State's capacity to conduct routine microplastics monitoring in drinking water and the coastal ocean. Consequently, these two State agencies have a pressing need to achieve standardization of microplastic measurement methods and to design robust, science-informed microplastic monitoring strategies around these methods.

In response to that need, leading scientific experts on microplastics convened in California in April 2019 for a two-day workshop at the Southern California Coastal Water Research Project Authority (SCCWRP) to begin laying the groundwork to build scientific consensus around methods for monitoring microplastic particles in aquatic environments. From that workshop, participants worked to develop a method evaluation study that would provide California with a scientific foundation for selecting among, and standardizing, microplastic measurement methods. This document describes the study plan, and an implementation schedule, that evolved from that workshop.

Study Design

This microplastics methods evaluation study will examine the performance of five microplastic measurement methods across a range of four matrices. Three key pieces of information will be provided about each microplastic measurement method: 1) Accuracy of the method, 2) Repeatability/reproducibility within and among laboratories, and 3) Resources necessary to perform the method (i.e. people, equipment, time and consumables).

The study is divided into two main parts:

- **Study Core**: Focus is around assessing accuracy, reproducibility and cost for five analytical methods in four frequently-encountered matrices (clean water, dirty water, sediment, and tissue). Multiple laboratories from throughout the world will perform these methods using a series of standard operating procedures (SOPs).

- **Study Augmentations**: Smaller sub-study elements in which individual laboratories (or a small set of laboratories) will investigate how novel methods, or small permutations of the core study SOPs, affect method performance. The Study Augmentations will leverage the Study Core by using the same samples, as well as custom samples as applicable, to examine method variations.

Study Core

The Study Core is based on multiple laboratories processing the same set of created samples. These samples will have diverse microplastic contents that are known to the study coordinators, but blind to study participants. Method accuracy will be assessed as the difference from the known content.

Method precision will be assessed as repeatability/reproducibility, both within and across laboratories. At least three laboratories, to the extent possible, will process three replicates for each method/matrix combination (Table 1). Forty-one laboratories located in 6 countries are participating in the study, though not all laboratories will process each method/matrix combination (Appendix A). For each method/matrix combination, the study will include at least one highly experienced laboratory and at least one novice laboratory, which will enable the study to assess whether the method is transferable. Novice laboratories will be ones having experience measuring microplastics, but not necessarily experience with every specific protocol and/or instrumentation. Novice laboratories will receive the SOPs and training to ensure they have sufficient competency for inclusion in the study.

Costs for each method will be assessed by asking each laboratory to track the time necessary to process the sample, as well as the per sample cost for supplies and the capital cost for the equipment they employ. No attempt will be made to standardize instrument manufacturer, though participants will be asked to supply that information, and differences among manufacturer will be assessed.

Five analytical methods will be performed in this study (Table 1): stereoscopy, stereoscopy with dye staining, Fourier-transform infrared spectroscopy (FTIR), Raman spectroscopy, and pyrolysis-GCMS. Four matrices will be analyzed: clean water, dirty water (to simulate surface water or wastewater), sediment and tissue. Each one of these matrices will be extracted using defined SOPs and analyzed using each of the methods. Labs will follow SOPs developed by experts in the field (Appendices C-F).

Table 1. Number of laboratories that will process each matrix and method in the Study Core.

	Matrix			
Method	Clean Water	Dirty Water	Sediment	Tissue
Stereoscope	21	14	11	8
Stereoscope with dye staining	8	6	4	5
Fourier-transform infrared spectroscopy (FTIR)	21	15	10	8
Raman spectroscopy	19	14	9	5
Pyrolysis-GCMS	6	4	4	2

The blind identical samples are created by a single laboratory for distribution to participating laboratories, though there is the possibility that a second laboratory using identical sample creation procedures could be employed if regulations prohibit overseas shipping of some sample types. The samples consist of several types of plastic particles of different sizes, colors, and morphologies. The microplastics will be added to each of the four matrices. In addition, each sample may have a series of non-plastic materials that are intended as false-positive controls. Further information on the sample preparation can be found in the Sample Generation section below.

Study Augmentations

While the Study Core is based on assessing accuracy, precision, time and cost for five common analytical methods in four frequently-encountered matrices, many of the scientists involved in the study employ methods or protocols that differ slightly from the standard operating procedures that are part of the Study Core. Thus, the Study Augmentations will enable individual researchers (or small research groups) to leverage the core program by using the same samples to assess how these variations affect method performance. These augmentation studies fall into three general categories: 1) Alternative extraction methods, 2) Alternative measurement methods, and 3) Matrix permutations. Each of these study augmentations are described in brief below and are more fully described in Appendix L.

Extraction method augmentations

Altering the concentration of KOH in the digestion process

The method for extraction of microplastics from fish tissue in the core study uses a 10% KOH digestion, which is most commonly used by researchers in the field. This study augmentation will assess the effect of using different KOH concentrations: 5%, 10%, and 20% on microplastic recovery.

Methods used: Raman and FTIR
Matrices used: Tissue
Lead scientist: Amy Lusher
Labs participating: NIVA, California Department of Public Health

Evaluating the effectiveness of different digestion salts

The extraction of microplastics from sediment in the core study is based on the use of $CaCl_2$ salts to separate them from sediment and organic materials. This augmentation will assess the effectiveness of using sodium iodide (Nal) as an alternative salt for extracting microplastics from sediment.

Methods used: Raman and FTIR
Matrices used: Sediment
Lead scientist: Amy Lusher
Labs participating: NIVA, Chinese Academy of Sciences

Testing an alternative sediment extraction method

This augmentation will involve testing an extraction method that has three primary differences from the core study sediment extraction method: 1) Use of sodium bromide (NaBr) as the density salt, 2) Re-extracting sediments with a denser concentration of NaBr to ensure retention of denser particles and 3) Use of separatory funnels which allow rinsing to ensure complete removal of particles, no scooping or decanting from beakers.

Methods used: Raman

Matrices used: Sediment
Lead scientist: Kay Ho
Labs participating: USEPA, SCCWRP

Measurement method augmentations

Automation of Raman spectroscopy
The core study will employ Raman spectroscopy in which the stage is manually moved by the operator to select individual particles. This study augmentation will employ automation procedures in which particles are selected for Raman identification through a computer algorithm driving a motorized stage. This augmentation will quantify the speed advantages of automation and assess whether that is offset by any losses in accuracy.

Methods used**:** Raman
Matrices used: Clean water and dirty water
Lead scientist: Silke Christiansen
Labs participating: Innovationsinstitut für Nanotechnologie und korrelative Mikroskopie (INAM), Germany; HORIBA Scientific

Chemical Identification of Microplastics Using a Raman Touch Probe
The samples used in the core study range in size from 1 µm up to 1 mm in size. While Raman microscopy is the only defined method that can measure particulate down to 1 µm in size, drawbacks include the sizable investment to own and operate Raman microscopes. This augmentation will use a Raman touch probe and fixed spectrograph as a low cost, small footprint, rugged system with simple operation capable of field measurements of meso to macro plastic particulates. Results will be compared with Raman microscope measurements, and a lower size limit will be determined for analysis of microplastics using this technique.

Methods used: Raman, Probe
Matrices used: Clean water
Lead scientist: Bridget O'Donnell
Labs participating: HORIBA Scientific

Macroscopic FTIR as a rapid screening technique
The core study is based on quantifying the types and sizes of each plastic particle in the sample, but California's drinking water monitoring requirements could potentially be based on assessing total microplastic concentrations relative to a threshold value, rather than a particle-by-particle type tabulation. This augmentation will assess the utility of macroscopic FTIR as a screening technique that may be used 'upstream' of IR

microscopy, allowing a determination of total microplastic content along with constituent plastic types in a much shorter time period than required for complete tabulation.

Methods used: FTIR
Matrices used: Clean water
Lead scientist: Simon Nunn
Labs participating: Thermo Fisher Scientific

Evaluating the effect of filters for FTIR and Raman microscopy

The core study includes filtration steps prior to FTIR and Raman spectroscopy, but the type of filter material used is not specified. The type of material is particularly important if the particles are to be analyzed in situ on the filter. This augmentation will evaluate the effect of filter type by analyzing triplicate samples that were prepared using a number of filter materials e.g., gold-coated polycarbonate, polycarbonate, silicon, Teflon filters, as well as a proprietary filter material from SiMPore, and subsequently measured on FTIR and Raman microscopes.

Methods used: FTIR, Raman
Matrices used: Clean Water
Lead scientist: Suja Sukumaran
Labs participating: Thermo Fisher Scientific, SiMPore

Effects of subsampling and density of microplastic particles

The core study includes a certain number of microplastic particles of each polymer type and a determined subsampling strategy – e.g., pick 30 of each color/morphology category combination and chemically analyze, image and measure these. The rest are simply counted and visually characterized. This augmentation will determine best practices for subsampling by simulating different subsampling procedures with various samples that are analyzed in full. We will also test out some of the methods we determine to be best practice on real samples.

Methods used: Raman
Matrices used: Clean water
Lead scientists: Chelsea Rochman; Hannah De Frond
Labs participating: University of Toronto

Monitoring pressure changes during filtration as a rapid screening technique

The core study is based on quantifying the types and sizes of each plastic particle in the sample, but California's drinking water monitoring requirements could potentially be based on assessing total microplastic concentrations relative to a threshold value, rather than a particle-by-particle type tabulation. This augmentation will assess the utility of monitoring pressure changes during filtration as an alternative screening technique that could even be used inline during drinking water treatment. This would allow a

determination of total particle content in a much shorter time period than required for other extraction and measurement techniques.

Methods used: Proprietary transmembrane filtration equipment; visual microscopy
Matrices used: Clean water
Lead scientist: James Roussie
Labs participating: SiMPore

Evaluating laser direct infrared chemical imaging as a quantification and identification method

Laser direct infrared (LDIR) chemical imaging is a newly developed instrumental technique that has the potential to provide rapid, automated quantification of particle count, size, and chemical makeup. This study augment will assess the efficacy of LDIR in analysis of processed samples of the four matrices studied in this intercalibration exercise, to determine its advantages and limitations.

Methods used: LDIR
Matrices used: Clean water, dirty water, sediment
Lead scientist: Charles Wong
Labs participating: Eurofins Australia, Agilent

Evaluating pyrolysis-GC/MS as a quantification tool for microplastics

The mass concentration of microplastics in an environmental sample may be a key factor in assessing environmental exposure of this class of contaminants. Spectroscopic techniques provide a number count, but cannot provide mass concentration, a complementary metric to number count. This augmentation will assess the capability of pyrolysis-GC/MS to measure mass-based concentrations of microplastics in the matrices studied in this intercalibration exercise, to determine its advantages and limitations.

Methods used: Pyrolysis-GC/MS
Matrices used: Clean water, dirty water, sediment
Lead scientist: Charles Wong
Labs participating: Eurofins Norway, Penn State

Evaluating effects of new Raman imaging methods in microplastics measurement

Conventional Raman spectroscopy can be time-consuming when applied specifically to microplastics measurement in waters. This may be due to a number of factors related to samples (e.g., hetereogeneity and complexity) but may also be due to the way conventional Raman imaging instruments operate (e.g., raster scanning). This study augment will compare the performance of unmodified Raman with instruments that use new, and potentially faster, imaging techniques. Both technical performance (e.g., accuracy, precision) as well as operational performance (e.g., time and resources expended) will be evaluated.

Methods used: Raman
Matrices used: Clean water
Lead scientist: Maria Navas-Moreno and James Chan
Labs participating: Lever Photonics/UC Davis, SCCWRP

Evaluating effects of tailoring Raman instrumentation specifically for microplastics measurement

Conventional Raman spectroscopy commonly results in a large capital investment because of the technical specifications required for microplastics measurement in waters. This may be due to a number of factors related to samples (e.g., hetereogeneity and complexity) but may also be due to conventional Raman instruments not being optimized for this specific purpose (e.g., tailored selection of technical parameters). This study augment will compare the performance of unmodified Raman microscope with instruments that have been tailored specifically for environmental microplastics analysis. Both technical performance (e.g., accuracy, precision) as well as operational performance (e.g., time and resources expended) will be evaluated.

Methods used: Raman
Matrices used: Clean water
Lead scientist: Maria Navas-Moreno
Labs participating: Lever Photonics, SCCWRP

Evaluating efficacy of optical photothermal infrared spectroscopy for microplastics measurement

Confirmation of individual microplastic particles is currently done by FTIR or Raman spectroscopy. Both these techniques have advantages and limitations. Recently, optical photothermal infrared spectroscopy (O-PTIR) has been developed. O-PTIR combines the two techniques together into a new micro-spectroscopic system that provides noncontact submicron simultaneous IR and Raman microscopy. This study augment will compare the performance of O-PTIR to conventional FTIR and Raman spectroscopy in evaluation of environmental microplastics. Both technical performance (e.g., accuracy, precision) as well as operational performance (e.g., time and resources expended) will be evaluated.

Methods used: O-PTIR and simultaneous Raman spectroscopy
Matrices used: Clean water
Lead scientist: Eoghan Dillon
Labs participating: Photothermal Spectroscopy Corp., SCCWRP

Anticipated Key Products

This section lists some of the key graphics that will be created to meet the core study goals of determining accuracy, repeatability and the costs of each method. Additional graphics not listed here will be produced to show results of the augmentation studies.

Comparison of the results for a given method and matrix (Figure 1) will be made to show how accurate (close to the known number) labs were, as well as how much variability there was in the results. Comparisons will be made both within and among labs.

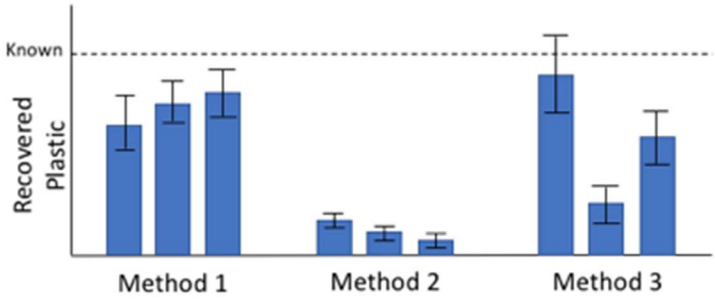


Figure 1. Example graphic showing the amount of recovered plastic among labs using different methods relative to a known quantity of microplastics in a given sample. Each bar represents a different lab.

The ability of each method to detect different sizes of microplastics will also be evaluated (Figure 2). The size ranges analyzed are dependent on the method used, with the goal of >212 µm being the size range for microscopy alone. Smaller sizes can be analyzed via chemical analysis methods with or without initial microscopy.

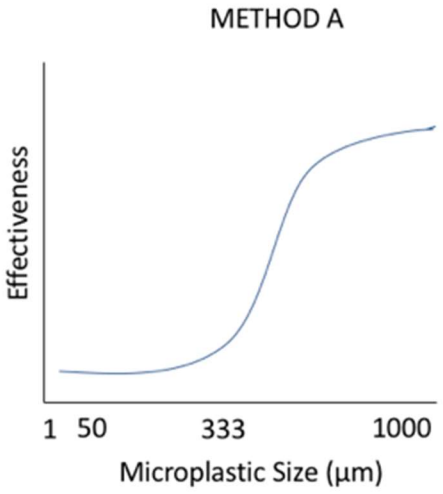


Figure 2. Example graphic showing the effectiveness of a given method at identifying plastic of different sizes.

Looking at differences in polymers and the recovery rates will identify any polymers that were particularly difficult to analyze (Figure 3). Similarly, differences in matrices can be determined by looking at the percent recovery by matrix and lab (Figure 4).

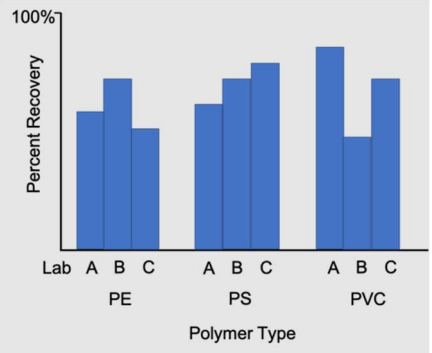


Figure 3. Example graphic showing differences in recovery of microplastics by polymer and lab.

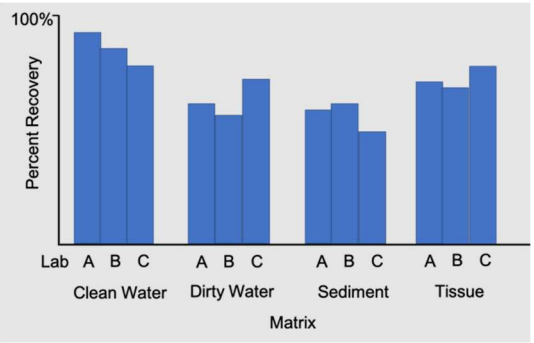


Figure 4. Example graphic showing the recovery of microplastics within a given matrix by lab.

This information will be summarized in a table that shows how each of the parameters of interest performed by method and matrix (Table 2).

Table 2. Summary of the performance (accuracy, repeatability, resources) of each combination of matrix and method.

Method	Accuracy	Repeatability	Resources
Clean Water			
Stereoscope			
Stereoscope with stain			
Fourier-transform infrared spectroscopy (FTIR)			
Raman spectroscopy			
Pyrolysis-GCMS			
Dirty Water			
Stereoscope			
Stereoscope with stain			
Fourier-transform infrared spectroscopy (FTIR)			
Raman spectroscopy			
Pyrolysis-GCMS			

Sediment

Stereoscope			
Stereoscope with stain			
Fourier-transform infrared spectroscopy (FTIR)			
Raman spectroscopy			
Pyrolysis-GCMS			

Tissue

Stereoscope			
Stereoscope with stain			
Fourier-transform infrared spectroscopy (FTIR)			
Raman spectroscopy			
Pyrolysis-GCMS			

Tracking and interpreting metadata

While the SOPs have substantial specificity, there also remains flexibility regarding a number of methodological details. The effects of some of that flexibility, such as types of filters used, are being examined explicitly through Augmentation studies (Appendix L). In addition, laboratories will be asked to provide detailed reporting of laboratory protocols (e.g., type of water used (RO, DI, Milli-Q), brand of chemical ID instrument, laser power on Raman, spectral libraries used, etc…) as per Appendix J so that we have the metadata to assess the importance of such factors in the data analysis stage. In addition, participants will be asked to provide raw spectra and images of plastics so that the effects of data interpretation on method variability can be assessed.

Samples will be distributed to participating laboratories, which will be asked to complete processing and to submit data on the simulated clean water samples for the Core Study by November 23, 2020, and for the other matrices in the Core Study by December 15, 2020. Following data compilation, participating laboratories will be invited to a series of webinars, scheduled between mid-January and early-March, 2021, for collaborative data analysis and interpretation of the study outcomes. The target product is a report that provides recommendations to the State of California about which methods are most appropriate for standardization. In addition, we will target a special issue of a scientific journal to capture not only the core results, but the outcomes from each of the Augmentation studies. The aim for the latter is to have as many Augmentation studies complete by March 2021, to provide data and guidance for the State of California moving forward.

Timeline

Activity	Due Date
Task 1. Finalize study plan and list of study participants	December 2019
Task 2. Train less experienced laboratories	November 2019
Task 3. Create blind test samples	February-September 2020
Task 4. Distribute blind samples to participating laboratories	February-September 2020
Task 5. Laboratories complete laboratory analyses	November 23, 2020 (clean water) December 15, 2020 (other matrices)
Task 6. Data analysis	November 2020-January 2021
Task 7. Webinars/videoconference workshop series among participating laboratories to discuss results and begin drafting report	January-March, 2021
Task 8. Final report	after completion of workshop series

Sample Generation

The blind samples are created using a two-step process. The first step is the generation of the microplastics of known size, which have been created by the Norwegian Institute for Water Research (NIVA). Plastics of a known type are ground into microplastics within a known size range and formed into a pill. Each pill contains one type of microplastic within a designated size range. The pills are sent to SCCWRP, where they are added to the reference matrices. Other polymers may also be added at SCCWRP. In addition, other materials are added to the matrices to allow assessment of false positive identification rate. Laboratories processing the samples will not know the types, sizes and amounts of plastic or false positives contained within each sample.

Sample Matrix Preparation

All matrix preparation will take place in the SCCWRP laboratory following adherence to cleanliness guidelines.

Clean Water

The clean water sample will simulate drinking water and also operate as the “easiest” sample. The pill(s) containing the plastic will be disintegrated into 450 mL clean water. Three samples of 450 mL each will be sent to each lab for the core study, with additional samples being sent for Augmentation studies as appropriate. for each method a lab is performing. A small amount of surfactant is added to help solubilize some of the plastic materials. This water may not necessarily be clear; this is not an issue. These samples can be stored at room temperature, away from direct sunlight or bright light. Please see the SOP (Appendix C) for more details on handling these samples during processing.

Dirty Water

Dirty water samples will be simulated by taking a slurry of plastic-free algae, then diluting it in clean water. Olive oil may be present in addition to algae. The pill(s) containing the plastics will be disintegrated in clean water then mixed with the dirty water to create a 450 mL sample. A 10% volume of isopropyl alcohol will be added for preservation, and a small amount of surfactant may be present to help solubilize some of the plastic materials. Other materials simulating a surface or groundwater matrix may also be present e.g., sand, plant material. Three samples of 450 mL each will be sent to each lab for the core study, with additional samples being sent for Augmentation studies as appropriate. These samples should be stored refrigerated (4 °C). You may notice solid material forming under cold storage; these will go away when the sample is brought back to ambient temperatures. Please see the SOP (Appendix F) for more details on handling these samples during processing.

Sediment

Sediment samples will be created using radio-dated pre-industrial cores, which should contain minimal amounts of plastic. A density separation will be performed, with the floating sediment and particulate extracted. Prior to adding the plastic, samples of the density-separated sediment will be dried and sent to at least two labs to test to ensure the sediment is free of plastic. Once that is accomplished and the samples are confirmed as clean, the pill(s) containing the plastic will be disintegrated in clean water and then mixed with the sediment. Three samples of dry sediment each in 500 mL glass containers will be sent to each lab for the core study, with additional samples being sent for Augmentation studies as appropriate.

Tissue

Clean fish tissue samples will be created using fresh whole fish (salmon). We assume that this fish will be free of microplastics. The fish will be filleted in the lab, then

homogenized. The pill(s) containing the plastic will be disintegrated in clean water and mixed with an aliquot (50 mL) of the fish tissue homogenate, then freeze-dried. Three samples of 50 mL each will be sent to each lab for the core study, with additional samples being sent for Augmentation studies as appropriate.

Sample Distribution

Samples will be distributed in February-September 2020. They will be shipped in glass jars to minimize plastic contamination and will be packaged sufficiently to discourage breakage in transit. SCCWRP will distribute all samples. Core Study samples will be prioritized with regards to creation and dissemination to study participants over Augmentation samples, some of which may require customization according to the parameters of the Augmentation study. Such issues will be discussed with Augmentation participants at the appropriate time.

Sample distribution, as well as other aspects of the study, has been affected by COVID-19. We will do our best to accommodate such issues.

Analytical Methods

There are two types of standard procedures for processing microplastics samples. The first is extraction of the microplastic from the matrix, which typically involves filtering, density separation or chemical digestion. Some good laboratory procedures for working with microplastics are found in Appendix B. Both the extraction and measurement methods are described in the Standard Operating Procedures (SOPs) for each matrix found in Appendices C-F. Within these SOPs, strict QA/QC guidelines have been outlined to ensure quality measurements. These are to be followed and each lab must take clear notes about what precautions are taken in the laboratory. Basic SOPs for instrumental analysis by FTIR and Raman are found in Appendices G and H respectively. Morphologies and colors to use for describing microplastics are listed in Appendices M and N, respectively.

Sample Extraction

Extractions will be performed by each laboratory participating in the study and will be matrix dependent. Clean water samples will be filtered (see Appendix C). Sediment extractions will be conducted via density separation using $CaCl_2$. A minimum of two extractions will be required, followed by filtration (Appendix D). Fish tissue extractions will use a 10% KOH digestion followed by filtration (Appendix E). Extraction of dirty water will use Fenton's reagent (Fe^{2+} + H_2O_2), controlling the temperature of the sample with an ice bath, followed by filtration (Appendix F). Once the microplastics have been extracted from the matrix, they will be analyzed using one or more of five identification methods. Videos on the extraction process will be made available by SCCWRP to participating laboratories, which will aid in training of study participants. Please contact Charles Wong (see Contact Information at beginning of document) for details.

Identification Methods

Five methods will be evaluated in this study: 1) visual microscopy; 2) fluorescence microscopy with Nile Red staining; 3) FTIR; 4) Raman; and 5) pyrolysis-GCMS.

Visual microscopy and Fluorescence microscopy with Nile Red staining

For these methods, a simple stereoscope (with/without fluorescence attachments) is used to count and identify objects as either plastic or non-plastic. Nile Red dye is used to stain the sample/filter so that synthetic materials fluoresce when irradiated by fluorescent light. In addition to ease of use, other advantages to using these methods include low cost and high availability. Cons of these methods include: 1) microplastics may be more difficult to measure in the smaller size ranges (e.g., <212 µm); 2) labs can only visually identify plastics and quantify the number, but not chemically identify the type of plastic; and 3) increased likelihood of false positive and negative identification of materials compared to other methods.

Fourier-transform Infrared Spectroscopy (FTIR)

Fourier-transform infrared spectroscopy (FTIR) is used to obtain an infrared spectrum of absorption or emission of a solid material, such as plastic. Once the spectrum is obtained, it is compared to a selection of known libraries to determine the material. Measurements can typically be made down to about 10 µm. Drawbacks include the expense of the machine and the amount of time it takes to process a sample. Some basic SOPs for operating a Thermo Nicolet iN10 FTIR MX infrared microscope are in Appendix H.

Raman Spectroscopy

Raman spectroscopy is based on inelastic scattering of monochromatic light from a laser source. As with FTIR, the laser excites the particle to create a spectrum that can be compared to a selection of known libraries to determine the material. This method can measure microplastics down to 1 µm in size. Some basic SOPs for operating a Horiba XploRA Plus Raman spectrometer are found in Appendix I.

Pyrolysis-GCMS

Pyrolysis Gas Chromatography/Mass Spectrometry (Pyrolysis-GC-MS) involves heating a sample at a high temperature to gasify the particle. The pyrolyzates are then chromatographically separated in the same manner as traditional GC-MS. The process of pyrolysis completely destroys the sample. This technique has the advantage of being able to specifically identify polymers and plastic additives within a sample. Laboratories using this technique are free to use instrumental parameters that they deem appropriate, and should record these as per Appendix J.

Data Reporting

Data and meta-data will be reported through a spreadsheet template provided to study participants. The format of this spreadsheet and the data fields within it are discussed in Appendix J.

References

Carpenter, E. J. & Smith, K. L. Plastics on the Sargasso Sea surface. Science 175, 1240−1241 (1972).

Thompson, R. C.et al. Lost at sea: Where is all the plastic? Science 304, 838 (2004).

Appendices

Appendix A: Study Participants and Assignments

Study participants consist of all laboratories that have volunteered to process at least 3 replicates for at least one matrix using at least one method.

Table A1. Laboratories participating in this study. *Participating in Augmentation(s) only.

Laboratory (LabID code as per Appendix J)	Contact
Agilent Technologies, US (agilent)	Tarun Anumol
Alfred-Wegener-Institute, Helgoland/Bremerhaven, Germany (awi)	Sebastian Primpke
Algalita Marine Research and Education. US (algalita)	Charles Moore/Gwen Lattin
Barnett Technical Services/Ostec (Barnett)	Steve Barnett
BASF Corporation (basf)	Jeanne Hankett
California Department of Public Health, US (cdph)	Sutapa Ghosal
California State University, Bakersfield, US (csub)	Rae McNeish
California State University, Channel Islands, US (csuci)	Mary Woo/Clare Steele
Carollo Engineers, Inc. (carollo)	Cayla Cook
East China Normal University, China (ecnu)	Hahong Shi
Eastman Chemical Company (eastman)	Gustav Amarpuri
Environmental Protection Agency (EPA), US (epa)	Kay Ho
Eurofins, Australia (eurofins-aus)	Kane Vorwerk
Eurofins, Norway (eurofins-nor)	Joakim Skovly
Eurofins, US (eurofins-usa)	David Riggs/Amber Skaretka
Innovationsinstitut für Nanotechnologie und korrelative Mikroskopie (INAM), Germany (inam)	Silke Christiansen
*HORIBA Scientific, US (horiba)	Bridget O'Donnell
Institute of Hydrobiology, Chinese Academy of Sciences, China (cas)	Chenxi Wu
Jinan University, China (jinan)	Eddy Zeng
*Lever Photonics, US (lever) (in conjunction with UC Davis)	Maria Navas-Moreno
Metropolitan Water District, US (mwd)	Theresa Slifko
National Oceanic and Atmospheric Administration, US (noaa)	Ashok Deshpande

Laboratory (LabID code as per Appendix J)	Contact
NatureWorks LLC (natureworks)	Joseph Schroeder
Norwegian Institute for Water Research, Norway (niva)	Amy Lusher
Ontario Ministry of the Environment, Conservation and Parks, Canada (moe)	Paul Helm
Orange County Sanitation District, US (ocsd)	Violet Renick
Oregon State University, US (osu-brander)	Susanne Brander
Pennsylvania State University, US (psu)	Odette Mina/Josh Stapleton
Photothermal Spectroscopy Corp, US (photo)	Jay Anderson/Eoghan Dillon
RJ Lee Labs, US (rjlee)	Keith Rickabaugh
Southern California Coastal Water Research Project Authority, US (sccwrp)	Charles Wong
*SiMPore, Inc., US (simpore)	James Roussie
*ThermoFisher, US (thermo)	Simon Nunn
*University of California, Davis (ucd) (in conjunction with Lever Photonics)	James Chan
University of California, Riverside, US (ucr)	Win Cowger
University of California, Santa Barbara, US (ucsb)	Timnit Kefela
University of Minnesota, Duluth, US (umd)	Elizabeth Austin-Minor
University of Quebec at Rimouski, Canada (uqar)	Zhe Lu
University of Toronto, Canada (uoft-andrews)	Bob Andrews
University of Toronto, Canada (uoft-rochman)	Chelsea Rochman

Table A2. Laboratories analyzing microplastics using stereoscope and stereoscope with dye staining.

	Stereoscope				Stereoscope with dye stain			
	Water				Water			
Lab	Clean	Dirty	Sediment	Tissue	Clean	Dirty	Sediment	Tissue
Amy Lusher (NIVA)	✓	✓	✓	✓	-	-	-	-
Bob Andrews (U of T)	✓	-	-	-	✓	-	-	-
Cayla Cook (Carollo)	-	✓	-	-	-	-	-	-
Charles Wong (SCCWRP)	✓	✓	✓	✓	✓	✓	✓	✓
Chelsea Rochman (U of T)	✓	-	-	-	-	-	-	-
Chenxi Wu (Chinese Acad. Sci)	✓	✓	✓	-	-	-	-	-
David Riggs/Amber Skaretka (Eurofins US)	✓	✓	-	-	✓	✓	-	-

Eddy Zeng (Jinan University)	✓	✓	-	-	✓	✓	-	-
Elizabeth Austin-Minor (UMD)	✓	✓	-	-	-	-	-	-
Gwen Lattin (Algalita MRE)	✓	✓	✓	✓	✓	-	-	-
Jeanne Hankett (BASF)	✓	-	✓	-	-	-	-	-
Joseph Schroeder (NatureWorks)	✓	-	-	-	-	-	-	-
Kane Vorwerk (Eurofins Australia)	✓	✓	✓	-	-	-	-	-
Mary Woo/Clare Steel (CSUCI)	✓	-	-	✓	✓	-	-	✓
Paul Helm (MECP)	✓	✓	✓	✓	-	-	-	-
Rae McNeish (CSUB)	✓	✓	✓	✓	-	✓	-	-
Steve Barnett (Barnett)	✓	-	-	-	-	-	-	-
Susanne Brander (OSU)	✓	-	-	✓	-	-	-	✓
Theresa Slifko (MWD)	✓	-	-	-	✓	-	-	-
Timnit Kefela (UCSB)	-	✓	✓	-	-	✓	✓	-
Violet Renick (OCSD)	✓	✓	✓	-	✓	✓	✓	-
Win Cowger (UCR)	✓	✓	✓	✓	-	-	✓	✓
Zhe Lu (UQAR)	✓	-	-	-	-	-	-	-
Number of Labs	21	14	11	8	8	6	4	4
Total Samples (3/Lab/Matrix)	63	42	33	24	24	18	12	12

Table A3. Laboratories analyzing microplastics using Fourier-Transform Infrared Spectroscopy (FTIR).

	FTIR			
	Water			
Lab	Clean	Dirty	Sediment	Tissue
Amy Lusher (NIVA)	✓	✓	✓	✓
Cayla Cook (Carollo)	-	-	-	-
Charles Wong (SCCWRP)	✓	✓	✓	✓
Chelsea Rochman (U of T)	✓	-	-	-
David Riggs/Amber Skaretka (Eurofins US)	✓	✓	-	-
Eddy Zeng (Jinan University)	✓	✓	-	-
Elizabeth Austin-Minor (UMD)	✓	✓	-	-
Gaurav Amarpuri (Eastman)	✓	-	-	-
Gwen Lattin (Algalita MRE)	✓	✓	✓	✓
Huahong Shi (East China Normal U)	✓	✓	-	✓
Joseph Schroeder (NatureWorks)	✓	-	-	-
Josh Stapleton (PSU)	✓	✓	-	-
Keith Rickabaugh (RJ Lee)	✓	✓	✓	-
Mary Woo/Clare Steele (CSUCI)	✓	-	-	✓
Paul Helm (MECP)	✓	✓	✓	✓
Rae McNeish (CSUB)	✓	✓	✓	✓

	FTIR			
	Water			
Lab	Clean	Dirty	Sediment	Tissue
Sebastian Primke (Alfred-Wegener)	✓	✓	✓	✓
Susanne Brander (OSU)	✓	-	-	✓
Theresa Slifko (MWD)	✓	-	-	-
Timnit Kefela (UCSB)	-	✓	✓	-
Violet Renick (OCSD)	✓	✓	✓	-
Win Cowger (UCR)	✓	✓	✓	-
Zhe Lu (UQAR)	✓	-	-	-
Number of Labs	21	15	10	8
Total Samples (3/Lab/Matrix)	63	45	30	24

Red=Training at SCCWRP

Italics=Using SCCWRP lab

Table A4. Laboratories analyzing microplastics using Raman Spectroscopy.

	Raman Spectroscopy			
	Water			
Lab	Clean	Dirty	Sediment	Tissue
Ashok Deshpande (NOAA)	✓	✓	✓	✓
Bob Andrews (U of T)	✓	-	-	-
Bridget O'Donnell (Horiba)	-	-	-	-
Cayla Cook (Carollo)	-	✓	-	-
Charles Wong (SCCWRP)	✓	✓	✓	✓
Charles Moore (Algalita MRE)	✓	✓	✓	✓
Chenxi Wu (Chinese Acad. of Sci.)	✓	✓	✓	-
Eddy Zeng (Jinan University)	✓	✓	-	-
Eoghan Dillon (Photothermal)	✓	-	-	-
Gaurav Amarpuri (Eastman)	✓	-	-	-
Huahong Shi (East China Normal U)	✓	✓	-	-
Josh Stapleton (PSU)	✓	✓	-	-
Keith Rickabaugh (RJ Lee)	✓	✓	✓	-
Kay Ho (EPA)	✓	-	✓	-
Rae McNeish (CSUB)	✓	✓	✓	✓
Silke Christiansen (INAM)	✓	✓	-	-
Steve Barnett (Barnett)	✓	-	-	-
Sutapa Ghosal (CDPH)	-	✓	-	✓
Theresa Slifko (MWD)	✓	-	-	-
Timnit Kefela (UCSB)	-	✓	✓	-
Violet Renick (OCSD)	✓	✓	✓	-
Win Cowger (UCR)	✓	-	-	-

Zhe Lu (UQAR)	✓	-	-	-
Number of Labs	19	14	9	5
Total Samples (3/Lab/Matrix)	57	42	27	15

Red=Training at SCCWRP

Italics=Using SCCWRP lab

Table A5. Laboratories analyzing microplastics using Pyrolysis-GCMS.

	Pyrolysis-GCMS			
	Water			
Lab	Clean	Dirty	Sediment	Tissue
Ashok Deshpande (NOAA)	✓	✓	✓	✓
Elizabeth Austin-Minor (UMD)	✓	✓	-	-
Gaurav Amarpuri (Eastman)	✓	-	-	-
Jeanne Hankett (BASF)	✓	-	✓	-
Joakim Skovly (Eurofins Norway)	✓	✓	✓	-
Odette Mina (PSU)	✓	✓	✓	✓
Number of Labs	6	4	4	2
Total Samples (3/Lab/Matrix)	18	12	12	6

Table A6. Laboratories participating in Augmentation studies. Values indicate additional numbers of Blind Samples necessary for each lab to conduct its Augmentations. Numbers in parentheses indicate necessary numbers of custom samples with different composition(s) from those in the core study. No value indicates that the lab is not participating in Augmentations requiring that matrix. *SiMPore, Eurofins Norway, and SCCWRP will require an additional number of custom samples to be determined separate from this document.

	Augmentations			
	Water			
Lab	Clean	Dirty	Sediment	Tissue
Silke Christiansen (INAM)	0	0		
Bridget O'Donnell (Horiba)	0	0		
Simon Nunn (Thermo)	15			
Charles Wong (SCCWRP)	0			
Bob Andrews (U of T)	0			
Chelsea Rochman (U of T)	0	0	0	
Sebastian Primpke (Alfred-Wegener)	0			
James Roussie (SiMPore)	15(10)			
Kane Vorweck (Eurofins Australia)	0	0	0	0
Joakim Skovly (Eurofins Norway)	0(*)	0(*)	0(*)	
Maria Navas-Moreno (Lever Photonics)/James Chan (UCD)	3			
Eoghan Dillon (Photothermal)	3			

Odette Minna (Penn State)	0(*)	0(*)	0(*)	
Chenxi Wu (Chinese Acad. of Sci.)		6	6	
Amy Lusher (NIVA)			3	6
Kay Ho (EPA)			3	
Sutapa Ghosal (CDPH)				6
Number of Labs	13	7	7	3
Total Samples	36(10)	6	12	12

Appendix B: Some good laboratory practices for processing and analyzing microplastics

- *Work effectively but efficiently throughout all stages of sample processing to minimize chances of airborne sample contamination.*

- Keep surfaces clean. Wipe them down daily, as well as before and after using them for microplastics work.
- Keep floors clean. Mop down regularly (e.g., with clean water).
- Avoid synthetic fibers in the laboratory.
    - Wear cotton lab coats, ideally of a noticeable color, throughout all.
    - Avoid wearing synthetic clothing.
    - Avoid furniture (e.g., chairs, stools) with padding or fabric on them (i.e., all metal or wood construction).
- Clean all labware thoroughly with soap and water, and triple-rinse with RO water before use.
- Keep all samples and materials covered with clean aluminum foil as much as possible.
    - Use pressurized air to remove possible contamination on the surface of the foil.
    - As an extra measure, heavy duty aluminum foil can be ashed ≥ 450 °C for at least 1 hour to destroy all organic material. Ash heavy duty foil only, lightweight foil will disintegrate at high temperatures.
- If you can, process microplastics on a clean lab bench, in a biosafety cabinet, or a clean cabinet.
    - A fully enclosed space, even without active air convection, helps to reduce airborne particulates getting into and onto your samples.
    - Some procedures must be done in a fume hood e.g., peroxide digestion.
- Install a HEPA filtration system in your laboratory to minimize airborne particulates.
    - Portable consumer/residential units are inexpensive and effective.
    - Be sure to change the HEPA filter regularly.
- Minimize use of plastics in the laboratory. If you must use plastics, use items that are unlikely to disintegrate or shed particles.
    - Using tygon tubing to dispense RO (or 18 MΩ-cm nanopore/MilliQ/DI) water is ok, as it is unlikely to fragment.
    - Typical laboratory-grade solvent squeeze bottles are also suitable, but better when they do not have a label that may peel off.
- Any reagents or solvents (e.g., CaCl2, KOH) that will be added to a sample should be filtered to remove any contamination present.
- Use a natural sponge, not a synthetic one, for any cleaning where a flexible cleaner is needed.
- If you use compressed gas to blow-dry equipment or samples for microplastics, be sure that the air is clean (e.g., put a filter between the source and the outlet).

This is not necessarily an all-inclusive list.

Appendix C: SOP for Microplastic Extraction from Clean Water

PURPOSE

This SOP describes the procedure by which microplastics >1 µm will be extracted from clean water samples, picked, quantified, characterized and chemically identified. A laboratory blank will be run in addition to each set of test samples, used to monitor particles introduced via procedural contamination.

OVERVIEW

Here, the sample will be analyzed with little sample preparation. The sample will be size fractionated down to 1 µm to assist particle sorting by size. Where able, size fractions will be visually sorted by microscopy (this will vary by lab in accordance with technique and equipment/supplies available, and is easiest when >212 µm; some labs may automate at smaller size fractions or not be able to analyze certain size fractions). Particle ID will be analyzed using methods agreed upon by each lab. Each lab is expected to quantify the total number of suspected plastic particles within each size fraction, the morphology of each suspected plastic particle, and the color of each suspected plastic particle (where possible). A subset of particles will be imaged, measured and chemically identified (as per the subsampling rules below).

A graphical flowchart depiction of this procedure is found at the end of this Appendix.

MATERIALS

For extraction

Item	Suggested Materials
Low foam dish soap	Alcojet detergent Fisher Catalog no. 16-000-111
Natural sponge	Amazon - “Natural Sea Sponge 6-7"
Aluminum foil	-
Laboratory Labeling tape	Fisher Catalog No. 15901A
Fine-tip sharpie	Sold at stationary stores
Squirt bottle (polypropylene)	Amazon – “Highfive 250cc Scientific Safety Wash Bottle Narrow Mouth Polypropylene/Plastic Squeeze Bottle Medical Label Tattoo Wash Bottle”
RO water	Alternatives include; MilliQ (18 MΩ cm), Deionized water or water filtered through a 1 µm pore-size filter
1 µm pore-size filters	Material and diameter will vary based on analytical technique and filtering apparatus
20 µm pore-size filters	Material and diameter will vary based on analytical technique and filtering apparatus

Metal sieves	VWR Catalog no. 57334-568 (500 µm mesh size) VWR Catalog no. 57334-578 (212 µm mesh size)
Metal sieve pan	Same diameter as sieves
Glass mason jars	>500 mL size One for each size fraction that will be wet picked Non-plastic lids preferred
Vacuum filtration system: 1 x Vacuum pump 2 x Plastic tubing 2 x 1000 mL Glass filtering flasks with rubber stopper 1 x filtering funnel 1 x filter holder with glass support 1 x metal clamp	GAST model DOA-P704-AA Tygon S3™ Laboratory Tubing Filtration set-up VWR Catalog no. 89428-970 Secondary filtering flask VWR Catalog no. 10545-858

For counting

Item	**Suggested Materials**
Glass Petri Dishes for wet picking	VWR Catalog no. 25354-069
Small Glass Petri Dishes for dry picking from a filter	VWR Catalog no. 25354-025 (For use with a 47mm diameter filter)
Petri Dishes for picked particles	Size and material not specified
Superfine-tip forceps	VWR Catalog no. 63042-688
Petri dish grid stickers	Amazon - “Diversified Biotech PetriStickers PSTK-1070 Square Grid Label for Petri Dish, 70 Square Grid (Pack of 36)”
Laboratory labelling tape	-
Aluminum foil	-
Double sided tape	Available from stationary stores
Clear projector paper	Available from stationary stores
Metal teaspoon	Amazon - “4.5" Stainless Steel Teaspoon, Set of 6”
Stereoscope	Interchangeable black and white base preferable for picking
Microscope digital camera attachment	E.g. ToupTek touptek.com/product/product.php?lang=en&class2=56
Computer with software for images and measurements	E.g. - ImageJ imagej.nih.gov/ij/ (free to download)

	- ToupView touptek.com/product/product.php?lang=en&class2=74

For counting with Nile Red (in addition to counting materials above)

Item	Suggested Materials
Fluorescence stereoscope	- Fluorescence light attachment, excitation at 460-500 nm; emission at 535 nm.: nightsea.com/products/stereomicroscope-fluorescence-adapter/ **OR** - Crime Lite (Blue light) with orange filter; excitation at 450–510 nm; emission at 529 nm: fosterfreeman.com/forensic-light-sources/328-crime-lite-2.html
Nile Red ≥ 99% purity	VWR Catalog no. TCN0659-500MG CAS 7385-67-3
Acetone ≥ 99% purity	Technical grade or higher 10 mL per reagent stock solution VWR Catalog no. BDH1101-1LP
n-Hexane ≥ 95% purity	Technical grade or higher 100 mL per reagent stock solution VWR Catalog no. CAHX0295-6
15 mL Amber glass bottles	One per reagent stock solution VWR Catalog no. 11311-184
120 mL Amber glass bottles	One per reagent stock solution VWR Catalog no. 36319-770
1 mL glass pipettes	VWR Catalog no. 76003-572

Personal Protective Equipment (PPE)

The following PPE are mandatory for sample processing:

- Clean cotton lab coat
- Clean nitrile gloves
- Safety glasses, goggles or face shield when applicable (e.g. when working with reagents)
- Clean cabinet or covered enclosure to reduce contamination (if available)

PROCEDURE

Take notes on everything you do, especially any deviation from the wording of the SOP.

Procedural Blanks

- One laboratory blank will be sent with each set of test samples; the blank will consist of a 450 mL sample of clean water, provided with the shipment of blind samples, which will be run through the same protocol as the test samples; extracted, size fractioned, particles quantified, characterized and chemically identified.
- The samples can be kept at room temperature, away from direct sunlight or bright light.

A. Preparation

- Before using any glassware or tools, wash with soap and water (surfactant helps to remove contaminant microplastics). Rinse three times with tap water, then three times with RO water (or suitable equivalent).
- Clean sieves with soap and water using a natural sponge.
- When equipment/tools/labware are not being used, or when samples are not being analyzed, keep covered to prevent procedural contamination.

B. Extraction procedure - Filtering

1. Set up sieve stack with (from top to bottom; 500 µm sieve, 212 µm sieve and sieve pan).
   a) Pour the sample through the sieve stack
   b) Triple rinse the sample jar into the sieve stack using RO water. Tap the sieves gently to move everything through to its appropriate size fraction.
   c) Rinse the contents of each sieve into a separate (cleaned and labelled) glass jar using RO water.
   Alternatively, you can filter all size fractions onto filter paper. Either is acceptable, as long as you have them split into the relevant size classes dictated by the sieves (i.e. you can use wet or dry sorting for the larger size fractions).
   d) Pour the contents of the sieve pan into a clean beaker and cover.
2. Assemble vacuum filtration system without the filtering funnel and clamp. NOTE: During vacuum filtration, you may wish to heat the sample to no more than 55 °C and monitor the temperature with a thermometer. Then add a small amount of water to the filtering cup. This will help the intercalibration water samples filter a bit faster. If you fill the whole thing, the water cools down during the time it takes to filter and slows down the process.
   a) Turn on vacuum pump. Pour RO water onto the glass filter holder to clean the system.
   b) Turn vacuum pump off. Empty the waste from the bottom flask and rinse the flask with RO water, then reassemble.
   c) Place a 20 µm filter onto the glass filter holder and secure the filtering funnel on top using the metal clamp.

3. Turn the vacuum pump on and pour the remaining sample (sieve pan contents, transferred to a beaker) through the filtration system.
   Note: ensure not to overfill the filtering flask as this may lead to sample loss.
4. Keeping vacuum pump on, triple rinse the sides of the filtering funnel with RO water.
5. Turn off the vacuum pump, remove the metal clamp and carefully lift the filtering funnel away from the base.
   Note: Tweezers may be used to ensure the filter is not removed with the filtering funnel as you do this.
6. Turn on the vacuum pump and carefully rinse the base of the filtering funnel onto the 20 µm filter, using RO water.
7. Turn off the vacuum pump, remove the 20 µm filter from the filtration system, place it into a clean, labelled petri dish and cover.
8. Pour and triple rinse the contents of the filtering flask into a clean beaker and cover, reassemble the filtration system using a 1 µm filter and repeat steps 3-7.

Quantification and characterization
See Appendix G for guidance on order of processing

C. Visual microscopy
If particles are too small to manually count and identify, you may leave them on the filter and use automated quantification, characterization (morphology, color (where possible)) and chemical identification methods (see Section E of this Appendix).

1. Bring all four size fractions over to the microscope (i.e., >500 µm, >212 µm, >20 µm and >1 µm).
2. Using a systematic method of your choice, count all particles for each size fraction and record the color and morphology of each.
3. Pick (subsample) the first 30 particles identified from each color/morphology category (e.g. blue fiber, black fragment) within each size fraction. Store the subsampled particles on a substrate relevant to the method of chemical identification you will be using, e.g. double-sided tape for particles that will be analyzed via Raman or benchtop ATR-FTIR, a reflective surface for reflectance FTIR spectroscopy. This is a suggestion only; please store particles as you see fit. When using double-sided tape, the tape should not be laid directly into the base of a petri dish. Instead, we recommend using projector paper or a glass slide as a base to lay the tape on. The choice between the two may depend on the stage of the instrumentation you are using for chemical analyses. When using ATR-FTIR spectroscopy, double sided tape should only be used for larger size fractions to avoid the crystal coming into contact with the tape. If you are using Nile Red, do this by morphology only (see below). If less than 30 particles are identified for a certain particle category, pick as many as you find. After 30 particles have been picked, no longer pick from that category, but continue to count and characterize all other plastic particles you find. For smaller size fractions, particles do not need to be manually picked as this is not only challenging but provides a high likelihood for particle loss. These particles can instead be analyzed directly from the filter substrate.

4. For subsampled particles, image and measure each particle along the longest perpendicular axes (length and width). For fibers, do not measure frayed projections and use segmented/curved lines where necessary. If a particle has broken apart, use your best judgement e.g. measure three lengths and one width for a fragment that has fractured along its length. Make note of the method used for measurement in this case.
5. If you are quantifying and characterizing via microscopy only, your analysis is complete. If you are using a further analytical method, proceed to that section.

D. Nile Red fluorescence microscopy

1. Nile Red working solution preparation.
   Note: Always work with Nile Red solutions inside a functioning fume hood to avoid contact with acetone and n-*Hexane vapors.*
   a) If needed, prepare a 1 mg/mL stock solution of Nile Red in 10 mL acetone.
   b) Dilute stock solution with *n*-Hexane to create 100 mL of 10 µg/mL Nile Red working solution.
   c) Keep the stock and working solutions in amber glass bottles or covered in aluminum foil to prevent photodegradation. It is recommended to store the stock solution for no more than 6 months, and the working solution for no more than 2 months, providing it is kept in the dark at 4°C during this period.
2. Working in a fume hood, apply 0.5 mL of working Nile Red solution to each filter using a glass pipette, cover with the petri dish lid. Cover the petri dish with a layer of aluminum foil and allow to incubate at room temperature in the dark for 30 minutes.
3. After the incubation period, lift the petri dish lid and prop it open using the edge of the dish. Keep the dish and sample covered with aluminum foil and allow the *n*-Hexane to completely evaporate for a minimum of another 30 minutes.
   Alternatively: you may carefully transfer the filter back onto the filtration unit. Stop the vacuum and incubate the filter with 0.5 mL of the working solution of Nile Red. Cover the filtration unit with aluminum foil to avoid direct light. After 30 minutes, turn the vacuum back on to release the working solution, and rinse the filter three times with n-Hexane. Carefully remove the filter and transfer back to the petri dish. Record which method you have used to incubate and stain samples.
4. Bring samples to the microscope. Adjust settings dependent on fluorescence attachment, record fluorescence settings and magnification used:
   a) Crime-lite - Orange filter; excitation at 450–510 nm; emission at 529 nm.
   b) Fluorescence adapter (e.g. Night Sea) - excitation at 460-500 nm; emission at 535 nm.
5. Count and record the morphology of all brightly fluorescing particles observed in each size fraction.
6. Pick (subsample) the first 30 particles identified from each morphology category (e.g. fiber, fragment) for each size fraction. Store the subsampled particles on a substrate relevant to the method of chemical identification you will be using, e.g. double-sided tape for particles that will be analyzed via Raman or benchtop ATR-FTIR, a reflective surface for reflectance FTIR spectroscopy. This is a suggestion only; please store particles as you see fit. When

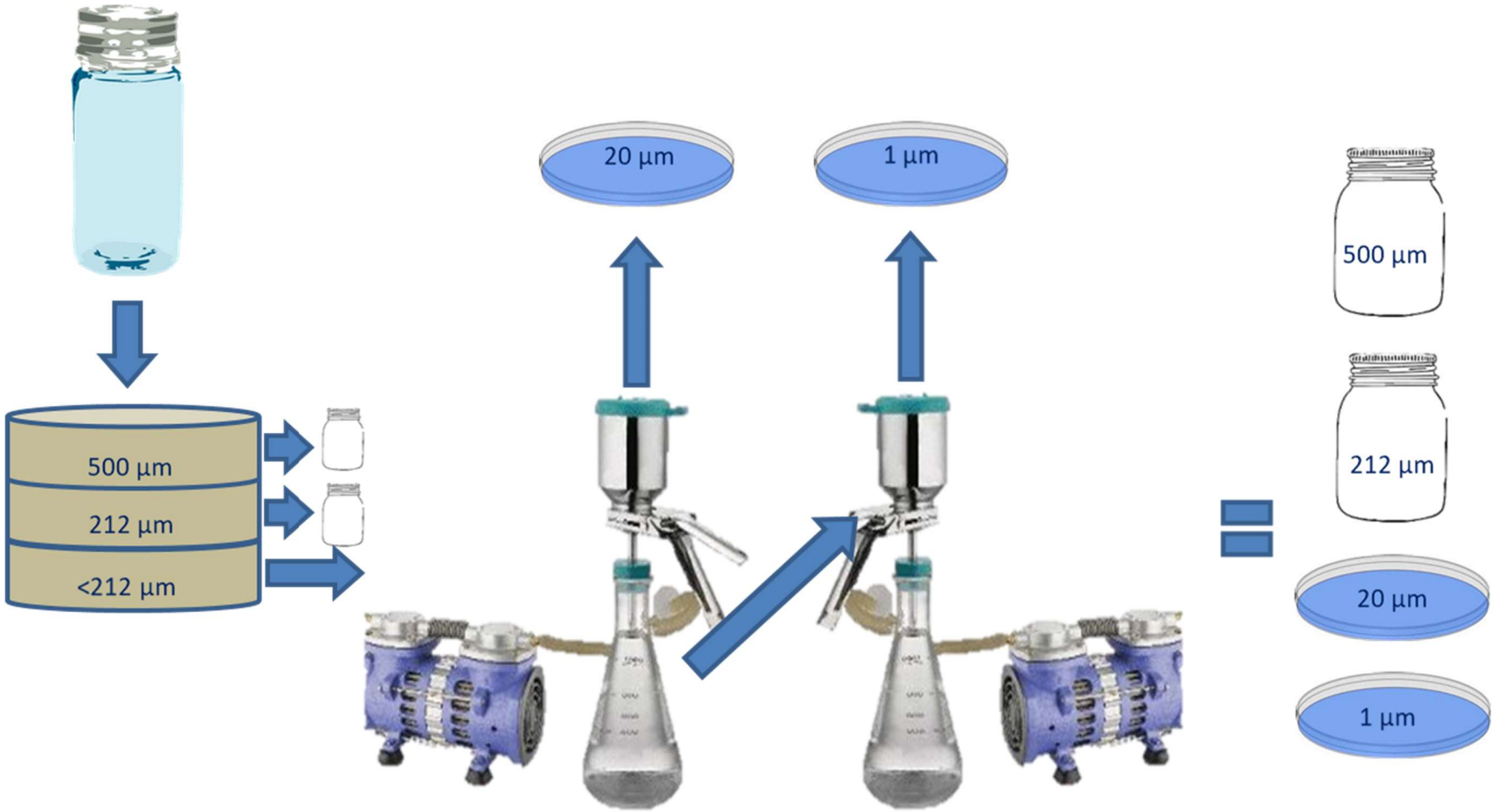
using double-sided tape, the tape should not be laid directly into the base of a petri dish. Instead, we recommend using projector paper or a glass slide as a base to lay the tape on. The choice between the two may depend on the stage of the instrumentation you are using for chemical analyses. When using ATR-FTIR spectroscopy, double sided tape should only be used for larger size fractions to avoid the crystal coming into contact with the tape. If less than 30 particles are identified for a certain category, pick as many as you find. After 30 particles have been picked, continue to count and characterize all other particles from that category. For smaller size fractions, particles do not need to be manually picked as this is not only challenging but provides a high likelihood for particle loss. These particles can instead be analyzed directly from the filter substrate.

7. For all subsampled particles, take an image of each particle and measure along the longest perpendicular axes (length and width). For fibers, do not measure frayed projections and use segmented/curved lines where necessary. If a particle has broken apart, use your best judgement e.g. measure three lengths and one width for a fragment that has fractured along its length. Make note of the method used for measurement in this case.
8. If you are quantifying and characterizing via Nile Red microscopy only, your analysis is complete. If you are using a further analytical method, proceed to that section.

E. Chemical Analysis (FTIR, Raman or Pyro-GC/MS)

1. If you have signed up to complete more than one method of chemical analysis, refer to the flow chart (Appendix G) for a recommended order of processing. SOPs for conducting chemical analysis can be found in Appendix H for FTIR spectroscopy and Appendix I for Raman spectroscopy.
2. All subsampled particles (≤30 of each category per size fraction) must be chemically identified.
3. Particles too small for manual picking and subsampling may be analyzed directly from a filter. The choice of method for this is yours to make (see Appendix G).
4. Whichever method is used for analysis, submit all details and references used.
5. Record all results in data sheets provided (Appendix J) and back up the data electronically.

SOP for Microplastic Extraction from Clean Water



Appendix D: SOP for Microplastic Extraction from Sediments

PURPOSE

This SOP describes the procedure by which microplastics >1 µm in longest length will be extracted from sediment samples, picked, quantified, characterized and chemically identified. A laboratory blank will be run in addition to each set of test samples, used to monitor particles introduced via procedural contamination.

OVERVIEW

The sample will be size fractionated down to 1 µm to assist particle sorting by size. Where able, size fractions will be visually sorted by microscopy (this will vary by lab in accordance with technique and equipment/supplies available, and is easiest when >212 µm; some labs may automate at smaller size fractions or not be able to analyze certain size fractions). Particle ID will be analyzed using methods agreed upon by each lab. Each lab is expected to quantify the total number of suspected plastic particles within each size fraction, the morphology of each suspected plastic particle, and the color of each suspected plastic particle (where possible). A subset of particles will be imaged, measured and chemically identified (as per the subsampling rules below).

A graphical flowchart depiction of this procedure is found at the end of this Appendix.

MATERIALS

For extraction

Item	Suggested Materials
Low foam dish soap	Alcojet detergent Fisher Catalog no. 16-000-111
Natural sponge	Amazon - “Natural Sea Sponge 6-7"
Aluminum foil	-
Laboratory Labelling tape	Fisher Catalog No. 15901A
Fine-tip sharpie	Sold at stationary stores
2 x Squirt bottles (Polypropylene)	Amazon – “Highfive 250cc Scientific Safety Wash Bottle Narrow Mouth Polypropylene/Plastic Squeeze Bottle Medical Label Tattoo Wash Bottle”
RO water	Alternatives include; MilliQ (18 MΩ cm), Deionized water or water filtered through a 1 µm pore-size filter
1 µm pore-size filters	Material and diameter will vary based on analytical technique and filtering apparatus
20 µm pore-size filters	Material and diameter will vary based on analytical technique and filtering apparatus
Metal sieves	VWR Catalog no. 57334-568 (500 µm mesh size) VWR Catalog no. 57334-578 (212 µm mesh size)

Metal sieve pan	Same diameter as sieves
Glass mason jars	>500 mL size One for each size fraction that will be wet picked Non-plastic lids preferred
Vacuum filtration system: 1 x Vacuum pump 2 x Plastic tubing 2 x 1000 mL Glass filtering flasks with rubber stopper 1 x filtering funnel 1 x filter holder with glass support 1 x metal clamp	GAST model DOA-P704-AA Tygon S3™ Laboratory Tubing Filtration set-up VWR Catalog no. 89428-970 Secondary filtering flask VWR Catalog no. 10545-858
Calcium Chloride anhydrous pellets	Approximately 800 g/sample VWR Catalog no. 97062-590
Glass beaker, 4 L	Used for mixing $CaCl_2$ solution Fisher Catalog no. 02-540T
Glass beakers, 500 mL	1 per sample (Beaker 1) Fisher Catalog no. S15441
Glass beakers, 1 L	1 per sample (Beaker 2) Fisher Catalog no. 02-540P
Magnetic stir bar	Fisher Catalog no. 14-513-67
Stir plate	Fisher Catalog no. S504631H
Hydrometer	VWR Catalog no. 34640-207
Small metal spoon	Amazon - “4.5" Stainless Steel Teaspoon,Set of 6”

For counting

Item	**Suggested Materials**
Glass Petri Dishes for wet picking	VWR Catalog no. 25354-069
Small Glass Petri Dishes for dry picking from a filter	VWR Catalog no. 25354-025 (For use with a 47mm diameter filter)
Petri Dishes for picked particles	Size and material not specified
Superfine-tip forceps	VWR Catalog no. 63042-688
Petri dish grid stickers	Amazon - “Diversified Biotech PetriStickers PSTK-1070 Square Grid Label for Petri Dish, 70 Square Grid (Pack of 36)”
Laboratory labeling tape	-
Aluminum foil	-
Double sided tape	Available from stationary stores
Clear projector paper	Available from stationary stores
Metal teaspoon	Amazon - “4.5" Stainless Steel Teaspoon,Set of 6”

Stereoscope	Interchangeable black and white base preferable for picking
Microscope digital camera attachment	E.g. ToupTek touptek.com/product/product.php?lang=en&class2=56
Computer with software for images and measurements	E.g. - ImageJ imagej.nih.gov/ij/ (free to download) - ToupView touptek.com/product/product.php?lang=en&class2=74

For counting with Nile Red (in addition to counting materials above)

Item	**Suggested Materials**
Fluorescence stereoscope	- Fluorescence light attachment, excitation at 460-500 nm; emission at 535 nm.: nightsea.com/products/stereomicroscope-fluorescence-adapter/ **OR** - Crime Lite (Blue light) with orange filter; excitation at 450–510 nm; emission at 529 nm: fosterfreeman.com/forensic-light-sources/328-crime-lite-2.html
Nile Red ≥ 99% purity	VWR Catalog no. TCN0659-500MG CAS 7385-67-3
Acetone ≥ 99% purity	Technical grade or higher 10 mL per reagent stock solution VWR Catalog no. BDH1101-1LP
n-Hexane ≥ 95% purity	Technical grade or higher 100 mL per reagent stock solution VWR Catalog no. CAHX0295-6
15 mL Amber glass bottles	One per reagent stock solution VWR Catalog no. 11311-184
120 mL Amber glass bottles	One per reagent stock solution VWR Catalog no. 36319-770
1 mL glass pipettes	VWR Catalog no. 76003-572

Personal Protective Equipment (PPE)

The following PPE are mandatory for sample processing:

- Clean cotton lab coat
- Clean nitrile gloves
- Safety glasses, goggles or face shield when applicable (e.g. when working with reagents)
- Clean cabinet or covered enclosure to reduce contamination (if available)
- Functioning fume hood (when working with reagents)

PROCEDURE

Take notes on everything you do, especially any deviation from the wording of the SOP.

Procedural Blanks

- One laboratory blank will be sent with each set of test samples; the blank will consist of a sample of un-spiked sediment, which will be run through the same protocol as the test samples; extracted, size fractioned, particles quantified, characterized and chemically identified.

C. Preparation

- Before using any glassware or tools, wash with soap and water (surfactant helps to remove contaminant microplastics). Rinse three times with tap water, then three times with filtered/RO water.
- Clean sieves with soap and water using a natural sponge.
  When equipment/tools/labware are not being used, or when samples are not being analyzed, keep covered to prevent procedural contamination.

Prepare $CaCl_2$

Instructions here make 1.2 L of $CaCl_2$ at a density of 1.4 g/mL, more can be prepared and stored when carrying out multiple density separations.

1. Wash stir bar, 2 L beaker and 500 mL beaker.
2. Prepare $CaCl_2$ solution of 1.4 g/mL
   a) Place stir bar in 2 L beaker. Place beaker on stir plate and cover with aluminum foil.
   b) Weigh 800 g of $CaCl_2$ in 500 mL beaker and add to 2 L beaker.
   c) Add 1.2 L of RO water to beaker.
   d) Switch the stir plate power on. Mix until all $CaCl_2$ has dissolved and the liquid becomes (almost) colorless (approximately 30 minutes).
   e) Once the $CaCl_2$ has dissolved, switch the stir plate off and allow solution to cool down to room temperature. Check the density using a (clean, air dried) hydrometer.
   f) If the density is at 1.4 g/mL (meniscus touching the 1.4 line), your $CaCl_2$ mixture is ready. If the density is below 1.4 g/mL, add $CaCl_2$ in small amounts (up to 50 g at a time) and repeat steps 2d and 2e until density reaches 1.4 g/mL
   g) Remove beaker from the stir plate, cover with aluminum foil.
3. Filter $CaCl_2$ solution before use. Set up filtration system (see filtering SOP) and filter solution using a 20 µm filter (any filter type). Ensure all equipment has been cleaned, air dried and/or rinsed with $CaCl_2$ before use.
4. Pour the contents of the filtering flask (20 µm filtered $CaCl_2$) into a clean, dry beaker and repeat filtering process using a 1 µm filter (glass fiber is recommended for this step).

B. Extraction Procedure

Density Separation

1. Rinse 500 mL beaker (referred to as Beaker 1) with $CaCl_2$ solution. Spoon the sediment sample into the Beaker 1. Rinse the shipping container and spoon with $CaCl_2$, into Beaker
   Note: Some sediment may appear stuck to the sides of the shipping container. If this occurs, after emptying the bulk of the sediment into Beaker 1, fill the 'empty' shipping jars with $CaCl_2$ solution and leave to sit (at least 1 hour recommended) before rinsing the remains into Beaker 1.
2. Add $CaCl_2$ solution to Beaker 1 until the beaker is approximately 300 mL full.
   a) Using the metal spoon, stir the solution vigorously for 2 minutes.
   b) Rinse the spoon and sides of the beaker with $CaCl_2$ and let settle. Record the time.
3. Cover Beaker 1 with aluminum foil and let sit for 2h. Do not move Beaker 1 during this time as this may re-suspend particles.
4. During this time, wash a 1 L beaker (referred to as Beaker 2) and rinse with $CaCl_2$.
5. After 2 hours, use the small metal spoon to scoop the top floating layer from Beaker 1 and transfer it to Beaker 2. Try not to disturb the water beneath the surface during this process and do not move Beaker 1 as this may re-suspend particles.
6. After the surface layer has been removed (i.e. no visible particles), rinse the spoon with $CaCl_2$ into Beaker 2.
   a) Carefully rinse the sides of Beaker 1 with $CaCl_2$ to remove particles stuck to the sides.
   b) Decant the remaining liquid from Beaker 1 into Beaker 2, stop decanting when sediment settled at the bottom of Beaker 1 begins to move towards the mouth of the beaker.
   c) Cover Beaker 2 with aluminum foil and leave until Step 9.
7. Repeat density separation steps 2-6 once more with the remaining sediment in Beaker 1. The floating layer can be transferred into Beaker 2 (the same 1 L beaker as the first density separation). Discard the remaining settled portion in Beaker 1.
8. Using the metal spoon, stir the solution in Beaker 2 for 2 minutes. Rinse the spoon into Beaker 2 with $CaCl_2$ solution after stirring. Re-cover Beaker 2 with aluminum foil and record the time.
9. Leave Beaker 2 overnight to separate (12-24 hrs). Do not move Beaker 2 during this time as this may re-suspend particles

Size fractioning

1. Clean sieves with soap, water and a natural sponge, then triple rinse with RO water before use. Set up sieve stack (from top to bottom; 500 µm, 212 µm and sieve pan). With a small metal spoon, scoop the surface floating layer from Beaker 2, this time transfer the contents of each spoonful into the sieve stack. Once the surface layer has been removed, carefully rinse the sides of Beaker 2 with $CaCl_2$ to move particles stuck to the sides, then decant the remaining solution from Beaker 2 into the sieve stack, leaving settled particles at the bottom. Discard the settled portion.

2. Tap the sieves gently to move everything through to its appropriate size fraction, then rinse the contents of each sieve into a separate clean, labelled sample jar using RO water. *Alternatively, you can filter all size fractions onto filter paper. Either is acceptable, as long as you have them split into the relevant size classes dictated by the sieves (i.e. you can use wet or dry sorting for the larger size fractions).*
3. Transfer the contents of the sieve pan (<212 µm size fractions) into a clean beaker (a full sieve pan holds approximately 1 L). Using RO water, triple rinse the sieve pan into the beaker and cover with clean aluminum foil.
4. Set up vacuum filtration system with glass or stainless-steel filtration parts (see filtering SOP - if you have one, do this in a clean cabinet). NOTE: During vacuum filtration, you may wish to heat the sample to no more than 55 °C and monitor the temperature with a thermometer. Then add a small amount of water to the filtering cup. This will help the intercalibration water samples filter a bit faster. If you fill the whole thing, the water cools down during the time it takes to filter and slows down the process.
    a) Turn on the vacuum pump. Pour RO water onto the glass filter holder to clean the system.
    b) Turn off the vacuum pump, empty the waste from the bottom flask, rinse the flask with RO water and reassemble the filtration system.
    c) Place 20 µm filter onto the glass filter holder platform and secure the filtration funnel on top using the clamp.
5. Turn on the vacuum pump and pour the remaining sample (<212 µm size fractions in a beaker) into the filtering funnel, pouring small amounts at a time.
   Note: Ensure not to overfill the filtering flask as this may lead to sample loss. To avoid filter clogging, sample volumes can be subdivided into smaller volumes before the data are pooled from each subsample.
    a) Rinse the beaker with RO water three times, continuing to pour into the filtration system.
    b) Rinse the sides of the filtering funnel with RO water three times.
    c) Turn off the vacuum pump and remove the filtering funnel.
       Note: Tweezers may be used to ensure the filter is not removed with the filtering funnel as you do this.
    d) Turn on the vacuum pump and carefully use RO water to move any particles stuck from the base of the filtering funnel onto the 20 µm filter.
6. Turn off the vacuum pump. Carefully remove the 20 µm filter and place it in a labeled, clean petri dish.
7. Pour the contents of the filtering flask into a clean beaker, triple rinse the filtering flask with RO water into the beaker to ensure all particles are transferred and repeat filtering process (steps 6 and 7) using a 1 µm filter.

Quantification and characterization

See Appendix G for guidance on order of processing

C. Visual microscopy

If particles are too small to manually count and identify, you may leave them on the filter and

use automated quantification, characterization (morphology, color (where possible)) and chemical identification methods (see Section E of this Appendix).

1. Bring all four size fractions over to the microscope (i.e., >500 µm, >212 µm, >20 µm and >1 µm).
2. Using a systematic method of your choice, count all particles for each size fraction and record the color and morphology of each.
3. Pick (subsample) the first 30 particles identified from each color/morphology category (e.g. blue fiber, black fragment) within each size fraction. If you are using Nile Red, do this by morphology only (see below). Store the subsampled particles on a substrate relevant to the method of chemical identification you will be using, e.g. double-sided tape for particles that will be analyzed via Raman or benchtop ATR-FTIR, a reflective surface for reflectance FTIR spectroscopy. This is a suggestion only; please store particles as you see fit. When using double-sided tape, the tape should not be laid directly into the base of a petri dish. Instead, we recommend using projector paper or a glass slide as a base to lay the tape on. The choice between the two may depend on the stage of the instrumentation you are using for chemical analyses. When using ATR-FTIR spectroscopy, double sided tape should only be used for larger size fractions to avoid the crystal coming into contact with the tape. If less than 30 particles are identified for a certain particle category, pick as many as you find. After 30 particles have been picked, no longer pick from that category, but continue to count and characterize all other plastic particles you find. For smaller size fractions, particles do not need to be manually picked as this is not only challenging but provides a high likelihood for particle loss. These particles can instead be analyzed directly from the filter substrate.
4. For subsampled particles, image and measure each particle along the longest perpendicular axes (length and width). For fibers, do not measure frayed projections and use segmented/curved lines where necessary. If a particle has broken apart, use your best judgement e.g. measure three lengths and one width for a fragment that has fractured along its length. Make note of the method used for measurement in this case.
5. If you are quantifying and characterizing via microscopy only, your analysis is complete. If you are using a further analytical method, proceed to that section.

D. Nile Red fluorescence microscopy

1. Nile Red working solution preparation.
   Note: Always work with Nile Red solutions inside a functioning fume hood to avoid contact with acetone and n-*Hexane vapors.*
   a) If needed, prepare a 1 mg/mL stock solution of Nile Red in 10 mL acetone.
   b) Dilute stock solution with *n*-Hexane to create 100 mL of 10 µg/mL Nile Red working solution.
   c) Keep the stock and working solutions in amber glass bottles or covered in aluminum foil to prevent photodegradation. It is recommended to store the stock solution for no more than 6 months, and the working solution for no more than 2 months, providing it is kept in the dark at 4°C during this period.

2. Working in a fume hood, apply 0.5 mL of working Nile Red solution to each filter using a glass pipette, cover with the petri dish lid. Cover the petri dish with a layer of aluminum foil and allow to incubate at room temperature in the dark for 30 minutes.
3. After the incubation period, lift the petri dish lid and prop it open using the edge of the dish. Keep the dish and sample covered with aluminum foil and allow the *n*-Hexane to completely evaporate for a minimum of another 30 minutes.
   Alternatively: you may carefully transfer the filter back onto the filtration unit. Stop the vacuum and incubate the filter with 0.5 mL of the working solution of Nile Red. Cover the filtration unit with aluminum foil to avoid direct light. After 30 minutes, turn the vacuum back on to release the working solution, and rinse the filter three times with n-Hexane. Carefully remove the filter and transfer back to the petri dish. Record which method you have used to incubate and stain samples.
4. Bring samples to the microscope. Adjust settings dependent on fluorescence attachment, record fluorescence settings and magnification used:
   a) Crime-lite - Orange filter; excitation at 450–510 nm; emission at 529 nm.
   b) Fluorescence adapter (e.g. Night Sea) - excitation at 460-500 nm; emission at 535 nm.
5. Count and record the morphology of all brightly fluorescing particles observed in each size fraction.
6. Pick (subsample) the first 30 particles identified from each morphology category (e.g. fiber, fragment) for each size fraction. If less than 30 particles are identified for a certain category, pick as many as you find. After 30 particles have been picked, continue to count and characterize all other particles from that category. For smaller size fractions, particles do not need to be manually picked as this is not only challenging but provides a high likelihood for particle loss. These particles can instead be analyzed directly from the filter substrate.
7. Store the subsampled particles on a substrate relevant to the method of chemical identification you will be using, e.g. double-sided tape for particles that will be analyzed via Raman or benchtop ATR-FTIR, a reflective surface for reflectance FTIR spectroscopy. This is a suggestion only; please store particles as you see fit. When using double-sided tape, the tape should not be laid directly into the base of a petri dish. Instead, we recommend using projector paper or a glass slide as a base to lay the tape on. The choice between the two may depend on the stage of the instrumentation you are using for chemical analyses. When using ATR-FTIR spectroscopy, double sided tape should only be used for larger size fractions to avoid the crystal coming into contact with the tape.
8. For all subsampled particles, take an image of each particle and measure along the longest perpendicular axes (length and width). For fibers, do not measure frayed projections and use segmented/curved lines where necessary. If a particle has broken apart, use your best judgement e.g. measure three lengths and one width for a fragment that has fractured along its length. Make note of the method used for measurement in this case.
9. If you are quantifying and characterizing via Nile Red microscopy only, your analysis is complete. If you are using a further analytical method, proceed to that section.

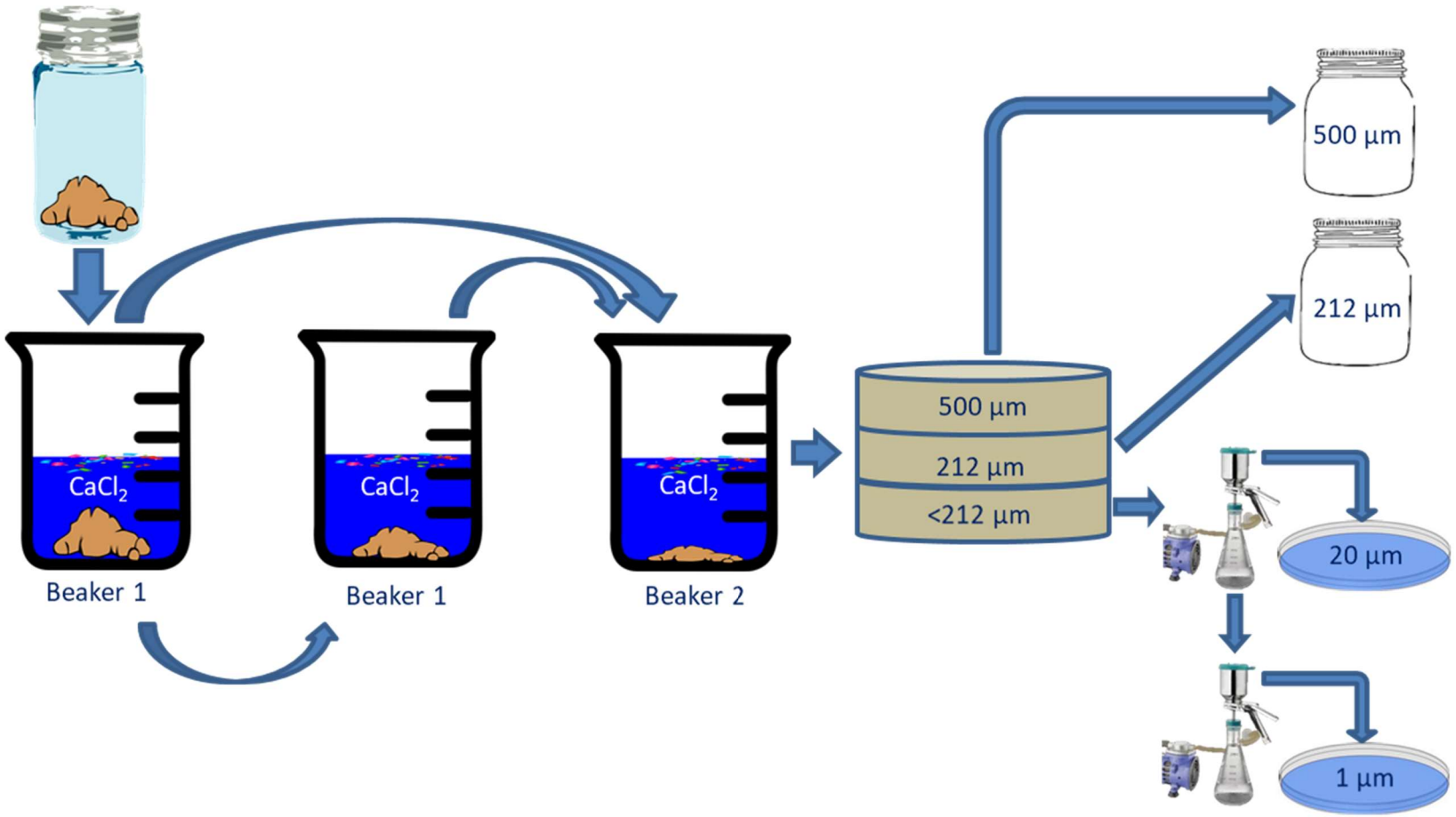
E. Chemical Analysis (FTIR, Raman or Pyro-GC/MS)

1. If you have signed up to complete more than one method of chemical analysis, refer to the flow chart (Appendix G) for a recommended order of processing. SOPs for conducting

chemical analysis can be found in Appendix H for FTIR spectroscopy and Appendix I for Raman spectroscopy.

2. All subsampled particles (≤30 of each category per size fraction) must be chemically identified.
3. Particles too small for manual picking and subsampling may be analyzed directly from a filter. The choice of method for this is yours to make (see Appendix G).
4. Whichever method is used for analysis, submit all details and references used.
5. Record all results in data sheets provided (Appendix J) and back up the data electronically.

Appendix D: SOP for Microplastic Extraction from Sediment



Appendix E: SOP for Microplastic Extraction from Fish Tissue

PURPOSE

This SOP describes the procedure by which microplastics >1 µm in longest length will be extracted from fish tissue samples, picked, quantified, characterized and chemically identified. A laboratory blank will be run in addition to each set of test samples, used to monitor particles introduced via procedural contamination.

OVERVIEW

Here, the sample will be size fractionated down to 1 µm to assist particle sorting by size. Where able, size fractions will be visually sorted by microscopy (this will vary by lab in accordance with technique and equipment/supplies available, and is easiest when >212 µm; some labs may automate at smaller size fractions or not be able to analyze certain size fractions). Particle ID will be analyzed using methods agreed upon by each lab. Each lab is expected to quantify the total number of suspected plastic particles within each size fraction, the morphology of each suspected plastic particle, and the color of each suspected plastic particle (where possible). A subset of particles will be imaged, measured and chemically identified (as per the subsampling rules below).

A graphical flowchart depiction of this procedure is found at the end of this Appendix.

MATERIALS

For extraction

Item	Details
Low foam dish soap	Alcojet detergent Fisher Catalog no. 16-000-111
Natural sponge	Amazon - “Natural Sea Sponge 6-7"
Aluminum foil	-
Laboratory Labelling tape	Fisher Catalog No. 15901A
Fine-tip sharpie	Sold at stationary stores
Squirt bottles (polypropylene)	Amazon – “Highfive 250cc Scientific Safety Wash Bottle Narrow Mouth Polypropylene/Plastic Squeeze Bottle Medical Label Tattoo Wash Bottle”
RO water	Alternatives include; MilliQ (18 MΩ cm), Deionized water or water filtered through a 1 µm pore-size filter
1 µm pore-size filters	Material and diameter will vary based on analytical technique and filtering apparatus
10 µm pore-size filters	Material and diameter will vary based on analytical technique and filtering apparatus
20 µm pore-size filters	Material and diameter will vary based on analytical technique and filtering apparatus

Metal sieves	VWR Catalog no. 57334-568 (500 µm mesh size) VWR Catalog no. 57334-578 (212 µm mesh size)
Metal sieve pan	Same diameter as sieves
Glass mason jars	>500 mL size One for each size fraction that will be wet picked Non-plastic lids preferred
Vacuum filtration system: 1 x Vacuum pump 2 x Plastic tubing 2 x 1000 mL Glass filtering flasks with rubber stopper 1 x filtering funnel 1 x filter holder with glass support 1 x metal clamp	GAST model DOA-P704-AA Tygon S3™ Laboratory Tubing Filtration set-up VWR Catalog no. 89428-970 Secondary filtering flask VWR Catalog no. 10545-858
Polypropylene sample jars	500 mL capacity One per sample VWR Catalog no. 30617-164
2 x Glass Beakers, 2 L	VWR Catalog no. 10754-760
Magnetic Stir bar	Fisher Catalog no. 14-513-67
KOH pellets	CAS 1310-58-3 Fisher Catalog no. P250-500
Alcojet detergent	Fisher Catalog no. 16-000-111
70% Ethanol	Fisher Catalog no. BP8201500
Weighing Balance	Satorius Item no. ENTRIS2201I-1SUS
Stir plate	Fisher Catalog no. S504631H
Drying oven (set to 45°C)	-

For counting

Item	**Suggested Materials**
Glass Petri Dishes for wet picking	VWR Catalog no. 25354-069
Small Glass Petri Dishes for dry picking from a filter	VWR Catalog no. 25354-025 (For use with a 47mm diameter filter)
Petri Dishes for picked particles	Size and material not specified
Superfine-tip forceps	VWR Catalog no. 63042-688
Petri dish grid stickers	Amazon - “Diversified Biotech PetriStickers PSTK-1070 Square Grid Label for Petri Dish, 70 Square Grid (Pack of 36)”
Laboratory labeling tape	-

Aluminum foil	-
Double sided tape	Available from stationary stores
Clear projector paper	Available from stationary stores
Metal teaspoon	Amazon - “4.5" Stainless Steel Teaspoon,Set of 6”
Stereoscope	Interchangeable black and white base preferable for picking
Microscope digital camera attachment	E.g. ToupTek touptek.com/product/product.php?lang=en&class2=56
Computer with software for images and measurements	E.g. - ImageJ imagej.nih.gov/ij/ (free to download) - ToupView touptek.com/product/product.php?lang=en&class2=74

For counting with Nile Red (in addition to counting materials above)

Item	**Suggested Materials**
Fluorescence stereoscope	- Fluorescence light attachment, excitation at 460-500 nm; emission at 535 nm.: nightsea.com/products/stereomicroscope-fluorescence-adapter/ **OR** - Crime Lite (Blue light) with orange filter; excitation at 450–510 nm; emission at 529 nm: fosterfreeman.com/forensic-light-sources/328-crime-lite-2.html
Nile Red ≥ 99% purity	VWR Catalog no. TCN0659-500MG CAS 7385-67-3
Acetone ≥ 99% purity	Technical grade or higher 10 mL per reagent stock solution VWR Catalog no. BDH1101-1LP
n-Hexane ≥ 95% purity	Technical grade or higher 100 mL per reagent stock solution VWR Catalog no. CAHX0295-6
15 mL Amber glass bottles	One per reagent stock solution VWR Catalog no. 11311-184
120 mL Amber glass bottles	One per reagent stock solution VWR Catalog no. 36319-770
1 mL glass pipettes	VWR Catalog no. 76003-572

Personal Protective Equipment (PPE)

The following PPE are mandatory for sample processing:

- Clean cotton lab coat
- Clean nitrile gloves
- Safety glasses, goggles or face shield when applicable (e.g. when working with reagents)
- Clean cabinet or covered enclosure to reduce contamination (if available)
- Functioning fume hood (when working with reagents)

PROCEDURE

Take notes on everything you do, especially any deviation from the wording of the SOP.

Procedural Blanks

- Run one laboratory blank with each set of test samples; the blank will consist of an empty 500 mL polypropylene jar, identical to those used in your laboratory for the digestion, run through the same protocol as the test samples; extracted, size fractioned, particles quantified, characterized and chemically identified.
- To account for possible contamination during sample mixing and/or shipping, each lab will receive one blank, consisting of fish tissue without microplastics or false positives added.

A. Preparation

- Before using any glassware or tools, wash with soap and water (surfactant helps to remove contaminant microplastics). Rinse three times with tap water, then three times with filtered/RO water.
- Clean sieves with soap and water using a natural sponge.
- When equipment/tools/labware are not being used, or when samples are not being analyzed, keep covered to prevent procedural contamination.

Prepare KOH solution (200 g/L)

KOH is a caustic and irritant solvent. All researchers must use KOH in a ventilated fume hood, and wear laboratory gloves and eye protection at all times.

1. Clean 2 L beaker and stir bar. Place stir bar in 2 L beaker, cover with aluminum foil and place beaker on stir plate.
2. For the digestion you will require a volume of KOH solution approximately three times that of the sample volume. To make 1 L of 20% KOH solution, weigh 200 g KOH pellets and add to beaker.
3. Add 1 L of RO water to the beaker to create a solution of 200 g/L.

4. Re-cover with aluminum foil and mix on stir plate until KOH is fully dissolved.
5. Once dissolved, allow the solution to return to room temperature before filtering.
6. Filter the solution. Set up vacuum filtration system (see filtering SOP) using a 1 µm filter. A glass fiber filter is recommended for this purpose.
7. Store filtered KOH solution in a clean polypropylene jar for later use (KOH etches glass).

Prepare 10% Alcojet detergent solution

1. Place stir bar in 2 L beaker, cover with aluminum foil and place on stir plate.
2. Weigh 100 g Alcojet detergent and add to 2 L beaker.
3. Add 1 L of RO water to the beaker.
4. Turn on stir plate and mix until detergent is fully dissolved.
5. Filter the solution. For filtering procedure set up vacuum filtration system (see filtering SOP) and use a 1 µm filter. A glass fiber filter is recommended for this purpose.
6. Store filtered detergent solution in a clean glass jar for later use.

B. Extraction Procedure: KOH Digestion

1. Label clean 500 mL polypropylene sample jars with lids, 1 per sample
2. Place each sample of fish tissue in a separate sample jar
3. *Triple rinse the fish tissue container (used for shipping) into the sample jar, using 20% KOH.*
4. Add 20% KOH solution to the sample jar so that the volume of the liquid is roughly three times that of the sample (minimum 100 mL).
5. Cap sample jars loosely and place in a temperature-controlled oven at 45°C for 48 hours to digest. If the sample is left in the oven for longer than 48 hours due to incomplete digestion, please note this.

Phase II: Detergent Soak

1. Place the 212 µm sieve on top of the sieve pan.
2. Remove the digested sample from the oven and pour the contents of the jar into the sieve. Rinse the sample jar and lid into the sieve three times with RO water. Warming the RO water (max. 50 °C) will help dissolve fatty residues.
   Note: Sieve the sample as soon as possible after removal from the oven. As it cools the sample will begin to solidify which is problematic for sieving.
3. Rinse the contents of the sieve into a clean, labelled beaker using RO water.
4. Cover the contents of the sieve pan (<212 µm size fraction) and store until later filtering (step 8).
   If the sieve pan becomes full, pour the contents into a clean, labelled beaker and cover. The sieve pan may need to be emptied multiple times into the same beaker to prevent overflow during rinsing of the 212 µm sieve.
5. Add detergent solution to the beaker (>212 µm size fractions) at a ratio of 1:1 to the volume of water present.

6. Cover and leave until the fatty residue appears fully dissipated (minimum of 1 hour, this will likely require leaving the sample to soak overnight). Record the duration of the detergent soak.
7. Clean sieves with soap and water and triple rinse with RO water. Set up sieve stack (from top to bottom; 500 µm, 212 µm and sieve pan).
    a) Pour the detergent soaked sample through the sieve stack.
    b) Rinse the sample jar and lid into the sieve three times with RO water.
    c) Tap the sieves gently to move everything through to its appropriate size fraction.
    d) Rinse the contents of each sieve into a separate clean, labelled glass jar using RO water (heating the RO water (no more than 55°C) helps dissolve fatty residues, as in step 2).

    Alternatively, you can filter all size fractions onto separate filters. Either is acceptable, as long as you have them split into the relevant size classes dictated by the sieves (i.e. you can use wet or dry sorting for the larger size fractions).

    e) Retain the contents of the sieve pan from the detergent soak and combine with the contents of the sieve pan from step 4 (KOH digestion).
8. Set up vacuum filtration system with glass or stainless-steel filtration parts (See filtering SOP). NOTE: During vacuum filtration, you may wish to heat the sample to no more than 55 °C and monitor the temperature with a thermometer. Then add a small amount of sample to the filtering cup at a time. This will help the intercalibration water samples filter a bit faster. If you fill the whole thing, the water cools down during the time it takes to filter and slows down the process.
    a) Assemble system and turn on vacuum pump to drain excess water from glass filter.
    b) Turn off vacuum pump. Empty the waste from the bottom flask and rinse the flask with RO water after doing this.
    c) Reassemble filtration system and place 20 µm filter onto the glass filter
    d) Place filtering funnel on top of filter and secure with clamp
9. Turn on vacuum pump and pour the sieve pan contents from steps 4 and 7 (<212 µm size fraction) through the filtration system. Ensure not to overfill the filtering flask as this may lead to sample loss. If bubbles begin to fill the filtering flask, pour ethanol into the filtration system to reduce bubble formation.
    Note: To avoid filter clogging, sample volumes can be subdivided into smaller volumes for filtering and results combined for the whole size fraction.
10. Rinse the beaker and the sides of the filtering funnel three times with RO water.
11. Turn off the vacuum pump and remove the filtering funnel.
    Note: Tweezers may be used to ensure the filter is not removed with the filtering funnel as you do this.
12. Turn on the vacuum pump and carefully use RO water to rinse any particles stuck to the base of the filtering funnel onto the 20 µm filter.
13. Turn off the vacuum pump and carefully slide the 20 µm filter off the glass filter and place it in a labeled clean petri dish.
    Note: Tweezers may be used to ensure the filter is not removed with the filtering funnel as you do this.

14. Pour the contents of the filtering flask into a clean beaker, and rinse the filtering flask with RO water three times into the same beaker. Repeat filtering process (steps 8-13) using a 10 µm filter, then a 1 µm filter.
15. When filtering through the 1 µm filter, add small volumes of the sample at a time (100 mL). Heating the sample (maximum 55 °C) will increase filtering speeds.
    a) If the heated sample is moving slowly through the 1 µm filter, add heated detergent solution to the filtering funnel to dissolve fatty residues.
    b) If the filter remains clogged, allow the liquid currently in the filtering flask to move through the filtering system. Carefully remove the filtering flask, slide the 1 µm filter off the holder and place it in a labelled clean petri dish. Replace with a new 1 µm filter and continue.
    c) Add ethanol to the filtering flask to reduce excessive bubble formation when filtering.

    Note: If filters clog and require replacement, you will have multiple filters for this size fraction. Together, these must be treated as one complete 1 µm *size fraction for analysis.*

Quantification and characterization

See Appendix G for guidance on order of processing

C. Visual microscopy

If particles are too small to manually count and identify, you may leave them on the filter and use automated quantification, characterization (morphology, color (where possible)) and chemical identification methods (see Section E of this Appendix).

1. Bring all four size fractions over to the microscope (i.e., >500 µm, >212 µm, >20 µm, >10 µm and >1 µm).
2. Using a systematic method of your choice, count all particles for each size fraction and record the color and morphology of each.
3. Pick (subsample) the first 30 particles identified from each color/morphology category (e.g. blue fiber, black fragment) within each size fraction. If you are using Nile Red, do this by morphology only (see below). Store the subsampled particles on a substrate relevant to the method of chemical identification you will be using, e.g. double-sided tape for particles that will be analyzed via Raman or benchtop ATR-FTIR, a reflective surface for reflectance FTIR spectroscopy. This is a suggestion only; please store particles as you see fit. When using double-sided tape, the tape should not be laid directly into the base of a petri dish. Instead, we recommend using projector paper or a glass slide as a base to lay the tape on. The choice between the two may depend on the stage of the instrumentation you are using for chemical analyses. When using ATR-FTIR spectroscopy, double sided tape should only be used for larger size fractions to avoid the crystal coming into contact with the tape. If less than 30 particles are identified for a certain particle category, pick as many as you find. After 30 particles have been picked, no longer pick from that category, but continue to count and characterize all other particles you find. For smaller size fractions, particles do not need to be manually picked as this is not only challenging but provides a high likelihood for particle loss. These particles can instead be analyzed directly from the filter substrate.

4. For subsampled particles, image and measure each particle along the longest perpendicular axes (length and width). For fibers, do not measure frayed projections and use segmented/curved lines where necessary. If a particle has broken apart, use your best judgement e.g. measure three lengths and one width for a fragment that has fractured along its length. Make note of the method used for measurement in this case.
5. If you are quantifying and characterizing via microscopy only, your analysis is complete. If you are using a further analytical method, proceed to that section.

D. Nile Red fluorescence microscopy

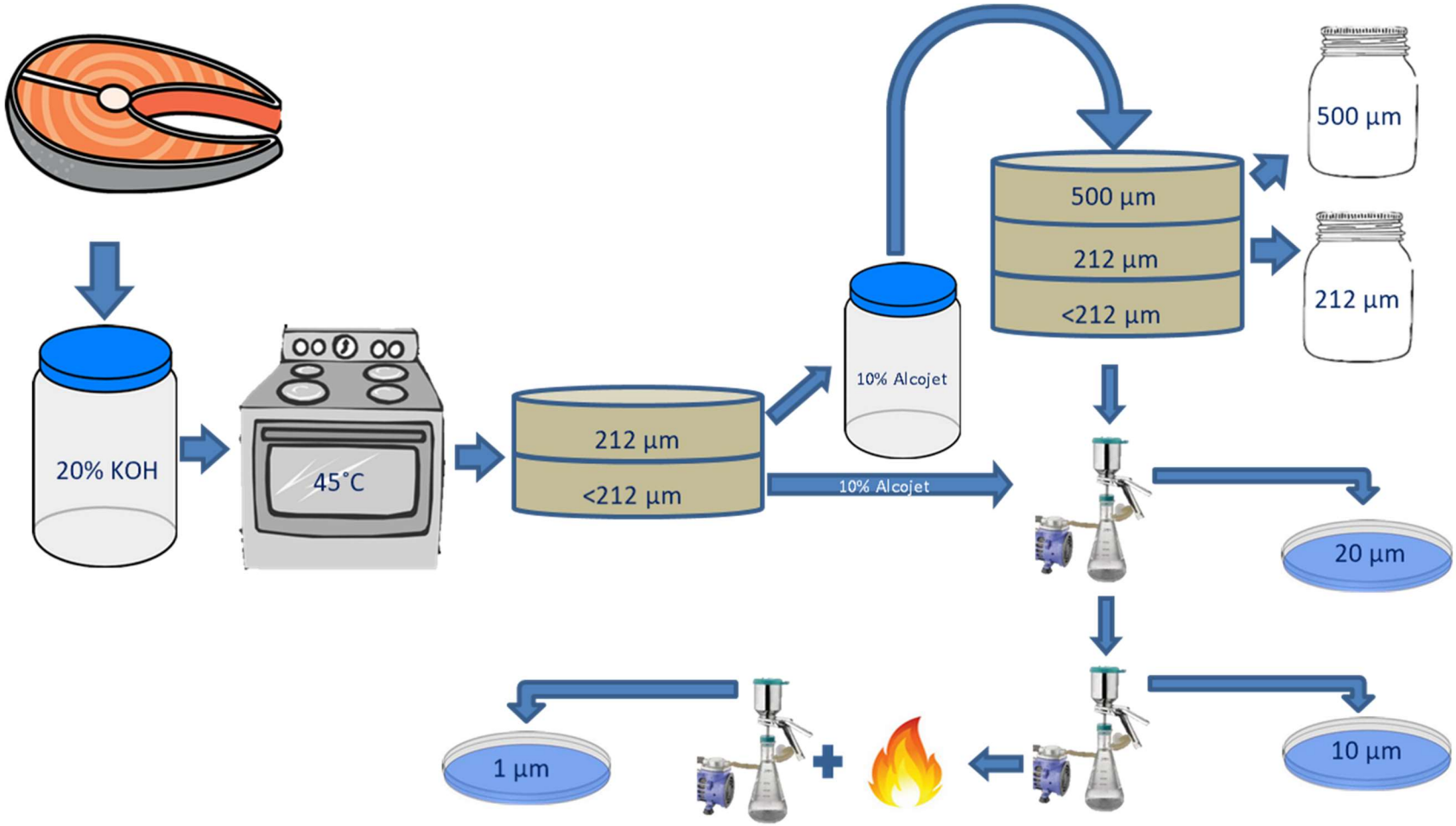
1. Nile Red working solution preparation.
   Note: Always work with Nile Red solutions inside a functioning fume hood to avoid contact with acetone and n-*Hexane vapors.*
   a) If needed, prepare a 1 mg/mL stock solution of Nile Red in 10 mL acetone.
   b) Dilute stock solution with *n*-Hexane to create 100 mL of 10 µg/mL Nile Red working solution.
   c) Keep the stock and working solutions in amber glass bottles or covered in aluminum foil to prevent photodegradation. It is recommended to store the stock solution for no more than 6 months, and the working solution for no more than 2 months, providing it is kept in the dark at 4°C during this period.
2. Working in a fume hood, apply 0.5 mL of working Nile Red solution to each filter using a glass pipette, cover with the petri dish lid. Cover the petri dish with a layer of aluminum foil and allow to incubate at room temperature in the dark for 30 minutes.
3. After the incubation period, lift the petri dish lid and prop it open using the edge of the dish. Keep the dish and sample covered with aluminum foil and allow the *n*-Hexane to completely evaporate for a minimum of another 30 minutes.
   Alternatively: you may carefully transfer the filter back onto the filtration unit. Stop the vacuum and incubate the filter with 0.5 mL of the working solution of Nile Red. Cover the filtration unit with aluminum foil to avoid direct light. After 30 minutes, turn the vacuum back on to release the working solution, and rinse the filter three times with n-Hexane. Carefully remove the filter and transfer back to the petri dish. Record which method you have used to incubate and stain samples.
4. Bring samples to fluorescent microscope. Adjust settings dependent on fluorescence attachment, record fluorescence settings and magnification used:
   a) Crime-lite - Orange filter; excitation at 450–510 nm; emission at 529 nm.
   b) Fluorescence adapter (e.g. Night Sea) - excitation at 460-500 nm; emission at 535 nm.
5. Count and record the morphology of all brightly fluorescing particles observed in each size fraction.
6. Pick (subsample) the first 30 particles identified from each morphology category (e.g. fiber, fragment) for each size fraction. If less than 30 particles are identified for a certain category, pick as many as you find. After 30 particles have been picked, continue to count and characterize all other plastic particles from that category. For smaller size fractions, particles do not need to be manually picked as this is not only challenging but provides a high likelihood for particle loss. These particles can instead be analyzed directly from the filter substrate.

7. Store the subsampled particles on a substrate relevant to the method of chemical identification you will be using, e.g. double-sided tape for particles that will be analyzed via Raman or benchtop ATR-FTIR, a reflective surface for reflectance FTIR spectroscopy. This is a suggestion only; please store particles as you see fit. When using double-sided tape, the tape should not be laid directly into the base of a petri dish. Instead, we recommend using projector paper or a glass slide as a base to lay the tape on. The choice between the two may depend on the stage of the instrumentation you are using for chemical analyses. When using ATR-FTIR spectroscopy, double sided tape should only be used for larger size fractions to avoid the crystal coming into contact with the tape.
8. For all subsampled particles, take an image of each particle and measure along the longest perpendicular axes (length and width). For fibers, do not measure frayed projections and use segmented/curved lines where necessary. If a particle has broken apart, use your best judgement e.g. measure three lengths and one width for a fragment that has fractured along its length. Make note of the method used for measurement in this case.
9. If you are quantifying and characterizing via Nile Red microscopy only, your analysis is complete. If you are using a further analytical method, proceed to that section.

E. Chemical Analysis (FTIR, Raman or Pyro-GC/MS)

1. If you have signed up to complete more than one method of chemical analysis, refer to the flow chart (Appendix G) for a recommended order of processing. SOPs for conducting chemical analysis can be found in Appendix H for FTIR spectroscopy and Appendix I for Raman spectroscopy.
2. All subsampled particles (≤30 of each category per size fraction) must be chemically identified.
3. Particles too small for manual picking and subsampling may be analyzed directly from a filter. The choice of method for this is yours to make (see Appendix G).
4. Whichever method is used for analysis, submit all details and references used.
5. Record all results in data sheets provided (Appendix J) and back up the data electronically.

Appendix E: SOP for Microplastic Extraction from Fish Tissue



Appendix F: SOP for Microplastic Extraction from Dirty Water

PURPOSE

This SOP describes the procedure by which microplastics >212 µm will be extracted from dirty water samples, picked, quantified, characterized and chemically identified. A laboratory blank will be run in addition to each set of test samples, used to monitor particles introduced via procedural contamination.

OVERVIEW

Here, a water sample will be manipulated to represent a “dirty” water sample similar to stormwater, wastewater, or surface water. The sample will be size fractionated down to 212 µm to assist particle sorting by size. Where able, size fractions will be visually sorted by microscopy. Particle ID will be analyzed using methods agreed upon by each lab. Each lab is expected to quantify the total number of suspected plastic particles within each size fraction, the morphology of each suspected plastic particle, and the color of each suspected plastic particle (where possible). A subset of particles will be imaged, measured and chemically identified (as per the subsampling rules below).

A graphical flowchart depiction of this procedure is found at the end of this Appendix.

MATERIALS

For extraction

Item	Details
Low foam dish soap	Alcojet detergent Fisher Catalog no. 16-000-111
Natural sponge	Amazon - “Natural Sea Sponge 6-7"
Aluminum foil	-
Laboratory Labelling tape	Fisher Catalog No. 15901A
Fine-tip sharpie	Sold at stationary stores
Squirt bottle (polypropylene)	Amazon – “Highfive 250cc Scientific Safety Wash Bottle Narrow Mouth Polypropylene/Plastic Squeeze Bottle Medical Label Tattoo Wash Bottle”
RO water	Alternatives include; MilliQ (18 MΩ cm), Deionized water or water filtered through a 1 µm pore-size filter
1 µm pore-size filters	Material and diameter will vary based on analytical technique and filtering apparatus
20 µm pore-size filters	Material and diameter will vary based on analytical technique and filtering apparatus
Metal sieves	VWR Catalog no. 57334-568 (500 µm mesh size)

	VWR Catalog no. 57334-578 (212 µm mesh size)
Metal sieve pan	Same diameter as sieves
Glass mason jars	>500 mL size One for each size fraction that will be wet picked Non-plastic lids preferred
Vacuum filtration system: 1 x Vacuum pump 2 x Plastic tubing 2 x 1000 mL Glass filtering flasks with rubber stopper 1 x filtering funnel 1 x filter holder with glass support 1 x metal clamp	GAST model DOA-P704-AA Tygon S3™ Laboratory Tubing Filtration set-up VWR Catalog no. 89428-970 Secondary filtering flask VWR Catalog no. 10545-858
Ice bath	Container e.g. glass or stainless-steel pan, EPS cooler filled with ice
Glass beakers, 500 mL	3 per sample (one for each size fraction)
Glass beaker, 2 L	Fisher Catalog no. 02-540R
Glass beaker, 1 L	Fisher Catalog no. 02-540P
Stir / hot plate	Fisher Catalog no. S504631H
Spatula (non-plastic)	Fisher Catalog no.14-357Q
Graduated glass cylinder	>20 mL Fisher Catalog no. S23905
Amber bottle, 1L	VWR Catalog no. 10861-786
Aluminum weigh boat	VWR Catalog no. 25433-010
Pyrex watch glass (optional)	1 for each beaker (when processing multiple samples / size fractions at once)
30-35% Hydrogen peroxide	100 mL per round of WPO per sample CAS 7722-84-1 Fisher Catalog no. H325-500
Sulfuric acid	3 mL per batch of digestion solution CAS 7664-93-9 VWR Catalog no. 470045-604
Iron (II) sulphate heptahydrate, ($FeSO_4 \cdot 7H_2O$)	7.5 g per batch of digestion solution CAS 7782-63-0 Fisher Catalog no. 1146-500
Pipette	Used to add 3 mL of sulfuric acid to digestion solution
Pipette tips	Of suitable size for pipette of choice
Small beaker	E.g. 10 mL, used as holding container to transfer sulfuric acid to digestion solution VWR Catalog no. 10754-696
Alcojet detergent	Fisher Catalog no. 16-000-111

70% Ethanol	Fisher Catalog no. BP8201500
Weighing balance	Satorius Item no. ENTRIS2201I-1SUS
Thermometer (2 per sample)	0-100°C range, small size to avoid tipping of beaker VWR Catalog no. 13201641

For counting

Item	**Suggested Materials**
Glass Petri Dishes for wet picking	VWR Catalog no. 25354-069
Small Glass Petri Dishes for dry picking from a filter	VWR Catalog no. 25354-025 (For use with a 47mm diameter filter)
Petri Dishes for picked particles	Size and material not specified
Superfine-tip forceps	VWR Catalog no. 63042-688
Petri dish grid stickers	Amazon - “Diversified Biotech PetriStickers PSTK-1070 Square Grid Label for Petri Dish, 70 Square Grid (Pack of 36)”
Laboratory labeling tape	-
Aluminum foil	-
Double sided tape	Available from stationary stores
Clear projector paper	Available from stationary stores
Metal teaspoon	Amazon - “4.5" Stainless Steel Teaspoon,Set of 6”
Stereoscope	Interchangeable black and white base preferable for picking
Microscope digital camera attachment	E.g. ToupTek touptek.com/product/product.php?lang=en&class2=56
Computer with software for images and measurements	E.g. - ImageJ imagej.nih.gov/ij/ (free to download) - ToupView touptek.com/product/product.php?lang=en&class2=74

For counting with Nile Red (in addition to counting materials above)

Item	**Suggested Materials**
Fluorescence stereoscope	- Fluorescence light attachment, excitation at 460-500 nm; emission at 535 nm.: nightsea.com/products/stereomicroscope-fluorescence-adapter/ **OR** - Crime Lite (Blue light) with orange filter; excitation at 450–510 nm; emission at 529 nm:

	fosterfreeman.com/forensic-light-sources/328-crime-lite-2.html
Nile Red ≥ 99% purity	VWR Catalog no. TCN0659-500MG CAS 7385-67-3
Acetone ≥ 99% purity	Technical grade or higher 10 mL per reagent stock solution VWR Catalog no. BDH1101-1LP
n-Hexane ≥ 95% purity	Technical grade or higher 100 mL per reagent stock solution VWR Catalog no. CAHX0295-6
15 mL Amber glass bottles	One per reagent stock solution VWR Catalog no. 11311-184
120 mL Amber glass bottles	One per reagent stock solution VWR Catalog no. 36319-770
1 mL glass pipettes	VWR Catalog no. 76003-572

Personal Protective Equipment (PPE)

The following PPE are mandatory for sample processing:

- Clean cotton lab coat
- Clean nitrile gloves
- Rubber dish gloves (when preparing digestion solution and conducting digestion)
- Safety glasses, goggles or face shield when applicable (e.g. when working with reagents)
- Clean cabinet or covered enclosure to reduce contamination (if available)
- Functioning fume hood (when working with reagents)

PROCEDURE

Take notes on everything you do, especially any deviation from the wording of the SOP.

Procedural Blanks

- One laboratory blank will be sent with each set of test samples; the blank will consist of 450 mL of dirty water, which will be run through the same protocol as the test samples; extracted, size fractioned, particles quantified, characterized and chemically identified.
- The samples should be kept refrigerated (4 °C). Solid blobs may form in the sample when it is cold. This is normal. These should go away once the sample is brought to room temperature. If they do not, warm the sample above ambient temperature, but to no more than 50 °C, until the solid blobs disappear.

D. Preparation

- Before using any glassware or tools, wash with soap and water (surfactant helps to remove contaminant microplastics). Rinse three times with tap water, then three times with filtered/RO water.
- Clean sieves with soap and water using a natural sponge.
- When equipment/tools/labware are not being used, or when samples are not being analyzed, keep covered to prevent procedural contamination.

Prepare digestion solution

1. Place stir bar in 1 L beaker, cover with aluminum foil and place on stir plate.
2. Using the metal spatula, weigh 7.5 g of $FeSO_4 \cdot 7H_2O$ into weigh boat and add to 1 L beaker.
3. Add 500 mL of RO water to 1 L beaker.
4. Add 3 mL of concentrated sulfuric acid to 1 L beaker. This can be done by pouring a small amount of sulfuric acid into a small beaker and pipetting 3 mL into the 1 L mixing beaker.
5. Turn on stir plate and mix until all particulate matter has dissolved.
6. Filter the solution before use. For filtering procedure set up vacuum filtration system (see filtering SOP) and use a 1 µm filter. A glass fiber filter is recommended for this.
7. Store filtered digestion solution in a clean amber bottle for later use.

Prepare 10% Alcojet detergent solution

1. Place stir bar in 2 L beaker, cover with aluminum foil and place on stir plate.
2. Weigh 100 g Alcojet detergent and add to 2 L beaker
3. Add 1 L of RO water to 2 L beaker
4. Turn stir plate on and mix until detergent is fully dissolved.
5. Filter the solution before use. For filtering procedure set up vacuum filtration system (see filtering SOP) and use a 1 µm filter. A glass fiber filter is recommended for this.
6. Store filtered detergent solution in a clean jar for later use.

A. Extraction procedure: Fenton's reagent

1. Set up sieve stack with the 500 µm sieve at the top and the 212 µm sieve beneath.
   Note: The liquid beneath the sieve stack does not need to be retained for this extraction, so there is no need for a sieve pan.
2. Pour the dirty water sample through the sieve stack.
3. Triple rinse the sample container with RO water, preheated to no higher than 50 °C, into the sieve stack. This will help get rid of some of the matrix for this specific intercalibration sample.
4. Use tweezers to remove any large organic matter (e.g. sticks and leaves) within the 500 µm sieve, as these will not digest easily. Triple rinse the removed organic matter with RO into the sieve stack before disposal.

5. Tap the sieves gently to move everything through to its appropriate size fraction. Rinse the contents of each sieve into a separate, clean, labelled glass 500 mL beaker using RO water, add a glass thermometer and cover.
6. Prepare ice bath and set up hot plate in fume hood.
7. Using the graduated cylinder, add 20 mL of Fe_2SO_4 solution to each beaker.
8. Using the graduated cylinder, add 20 mL of H_2O_2 to each beaker.
9. Cover the beakers using aluminum foil or a watch glass as the reaction progresses.
10. Monitor the temperature of the reaction using a thermometer. If the reaction appears slow (e.g. no bubbles forming and minimal color change), place the beaker on the hot plate, heating gently to initiate the reaction. When the temperature reaches 40 ºC, remove from hot plate.
    Caution: do not use a stir bar here as it will heat rapidly and can cause steep spikes in temperature.
11. Ensure the temperature of the sample does not spike, or reach above 55 ºC. Temperatures in excess of 60 ºC begin to melt some plastics. If the temperature is rising quickly (>45 °C) place sample in ice bath to reduce temperature. Once the temperature has settled at around 40 °C, remove from the ice bath and continue to monitor the temperature. Repeat this process if necessary, to maintain a temperature <55 °C throughout the digestion.
12. Once the reaction appears to slow (i.e. no bubbles and minimal color change), wait until the sample settles back to room temperature (<30 °C) before adding another 20 mL of H_2O_2. Place beakers in the ice bath to cool the contents if necessary.
13. Repeat steps 8-12 until a total of 5 aliquots (5 x 20 mL) of H_2O_2 has been added to each beaker to achieve a Fe_2SO_4:H_2O_2 ratio of 1:5 by volume.
14. Gently rinse built-up material on the sides of the beaker with RO water between H_2O_2 additions, minimizing water use to limit dilution of the solution.
15. Once a 1:5 ratio has been reached, allow the solution to settle back to room temperature (<30 ºC). Use the ice bath to cool the beaker contents if necessary.
16. Pour the contents of each beaker through the appropriate sieve with RO water. A rinse with hot water (RO heated on the hot plate to up to 55 °C) may be necessary to remove sticky/fatty residues from the sides of the beaker. For each beaker, rinse the aluminum foil/watch glass once and the beaker three times.
    a) Pour the 212 µm size fraction through the 212 µm sieve only.
    b) Add the 500 µm sieve on top and pour the 212 µm size fraction through both the 500 µm and 212 µm sieves.
    The reason for this process is to capture any small particles that were previously trapped in organic matter/larger particle agglomerations.
    c) Rinse the contents of each sieve back into their separate digestion beakers using RO water, then cover.
17. Repeat digestion procedure (steps 7-15) until desired level of digestion is met (i.e., no visible loss of organic material). Record the number of times the sample goes through the digestion procedure.
    Note: After each 'round' of digestion (i.e. 1:5 ratio Fe_2SO_4:H_2O_2), the sample should be sieved and size fractioned before starting another round. Larger size fractions may require additional rounds of digestion depending on the quantity of organic material.

18. After the digestion is complete, repeat sieving process (step 16) and rinse the contents of each sieve back into separate digestion beakers.
19. Add filtered 10% Alcojet solution to each beaker, equal to the volume of water in the beaker. Rinse off any particles stuck to the side of the beaker with detergent as you do this.
20. Cover and leave to allow detergent solution to soak for at least three hours (longer for samples with fatty residue).
21. Following the detergent soak, repeat sieving process (step 16) once more. Tap the sieves gently to move everything through to its appropriate size fraction. Rinse the sieves thoroughly with RO water to remove all surfactant residue. Ethanol can also be used to help remove detergent bubbles. If you use ethanol, please log this.
22. Rinse the contents of each sieve into a separate clean and labeled glass jar to give you two extracted size fractions (>500 µm and >212 µm).
    Alternatively, you can filter all size fractions onto separate filters. Either is acceptable, as long as you have them split into the relevant size classes dictated by the sieves (i.e. you can use wet or dry sorting for the larger size fractions).

Quantification and characterization
See Appendix G for guidance on order of processing

C. Visual microscopy
If particles are too small to manually count and identify, you may leave them on the filter and use automated quantification, characterization (morphology, color) and chemical identification methods (see Section E of this Appendix).

1. Bring the two size fractions over to the microscope (>500 µm and >212 µm).
2. Using a systematic method of your choice, count all particles for each size fraction and record the color and morphology of each.
3. Pick (subsample) the first 30 particles identified from each color/morphology category (e.g. blue fiber, black fragment) within each size fraction. Store the subsampled particles on a substrate relevant to the method of chemical identification you will be using, e.g. double-sided tape for particles that will be analyzed via Raman or benchtop ATR-FTIR, a reflective surface for reflectance FTIR spectroscopy. This is a suggestion only; please store particles as you see fit. When using double-sided tape, the tape should not be laid directly into the base of a petri dish. Instead, we recommend using projector paper or a glass slide as a base to lay the tape on. The choice between the two may depend on the stage of the instrumentation you are using for chemical analyses. When using ATR-FTIR spectroscopy, double sided tape should only be used for larger size fractions to avoid the crystal coming into contact with the tape. If less than 30 particles are identified for a certain particle category, pick as many as you find. After 30 particles have been picked, no longer pick from that category, but continue to count and characterize all other particles you find. For smaller size fractions, particles do not need to be manually picked as this is not only challenging but provides a high likelihood for particle loss. These particles can instead be analyzed directly from the filter substrate.

4. For subsampled particles, image and measure each particle along the longest perpendicular axes (length and width). For fibers, do not measure frayed projections and use segmented/curved lines where necessary. If a particle has broken apart, use your best judgement e.g. measure three lengths and one width for a fragment that has fractured along its length. Make note of the method used for measurement in this case.
5. If you are quantifying and characterizing via microscopy only, your analysis is complete. If you are using a further analytical method, proceed to that section.

D. Nile Red fluorescence microscopy

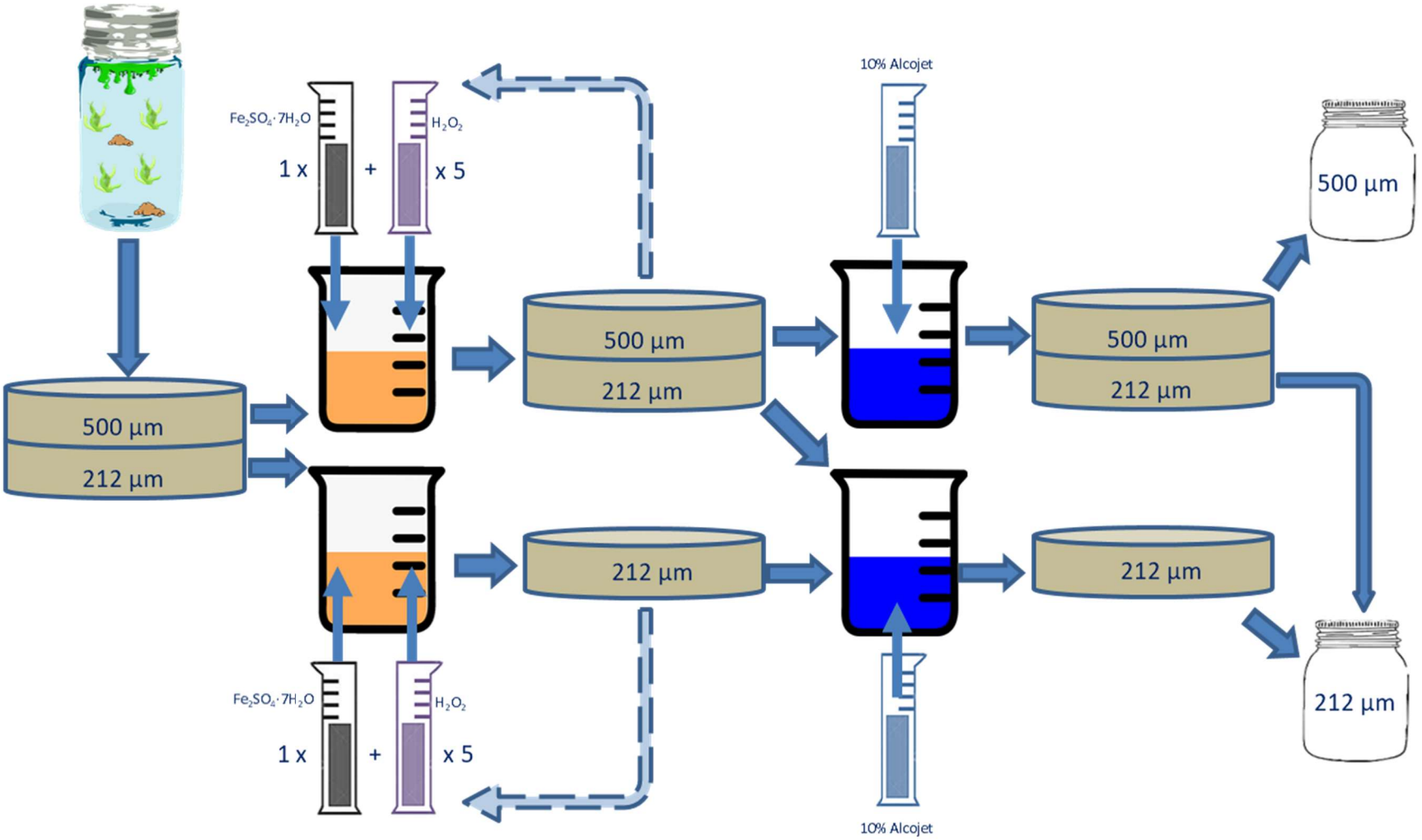
1. Nile Red working solution preparation.
   Note: Always work with Nile Red solutions inside a functioning fume hood to avoid contact with acetone and n-Hexane vapors.
   a) If needed, prepare a 1 mg/mL stock solution of Nile Red in 10 mL acetone.
   b) Dilute stock solution with n-Hexane to create 100 mL of 10 µg/mL Nile Red working solution.
   c) Keep the stock and working solutions in amber glass bottles or covered in aluminum foil to prevent photodegradation. It is recommended to store the stock solution for no more than 6 months, and the working solution for no more than 2 months, providing it is kept in the dark at 4°C during this period.
2. Working in a fume hood, apply 0.5 mL of working Nile Red solution to each filter using a glass pipette, cover with the petri dish lid. Cover the petri dish with a layer of aluminum foil and allow to incubate at room temperature in the dark for 30 minutes.
3. After the incubation period, lift the petri dish lid and prop it open using the edge of the dish. Keep the dish and sample covered with aluminum foil and allow the n-Hexane to completely evaporate for a minimum of another 30 minutes.
   Alternatively: you may carefully transfer the filter back onto the filtration unit. Stop the vacuum and incubate the filter with 0.5 mL of the working solution of Nile Red. Cover the filtration unit with aluminum foil to avoid direct light. After 30 minutes, turn the vacuum back on to release the working solution, and rinse the filter three times with n-Hexane. Carefully remove the filter and transfer back to the petri dish. Record which method you have used to incubate and stain samples.
4. Bring samples to the microscope. Adjust settings dependent on fluorescence attachment, record fluorescence settings and magnification used:
   a) Crime-lite - Orange filter; excitation at 450–510 nm; emission at 529 nm.
   b) Fluorescence adapter (e.g. Night Sea) - excitation at 460-500 nm; emission at 535 nm.
5. Count and record the morphology of all brightly fluorescing particles observed in each size fraction.
6. Pick (subsample) the first 30 particles identified from each morphology category (e.g. fiber, fragment) for each size fraction. If less than 30 particles are identified for a certain category, pick as many as you find. After 30 particles have been picked, continue to count and characterize all other plastic particles from that category. For smaller size fractions, particles do not need to be manually picked as this is not only challenging but provides a high likelihood for particle loss. These particles can instead be analyzed directly from the filter substrate.

7. Store the subsampled particles on a substrate relevant to the method of chemical identification you will be using, e.g. double-sided tape for particles that will be analyzed via Raman or benchtop ATR-FTIR, a reflective surface for reflectance FTIR spectroscopy. This is a suggestion only; please store particles as you see fit. When using double-sided tape, the tape should not be laid directly into the base of a petri dish. Instead, we recommend using projector paper or a glass slide as a base to lay the tape on. The choice between the two may depend on the stage of the instrumentation you are using for chemical analyses. When using ATR-FTIR spectroscopy, double sided tape should only be used for larger size fractions to avoid the crystal coming into contact with the tape.
8. For all subsampled particles, take an image of each particle and measure along the longest perpendicular axes (length and width). For fibers, do not measure frayed projections and use segmented/curved lines where necessary. If a particle has broken apart, use your best judgement e.g. measure three lengths and one width for a fragment that has fractured along its length. Make note of the method used for measurement in this case.
9. If you are quantifying and characterizing via Nile Red microscopy only, your analysis is complete. If you are using a further analytical method, proceed to that section.

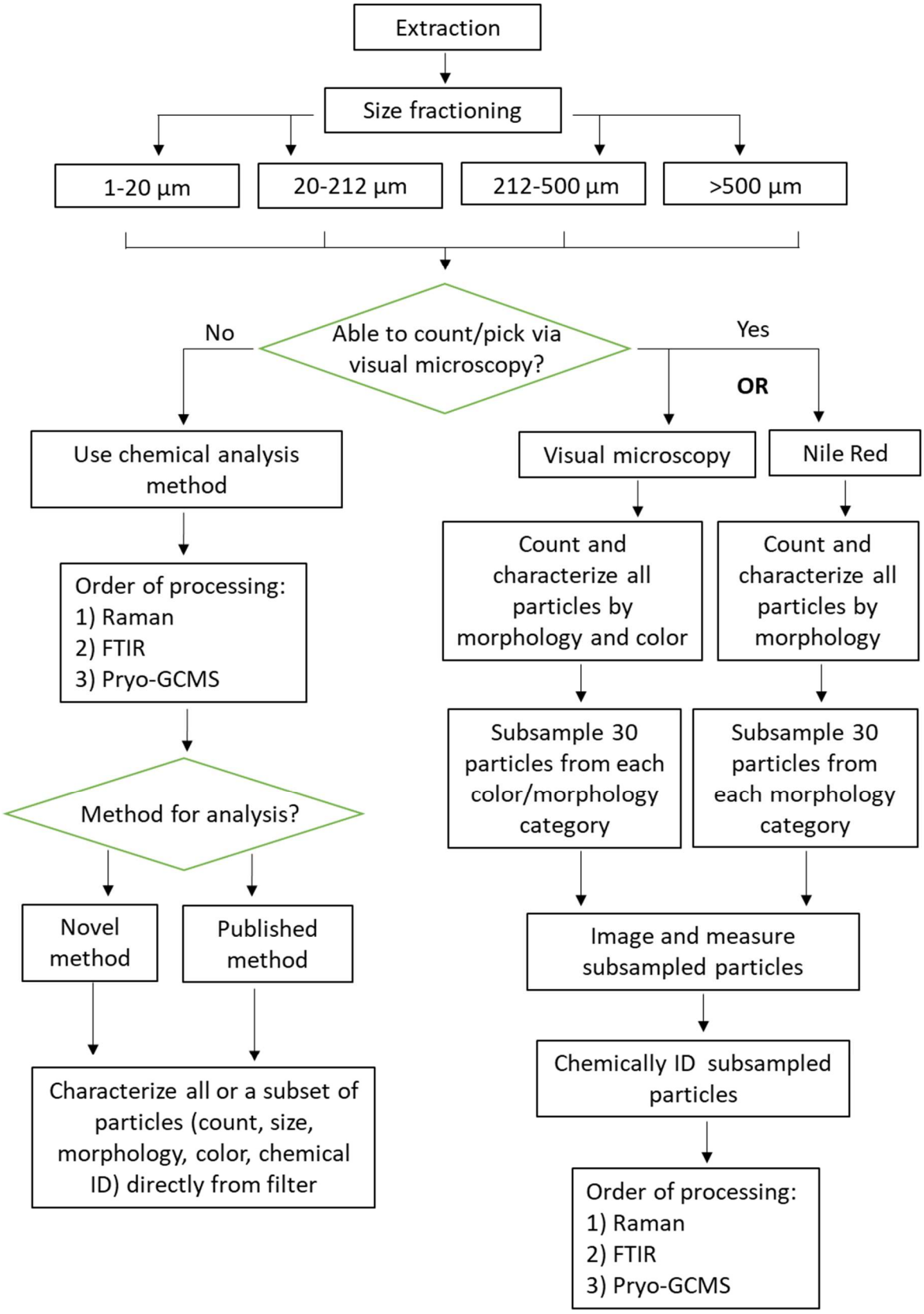
E. Chemical Analysis (FTIR, Raman or Pyro-GC/MS)

1. If you have signed up to complete more than one method of chemical analysis, refer to the flow chart (Appendix G) for a recommended order of processing. SOPs for conducting chemical analysis can be found in Appendix H for FTIR spectroscopy and Appendix I for Raman spectroscopy.
2. All subsampled particles (≤30 of each category per size fraction) must be chemically identified.
3. Record all results in data sheets provided (Appendix J) and back up the data electronically.

Appendix F: SOP for Microplastic Extraction from Dirty Water



Appendix G: Flow Chart for overview of sample processing



Appendix H: SOP for Microplastic Chemical Analysis Using FTIR spectroscopy (ThermoFisher Nicolet iN10)

PURPOSE

This SOP describes the procedure by which extracted microplastics >1 µm in size can be chemically identified using Fourier-transform infrared spectroscopy (FTIR) spectroscopy. This document is intended to provide basic guidance on operating this instrument, specifically as a refresher for those who have participated in the SCCWRP instrumental training course in November 2019. It is not intended to be a comprehensive reference.

OVERVIEW

The Thermo Scientific Nicolet iN10 MX infrared microscope allows the user to rapidly acquire microscopic images and simultaneously collect infrared spectra of solid specimens through both point-based analysis and comprehensive spectral mapping. Here, the whole sample will be analyzed with little sample preparation. This SOP assumes that the entire sample has been size fractionated down to 1 µm using the SOP outlined in Appendix B-E for microplastic extraction (>500 µm, 212 µm, 20 µm, >1 µm). Particles will either be sorted and mounted on double-sided sticky tape in a petri dish or dispersed across a filter membrane after vacuum filtration. Each lab is expected to identify the polymer type of each particle where possible and report the quantity of particles of each polymer type using FTIR spectroscopy.

MATERIALS

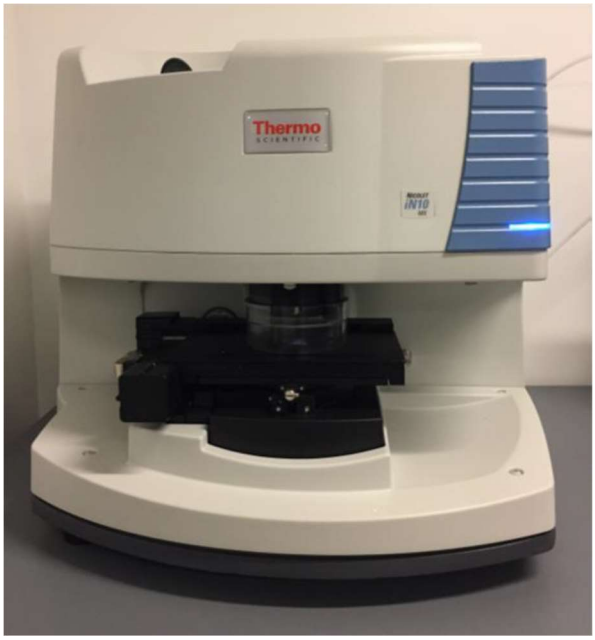


Figure 1. The ThermoFisher Nicolet iN10 MX infrared imaging microscope

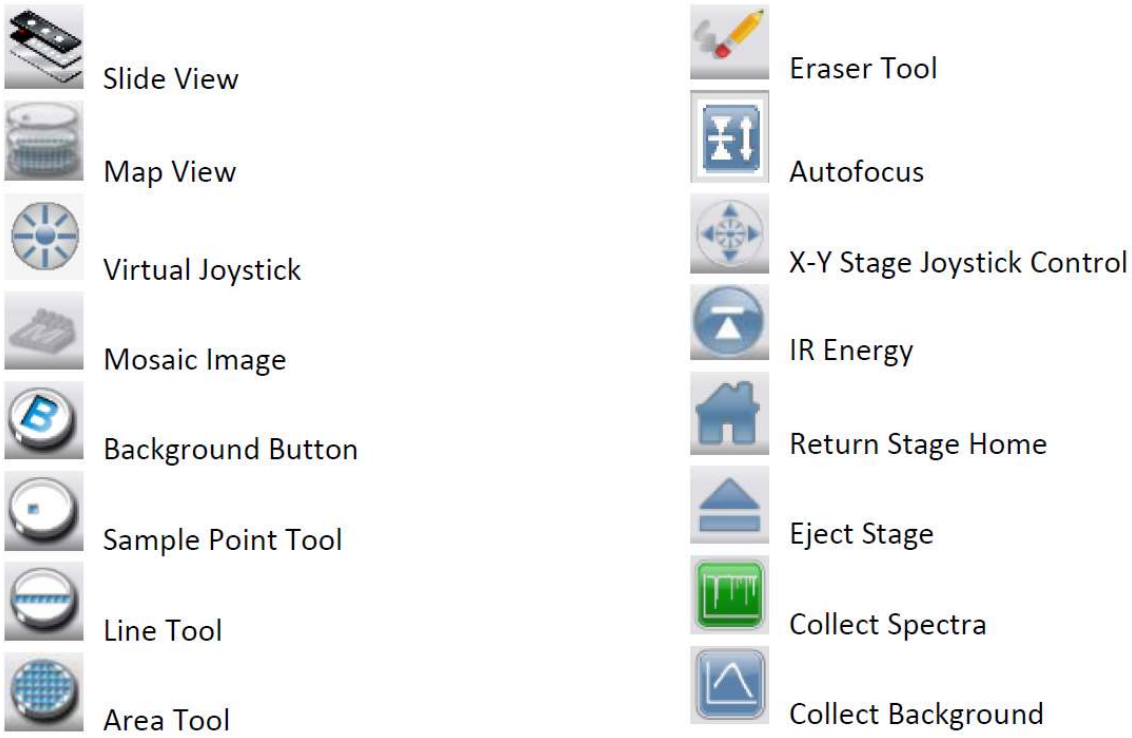
Personal Protective Equipment (PPE)
The following PPE are mandatory for all stages of sample measurement:

- Clean cotton lab coat
- Clean nitrile gloves

Safety and Precautions:

- Inspect the stage before moving it. Ensure that the stage, the ATR attachment, and your sample will not crash into the objective.
- Do not allow loose sample or particles to fall into the space under the stage.
- Follow the proper procedure utilized to cool the detectors.
- Do not move the stage when the ATR tip is in contact with a sample.
- Do not touch the tip of the ATR crystal with bare hands it will transfer finger oils. Do not twist or turn the metal plate of the ATR crystal, it will take it out of alignment.

Picta Buttons:



A. Preparation - Fill the Liquid Nitrogen Dewars

It is important to follow the cooling process listed below, or the detectors and/or regions around them can get damaged. Follow all PPE requirements for handling liquid nitrogen (LN_2).

1. Preliminary Cooling of the Detectors – Add two funnels worth of liquid LN_2 to each detector.
2. Allow the Detectors to Cool – Wait 3 minutes for the detectors to cool.
3. Fill the Detectors with LN_2 – Fill both detectors (roughly 700 mL / fill the funnel 10 times). Do not allow excessive LN_2 overflow.
4. Allow Further Cooling – Wait 20 minutes before operating the instrument.

B. Sample Placement and Visualization

1. Open Picta software – Picta controls the microscope's imaging and FTIR capabilities.
2. Select a collection mode from the "View and Collect" tab.
   a. Transmission – For transparent or translucent samples, or samples on a salt window. Window MUST be IR transparent
   b. Reflection – For solid, opaque samples, surface analysis, and for use with particle wizard.
   c. ATR (Attenuated Total Reflectance) – For samples requiring contact-based spectra. Requires an additional attachment to the micro-ATR, equipped with a germanium crystal.
3. Select an IR Detector – There are three detectors with varying acquisition speeds and capabilities.
   a. Room Temperature – For general analysis of samples from 4000-400 cm^{-1}. Better to use for particles of ≥50 µm in size. Lower sensitivity and slower.
   b. Cooled Detector – Facilitates point, line, and area analysis of samples from 4000-675 cm^{-1}. Detects less noise than the room temperature detector. High sensitivity use for small particles.
   c. Imaging Detector – For rapidly acquiring line and area scans which span large areas. Does not facilitate point scans. Detection range from 4000-715 cm^{-1}.
4. Select a Resolution – Select a spectral resolution, resolution must be set based on the library selection for identity (i.e., if the library spectra are collected at 8 cm^{-1}, you must use that resolution to use that library). Select Normal (8 cm^{-1}) or high (4 cm^{-1}). Higher resolution takes a longer time.
5. Select Number of Background Scans – The background is a coded from multiple scans. Same number as sample scan or higher.
6. Select Background Frequency and Type – Backgrounds can be taken before or after analysis of each sample or at regular time intervals (every 300 minutes).
7. Select a Spectra Format – The data can be formatted into multiple types, for microplastics analysis the most common formats are transmittance or absorbance.

8. Enter Aperture Size –The default aperture setting is 150 x 150 µm. Aperture size is selected based on the size of the particle. Ensure the aperture window encloses the particle of interest only, without background.
9. Enter Number of Points – The number of points can be tailored for area and line scans. Increasing the number of points will cause the instrument to collect more spectra from more locations across your sample.

C. Load and Locate Your Sample

1. Inspect the Stage – Check to make sure the stage and objective are free of obstructions.
2. Eject the Stage – Press the “Eject” button to make the stage more accessible.

 Eject

3. Insert your Sample – A sample can be placed on a microscope slide, the 3-hole slide, or the 12-spot slide. Clip the slide onto the stage to prevent movement.
4. Locate and Focus your Sample – Use the joystick, virtual joystick, autofocus, or the keyboard arrow keys to move the stage into position and focus on your sample.

 Autofocus  Virtual button

D. Capture an Image of your Sample

1. Select an Image Type – Press the “Map View” button. The detector can collect point, line, and area images.
2. Select an Area – Use the scroll button to determine the size of the area being analyzed. Draw a box, line, or point where you would like to collect an image.
3. Collect an Image – Press the “Capture Mosaic” button to collect an image. Right click to remove the area map ‘delete area map.
4. Save image – right click on the mosaic image and click save mosaic.

 Map View;  Point Tool;  Line Tool;  Area Tool;  Capture Mosaic

E. Collect a Background Spectrum

1. To Automatically Collect a Background – Select “Collect Backgrounds at Reference Location”. The sample holder has preset positions for collecting background spectra. The gold disk is used for reflection mode and the open hole is used for transmission mode.

2. To Manually Collect a Background – Move your sample to an area of interest, focus the image, press the “IR Energy” button and then the “Background” button. When using ATR mode, insert the ATR attachment and choose manual background collection. The background should be taken of the crystal itself.
3. Collect the Background – After a location has been marked, press the “Collect Background” button.

 IR Energy;  Background;  Collect Background

F. Collect a Spectrum

1. Focus the Sample – Use the joystick or virtual joystick to bring the image into focus.
2. Select a Spectra Tool – Either a point, line, or area scan can be produced.
3. Select a Spectra Type – Either a single spectrum or a map can be produced by selecting from the options in the bottom right hand corner.
4. Ultra-Fast Mapping – Press “Ultra-Fast Mapping” for a fast scan with increased noise.
5. Collect Spectra – When in reflection mode - Optimize the “IR Energy” with the IR energy tool then press “Collect Spectra” button in the bottom-right corner. When in ATR mode - press “Collect Spectra” button in the bottom-right corner.
6. To save the raw spectrum – Click “File” >> “Save As” >> Name your spectrum. Save as a ‘.SPA’ file.

 Collect Spectra

G. Data Analysis and Library Searching

1. Set up a Library – In the “Analyze Spectra and Maps” tab scroll down to “Library Set-Up” and “Select Library”.
2. Select Libraries to Include – Highlight libraries related to your sample and press “Add >>”, Press “Ok”, scroll down to the analyze section and press “Search”.
3. Analyze Spectra – Picta also allows spectral mapping of your sample and 3D Mapping. This is accomplished by selecting a peak of interest in the spectral window.

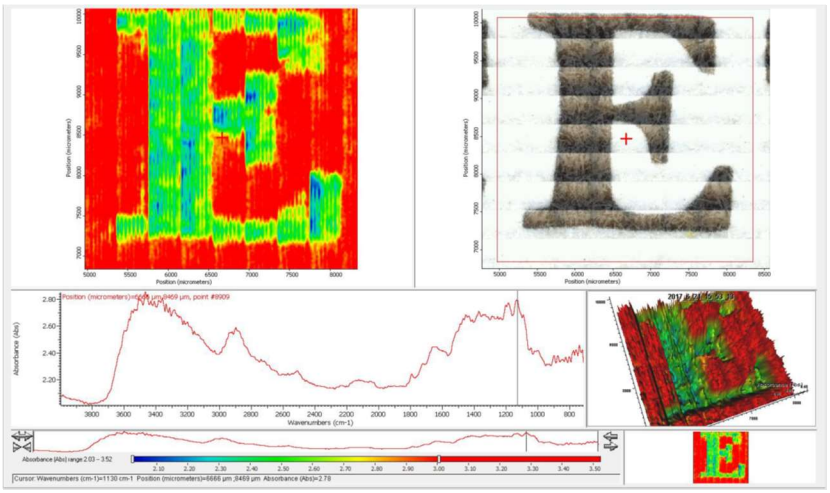


Figure 2. FTIR mapping of printed media.

H. Instrument Shutdown

1. Inspect the Stage – Check to make sure the stage is free of obstructions.
2. Eject the Stage – Press the “Eject” button: to move the stage from the Home position.
3. Remove your Sample – Remove your sample from the holder. Replace the slide with a glass slide, to avoid any dust from getting into the condenser.
4. Return the Stage Home – Press the “Home” button: to return the stage.
5. Turn off the Illumination – Use the illumination sliders to lower the brightness to zero.

Appendix I: SOP for Microplastic Chemical Analysis Using Raman Spectroscopy (HORIBA Xplora Plus)

PURPOSE

This SOP describes the procedure by which extracted microplastics >1 µm in size can be chemically identified using confocal Raman microscopy. A silicon reference sample will be used to calibrate the instrument prior to microplastic sample measurements. This document is intended to provide basic guidance on operating this instrument, specifically as a refresher for those who have participated in the SCCWRP instrumental training course in November 2019. It is not intended to be a comprehensive reference.

OVERVIEW

Here, the whole sample will be analyzed with little sample preparation. This SOP assumes that the entire sample has been size fractionated down to 1 µm using the SOP outlined in Appendix B-E for microplastic extraction (>500 µm, >212 µm, >20 µm, >1 µm). Particles will be sorted and mounted with double-sided sticky tape in a petri dish or dispersed across a filter membrane after vacuum filtration. Each lab is expected to identify the polymer type of each particle where possible and report the quantity of particles of each polymer type using Raman spectroscopy.

MATERIALS

Item	Suggested Materials
Filter holder	Marzhauser Wetzlar, filter Holder Ø 47 mm, closed version, bayonet cap
Filter holder stage insert	Marzhauser Wetzlar, stage insert for one filter holder, size dependent on stage type

Personal Protective Equipment (PPE)

The following PPE are mandatory for all stages of sample measurement:

- Clean cotton lab coat
- Clean nitrile gloves
- Laser safety glasses (unless system is equipped with Class 1 safety enclosure)

PROCEDURE

NOTE – Calibrate the system before proceeding to sample measurements. The AC symbol in the status bar will be red when the system is not calibrated and green when the system is calibrated and ready for use.

A. Preparation

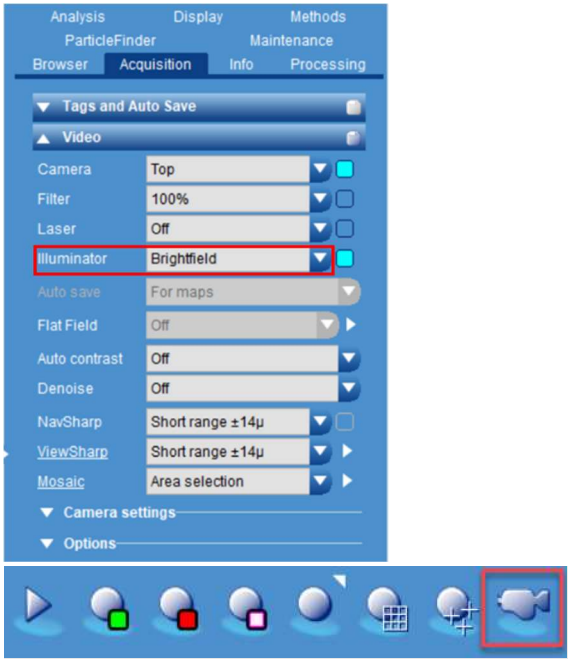
- For the two smallest size fractions (>20 µm and >1 µm), it is recommended to fix the filter flat and taut on a dedicated filter holder. Otherwise, the filter may become wrinkled or puckered after drying. If no filter holder is available, use a flat surface (e.g. a glass plate) to present the filter for measurement. If the filter dries completely before or during analysis, it can be rewet with a small amount of water to help flatten the filter.
- For the two smallest size fractions (>20 µm and >1 µm), if there are too many particles on the filter, they can become 'caked' together, and impossible to distinguish for individual analysis. Using dilution with RO water, the total number of particles can be divided into smaller volumes, and then filtered in succession on separate filters. Please note that this process requires analyzing multiple filters for each sample, and thus increases the analysis time.

B. Sample Placement and Visualization

1. Using the coarse Z adjustment on the microscope, rotate the knob by hand to lower the stage to the lowest setting (a). Select the 5× objective by rotating the objective turret (b). This is to secure the maximum space under the microscope, and minimize the risk of damaging or contaminating the objective lens.

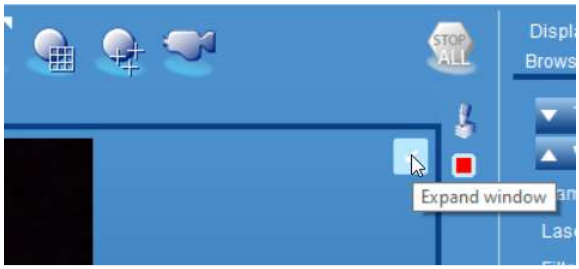
2. Place the sample on the stage. It is helpful to use an appropriate stage insert (e.g. glass plate for a Petri dish or filter holder and filter holder insert for a filter membrane).
3. Adjust the sample position laterally (by hand or using the stage's joystick) so that the targeted area of interest (i.e. particulates for analysis) is roughly under the objective lens.

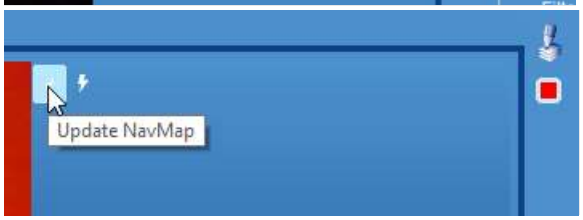
4. Using the coarse Z adjust (see step 1), raise the stage so that the distance between the objective lens and target area is approximately 1 cm (½”).
5. Set the illuminator under the video section of the acquisition tab to bright field (BF), and start a live video acquisition by clicking the video camera in the icon bar at the top of the screen. The light through the objective lens will become visible on the sample.



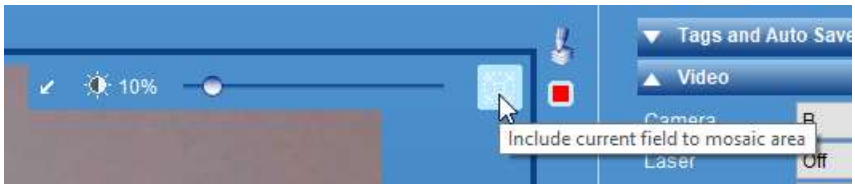
6. Using the joystick, move the stage in X and Y so the light that passes through the objective lens is focused on the target particle of interest. Focus the video image of the particle so that it is as clear as possible, first using the coarse adjust (see step 1 above) and then using the fine adjust (rotate joystick clockwise or counter-clockwise).

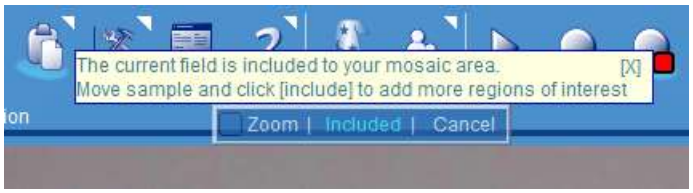
7. If the field of view (FOV) is larger than the region of interest, stop the live video ( ) to capture the image of the particle. Save the image by clicking the save button ( ) in the icon bar and update NavMap by expanding the NavMap window and clicking the “Update NavMap” icon in the Video tab:

8. 

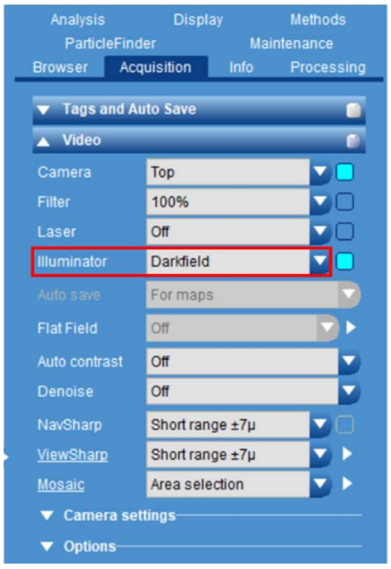
9. 

10. If the field of view is smaller than the region of interest (i.e. the particle extends beyond the viewable area), record a mosaic image to cover the entire area. To record a mosaic, click the mosaic icon from the upper right-hand corner of the video tab while the video image is live. A dialogue will appear stating that the current field is included in the mosaic area. Using the joystick, navigate around the region of interest, clicking “include” as each boundary is reached to add multiple areas to the mosaic. When the region of interest has been defined, click the mosaic icon again to record the final mosaic image. Save the image and update NavMap by expanding the NavMap window and clicking the “Update NavMap” icon in the Video tab.





11. Next, select the 10× objective by rotating the objective turret (see step 1 above). Start the live video acquisition by clicking the camera icon (), and use the joystick or click and drag the red box in the NavMap window to find the target area (i.e. a particle to analyze). Move the target particle to the center of the FOV so that it is aligned with the green dot in the video image. Bring the particle into focus using the fine z adjust (see step 6 above). Stop the live video image ().
12. Set the illuminator under the video section of the acquisition tab to dark field (DF), and start a live video acquisition by clicking the video camera () in the icon bar at the top of the screen. Stop video () to capture the image, and save it (this is DF image of the particle).

13. 

14. Select the 50× long working distance (LWD) objective by rotating the objective turret (see step 1 above). Start the live video acquisition by clicking the camera icon ( ), and use the joystick to navigate to a clean flat area on the particle (center it on the green dot in the video image). Click the NavSharp icon in the top right-hand corner of the video tab to perform autofocus, then click the ViewSharp icon to perform an extended autofocus. Stop the live video acquisition ( ) and save the resulting image (this is a dark field image of the measurement spot).

15. 

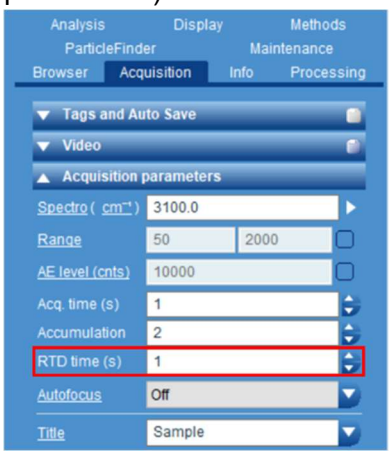
16. Note: Be careful not to hit the sample or sample container with the objective lens when rotating the objective turret to switch positions.

C. Spectral Data Acquisition and Analysis

1. Configure the instrument to record a survey spectrum. On the status bar at the bottom of LabSpec6 software, use the following settings:
   a. Grating: 600 gr/mm
   b. Laser: 532 nm
   c. Filter for laser power: 10 %
   d. AE: off
   e. DN: off
   f. ICS: on

g. SR: off
h. Spectro: 3100

2. Set the real time display (RTD) integration time to 1 s (Acquisition → Acquisition parameters).

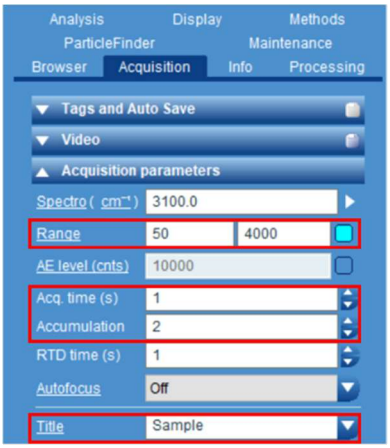


3. Start Real Time Display (RTD) by clicking the play button in the icon bar at the top of the screen.

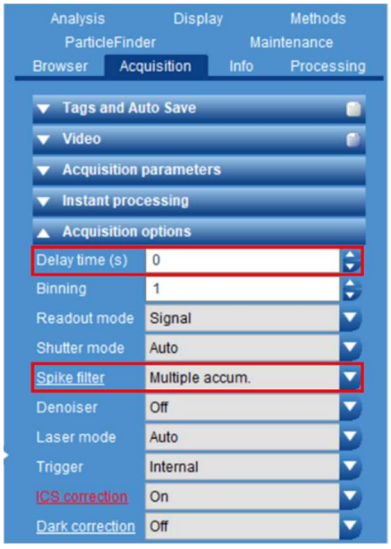
4. If the baseline is unchanging and small relative to the Raman signal, use the 532 nm laser for spectral measurement. If the baseline is high but decreases rapidly, wait until the baseline decreases and the Raman signal is more easily resolved. If the baseline stabilizes within a reasonable time period (e.g. ≤ 30 s), use the 532 nm laser for spectral measurement. If the baseline remains high, switch to progressively longer laser wavelengths (638 nm, 785 nm) until a laser is identified with a low fluorescent background.
5. Stop RTD by clicking the Stop symbol in the icon bar at the top of the screen. Start a live video acquisition, and check the sample for signs of damage (dark spot or small crater).

6. If there is no sign of damage, use 10% laser power for spectral measurement. If there is damage, reduce the laser power to 1%, navigate to a new spot on the same area using the joystick, and test for sample damage again using real time display.
7. If the signal to noise ratio (SNR) is acceptable for spectral measurement, maintain an RTD integration time of 1 s. If the SNR is not acceptable, increase the RTD time iteratively until a SNR of at least 10:1 is attained. It is recommended to cap RTD time at 60 s. If the integration time is long enough that the CCD saturates, decrease the integration time until it is no longer saturated.
8. Stop RTD by clicking the Stop symbol () in the icon bar at the top of the screen.
9. Configure the measurement to record a final spectrum:

a. Range: Turn on range mode by clicking the checkbox. Set the range from 50 cm^{-1} to 4000 cm^{-1} for 532 nm and 638 nm lasers, and from 50 cm^{-1} to 2000 cm^{-1} for the 785 nm laser.
b. Acq. time: e.g. 4
c. Accumulation: e.g. 4
d. Title: Text string for the data/file name

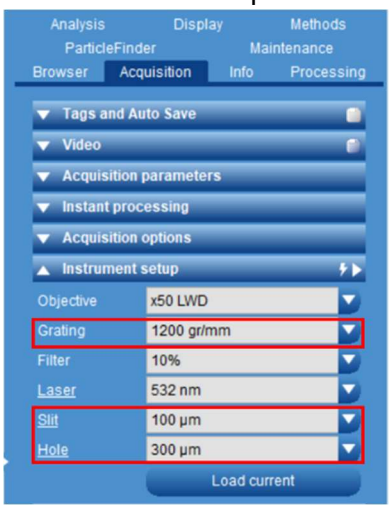


e. Delay time: 0 if baseline is stable, 30 if photobleaching is observed.
f. Spike filter: Multiple Accum.



g. Grating: 1200 gr/mm with the 532nm laser, 600 gr/mm with the 785 nm laser
h. Slit: Select 100 µm

i. Hole: Select 300 µm

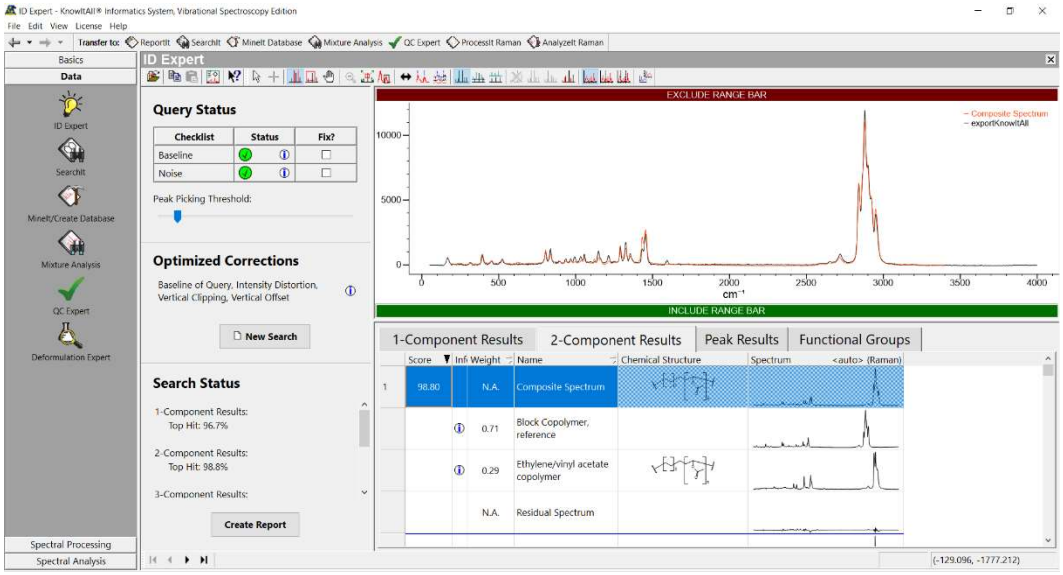


Note: If a parameter is not mentioned, do not change its setting. When changing a parameter setting, wait until the change is complete before changing the next parameter.

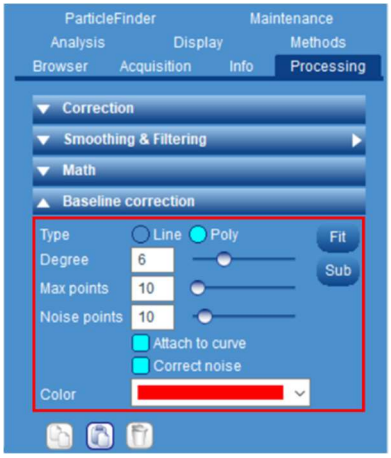
10. Acquire a Raman spectrum by clicking the record button from the icon bar at the top of the screen. Save the spectrum.

11. Initiate the library search by clicking the KnowitAll data link symbol in the icon bar at the top of the screen.

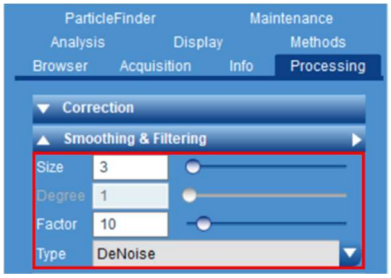
12. The spectrum is exported to ID Expert of KnowItAll, which searches the unknown spectrum with pure material spectra and then mixtures of multiple components as needed. ID Expert evaluates baseline level and noise of the spectrum, and performs baseline correction and smoothing automatically when deemed necessary.



13. If the baseline is highly complex and irregular in shape, it may be helpful to perform baseline correction within LabSpec6 (Processing → Baseline correction) before initiating the library search.

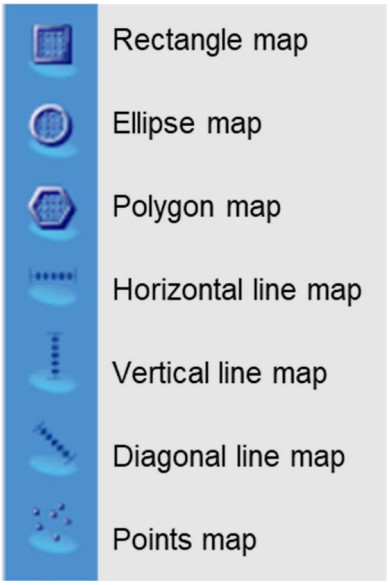


14. If preferred, it is possible to perform smoothing with DeNoise (Processing → Smoothing & Filtering) before initiating the library search.



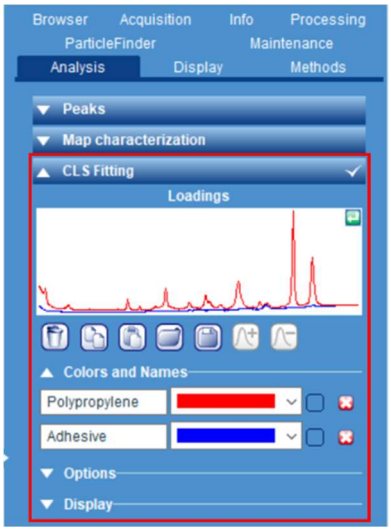
C. Multispectral (Mapping) Data Acquisition and Analysis

1. After visualizing the sample, select the shape of the desired map (e.g. rectangle, ellipse, lines, points), then draw the map area on the particle image.

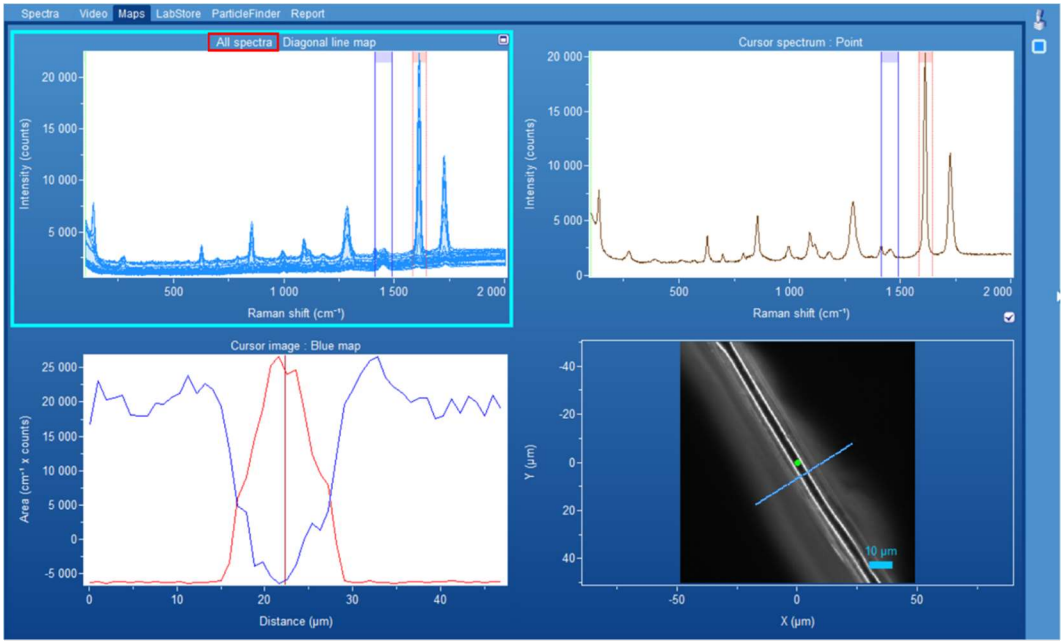


2. Use the same setup parameters defined above for either survey spectra (fast mapping) or spectrum acquisition (high SNR) above.
3. Acquire a Raman map by clicking map acquisition icon. Save the map.

4. Initiate a library search by selecting a spectrum in the map (i.e. a cursor spectrum), and clicking the KnowitAll data link symbol () in the icon bar at the top of the screen.
5. If the sample is completely unknown, explore individual spectra in the map to look for unique spectra.
6. If the sample exhibits visual characteristics of known polymers or products, explore spectra in the map for suspected materials.
7. After identifying unique or suspected spectra, it is possible to classify/identify the rest of spectra in the map based on the similarity to them using classical least square (CLS) fitting (Analysis → CLS).



8. If desired, it is possible to perform baseline correction and smoothing on all spectra in the map at once by applying operations to the All Spectra window.



Note: It is possible to acquire a map over time or along Z axis as well as X- and Y-axes.

Appendix J: Data Reporting

INTRODUCTION

Data reporting is often an arduous and difficult task; however, ensuring consistent and clean data when many data collectors are involved is necessary. This section describes the data tables necessary to produce understandable and easily analyzed data. The information in this report on what data is best to collect is adapted from a recent paper entitled “Reporting requirements to increase the reproducibility and comparability of research on microplastics” by Cowger et al., 2020 (Appl. Spectros., DOI:10.1177/0003702820930292). While it may seem complex, it can be broken down into simple tables. There are three main data tables and six additional metadata tables (Figure J1). The three main tables include information directly related to the sample, with the Raw Data Results table containing the main results relative to the amount, shape and type of microplastics contained within each sample. The Lab Information, Instrument Information and related instrument type tables include metadata on how procedures were done in the lab to prepare and extract the microplastics from various matrices, as well as how the instruments were used to analyze the microplastics samples. Recording this information is extremely important to determine what factors may alter the results from a given sample.

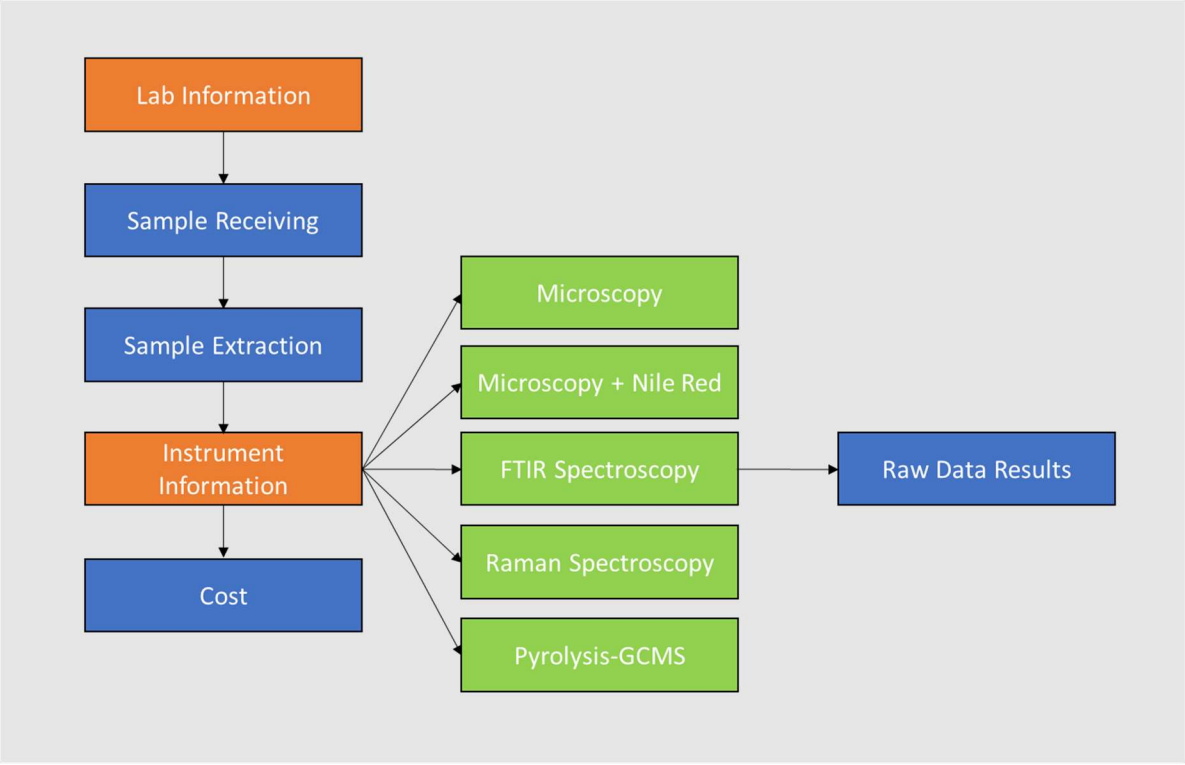


Figure J1. Basic diagram of microplastic data tables and flow.

Data will be entered into the different tables through a Microsoft Excel template workbook, provided at **http://microplastics.sccwrp.org**, along with a tally sheet for microscopy bench work (Appendix K). The sheets within the workbook will include the table names and necessary fields to fill in within each table (Figure J2). All project participants will submit their data through a web-based data system that will check the data for form and consistency. This section of the Study

Plan is meant to provide guidance on how to enter the data, show the relationships between the tables, and define each field and the values that go into them.

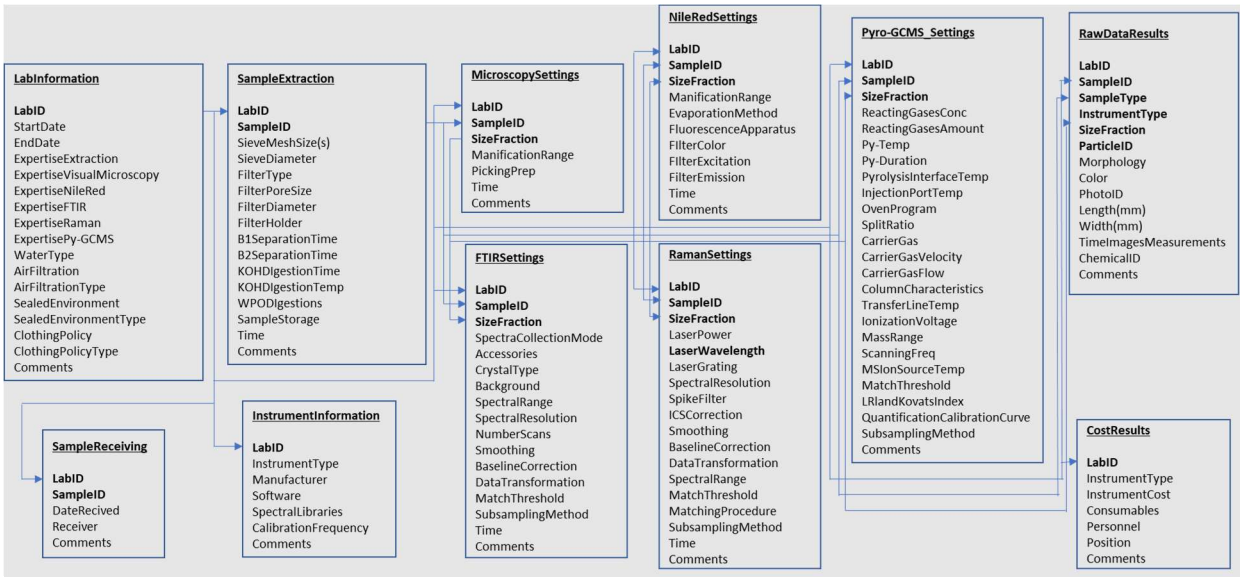


Figure J2. Entity relationship diagram showing microplastic data tables and structures.

Each required table has its own section below with a description of the purpose of each table as well as a table of the field names, types, sizes, and a definition. The order of the tables is 1) Lab Information, 2) Sample Receiving, 3) Sample Extraction, 4) Instrument Information, 5) Cost Results, 6) Microscopy Settings, 7) Nile Red Settings, 8) FTIR Settings, 9) Raman Settings, 10) Pyro-GCMS Settings, and 11) Raw data results.

SUBMISSION GUIDELINES

The data submission portal is **http://microplastics.sccwrp.org**.

This site contains the Microsoft Excel template to be filled out for submitting data, lookup lists that contain allowed values for some data (as described below), a link to the submission checker that will go through your spreadsheet to identify errors, and instructions including a how-to video. The checker and the how-to video were posted when the data portal was opened in late September 2020.

Your spreadsheet file needs to have a unique name; it should be named: “MethodEvaluationStudy_InstitutionUniqueIdentifier(IfSeveralLabsPerInstitution)”

e.g. “MethodEvaluationStudy_UniversityofTorontoRochman”.

Participants are advised to submit complete datasheets (results for all size fractions, test samples and blank samples) for each matrix. For multiple submissions, please provide a unique filename for each submission (e.g., MethodEvaluationStudy_UniversityofTorontoRochman_CleanWater, MethodEvaluationStudy_UniversityofTorontoRochman_Sediment, etc.). Be sure that other files being uploaded (e.g., image files of particles) all have unique names. Image files must be in either JPEG or PNG format.

Please name image files according to the following convention:

SampleID_SizeFraction_ParticleID#Range

Examples for images of individual particles are:

ACME_CW_1_above500_1

ACME_CW_1_above500_2, etc.

and for a range of particles in an image (e.g., particles 4-10 in the 1-20 um range of CW sample #1):

ACME_CW_1_1-20_4-10

The tab name must match the name of the table and the fields names must match the names and order listed below. In the submission checker, users will drag and drop their file over to the website and the file will be checked for errors. Errors include those associated with required fields that are not populated, fields that include the wrong data type, and values within the fields that do not match the list of allowed values. More information on data submission will be posted as the date nears for data submission.

In the data template, each tab to be completed has example lines of formatted “data” that will pass the submission checker. These are identified with LabID “ACME” with other names that follow, e.g., SampleID “ACME_CW_001”, ParticleID “ACME_CW__1_above500_1”. You can safely delete these lines upon submission; the database will ignore these lines if you leave them in. The data checker will add “-88” or other nonsensical entries to blank non-required cells to distinguish them easily from cells that have data, which you will see in the ACME sample lines.

DATA RECORDING GUIDELINES

Please use the tally sheet (Appendix K) for counting via microscopy, with or without Nile Red. This will ensure that everyone will use the same method. This data can then be entered into the Excel file for submission.

Data will be entered into tables as described below. Each data table is outlined in a table that consists of field names, data types, data sizes, descriptions of the field names, and whether the field is required. In addition, lookup lists are provided at the end of this Appendix for fields that have set values to ensure consistency. While some fields are not required you are encouraged to populate all that you are able to in order to provide any information that might influence the results.

Field names are required to match the lists below for each table. The type represents the data type need in the field, which includes Text (field can be up to 255 characters), Integer (whole number), Decimal (continuous number), Date/Time (for dates and/or times) and Yes/No. The required field is filled in with Yes, No or Conditional. Conditionally required fields are dependent on how other fields are filled out (e.g. if one field is a yes, another field may be required to fill out to describe the yes answer). Size is relative to the number of characters needed for answers for a given field. Typically, text fields are limited to a maximum of 255, but smaller limits are preferred if the choices for a given field can be smaller in size. Comments fields are usually 255 characters,

but it is recommended that comments be as brief and to the point as possible and also relate directly to information necessary to the record. A brief description is provided for each field to help the data provider determine the values to put into each filed. Where possible, constrained lookup lists are provided at the end of this Appendix. These lookup lists provide consistency in values and ensure proper spelling, etc. and are also available on the data submission website.

LAB INFORMATION TABLE

The purpose of this table is to document everything associated with the laboratory preparing the samples. Each record in this table represents a given time frame and the laboratory conditions during that time frame. The information in this table is meant to convey the measures taken to lessen the risk of contamination to the samples.

This table can be connected to the Sample Extraction and Raw Data Results tables via the LabID field.

PRIMARY KEY: To ensure no duplicates within the data set the following fields will be used as a primary key to create unique records:

- LabID

Lab Information table structure (primary key fields are indicated with bold text).

FIELD NAME	TYPE	REQUIRED	SIZE	DESCRIPTION
LabID	Text	Yes	25	This unique label for your lab is listed in Table A1.
StartDate	Date/Time	Yes		The date the sample was received (MM/DD/YYYY).
EndDate	Date/Time	Yes		The date the sample was received (MM/DD/YYYY).
ExpertiseExtraction	Integer	Yes		Level 0 – First microplastics study Level 1 – Less than 1 year experience Level 2 – Over 1 year experience.
ExpertiseVisualMicroscopy	Integer	Yes		Level 0 – First microplastics study Level 1 – Less than 1 year experience Level 2 – Over 1 year experience.
ExpertiseNileRed	Integer	Yes		Level 0 – First microplastics study

FIELD NAME	TYPE	REQUIRED	SIZE	DESCRIPTION
				Level 1 – Less than 1 year experience Level 2 – Over 1 year experience.
ExpertiseFTIR	Integer	Yes		Level 0 – First microplastics study Level 1 – Less than 1 year experience Level 2 – Over 1 year experience.
ExpertiseRaman	Integer	Yes		Level 0 – First microplastics study Level 1 – Less than 1 year experience Level 2 – Over 1 year experience.
ExpertisePy-GCMS	Integer	Yes		Level 0 – First microplastics study Level 1 – Less than 1 year experience Level 2 – Over 1 year experience.
WaterType	Text	Yes	25	Type of water used in the lab for rinsing and mixing (e.g. RO, DI, 1 µm filtered).
AirFiltration	Yes/ No	Yes	3	Is there an air filtration system in the lab?
AirFiltrationType	Text	Yes, if AirFiltration is Yes	100	Type of filtration used in the lab (e.g. HEPA filter). Required if AirFiltration is Yes.
SealedEnvironment	Yes/ No	No	3	Is a sealed environment being used to minimize contamination during sample preparation?
SealedEnvironmentType	Text	Yes, if SealedEnvironment is Yes	100	Type of sealed environment (e.g. laminar flow cabinet).
ClothingPolicy	Yes/ No	No	3	Is there a clothing policy in place in the lab?
ClothingPolicyType	Text	Yes, if ClothingPolicy is Yes	100	Type of clothing policy (e.g. cotton required).
Comments	Text	No	255	Any comments relative to the

FIELD NAME	TYPE	REQUIRED	SIZE	DESCRIPTION
				lab and procedures used.

SAMPLE RECEIVING TABLE

The purpose of this table is to document information regarding the lab receiving the samples. Information on the types of samples, when they were received and who received them will be collected.

This table can be connected to the Sample Extraction table via a combination of the SampleID and LabID fields.

PRIMARY KEY: To ensure each record is unique within the data set the following fields will be used as a primary key:

- SampleID
- LabID

Sample Receiving table structure (primary key fields are indicated with bold text).

FIELD NAME	TYPE	REQUIRED	SIZE	DESCRIPTION
LabID	Text	Yes	25	This unique label for your lab is listed in Table A1.
SampleID	Text	Yes		The ID assigned to the sample: '*Institution_Matrix_Number*' Clean water = CW Dirty water = DW Sediment = SD Fish Tissue = FT Sample numbers 1-3 will be test samples. Sample 4, or other samples with numbers divisible by 4, will be the blank sample. e.g. uoft-rochman_SD_2
DateReceived	Date/Time	Yes		The date the sample was received (MM/DD/YYYY).
Receiver	Text	Yes	100	The name of the person who received the samples for processing.
Comments	Text	No	255	Additional remarks relative to receiving the sample.

SAMPLE EXTRACTION TABLE

The purpose of this table is to document information regarding the lab receiving the samples. Information on the types of samples, when they were received and who received them will be collected.

This table can be connected to the Sample Extraction table via a combination of the SampleID and LabID fields.

PRIMARY KEY: To ensure each record is unique within the data set the following fields will be used as a primary key:

- SampleID
- LabID

Sample Extraction table structure (primary key fields are indicated with bold text).

FIELD NAME	TYPE	REQUIRED	SIZE	DESCRIPTION
LabID	Text	Yes	25	This unique label for your lab is listed in Table A1.
SampleID	Text	Yes	25	The ID assigned to the sample (see Sample Receiving Table).
SieveMeshSize(s)	Text	Yes	100	Sieve size(s) in µm used to extract the microplastics. Separate with commas (e.g., 212, 500 if you have a 212 um sieve and a 500 um sieve).
SieveDiameter	Text	Yes		The diameter of the sieve in inches.
FilterType	Text	Yes	100	Type of filter used (e.g. PCTE, Cellulose Acetate, Gold Coated, Aluminum Coated, Anodisc).
FilterPoreSize	Integer	Yes		The pore size of the filter in um.
FilterDiameter	Integer	Yes		The diameter of the filter in millimeters.
FilterHolder	Text	Yes		Glass or Stainless-steel.
B1SeparationTime	Decimal	Yes		The duration of the first density separation in Beaker 1 (hours).
B2SeparationTime	Decimal	Yes		The duration of the second density separation in Beaker 2 (hours).
KOHDigestionTime	Decimal	Yes		Incubation time for KOH digestion (hours).
KOHDigestionTemp	Decimal	Yes		Temperature at which digestion took place (°C).

FIELD NAME	TYPE	REQUIRED	SIZE	DESCRIPTION
WPODigestions	Text	Yes		The number of times the size fraction was WPO digested (1:5 ratio of Fe_2SO_4:H_2O_2 reached). Please state if one size fraction was put through more rounds of digestion than others.
SampleStorage	Text	Yes		Wet (stored in a glass container with RO water) or Dry (stored on a filter paper within a petri dish). If some size fractions are stored differently please state this.
Time	Decimal	Yes		Time taken for complete sample extraction and size fractioning (hours). If samples are left overnight e.g. density separation or digestion, please include this time.
Comments	Text	No	255	Additional remarks relative to the sample extraction.

INSTRUMENT INFORMATION TABLE

The purpose of this table is to document everything associated with the instrument(s) used to analyze microplastics samples. Each record in this table represents an instrument, its manufacturer, software and last calibration date.

This table can be connected to the Raw Data Results table via the LabID field.

PRIMARY KEY: To ensure no duplicates within the data set the following fields will be used as a primary key to create unique records:

- LabID
- Instrument Type

Instrument Information table structure (primary key fields are indicated with bold text).

FIELD NAME	TYPE	REQUIRED	SIZE	DESCRIPTION
LabID	Text	Yes	25	This unique label for your lab is listed in Table A1.
InstrumentType	Text	Yes	100	Type of instrument used for the analysis. (e.g. stereoscope, FTIR, Raman, Pyrolysis-GCMS) See InstrumentType lookup table
Manufacturer	Text	Yes	100	Manufacturer of the

FIELD NAME	TYPE	REQUIRED	SIZE	DESCRIPTION
				instrument.
Software	Text	Yes, for chemical ID instruments	100	Software and version used for the analysis on the instrument.
SpectralLibraries	Text	Yes, for chemical ID instruments		Spectral libraries used for spectral matching. In addition to commercial libraries e.g. Bio-Rad, HORIBA, Sigma-Aldrich. Please note all in-house and non-commercial libraries.
CalibrationFrequency	Text	Yes, for chemical ID insutruments	100	How often is instrument calibrated during sample analysis (e.g. Daily).
Comments	Text	No	255	Any comments relative to the instrument.

COST RESULTS TABLE

The purpose of this table is to document everything associated with the cost of processing samples. This table can be connected to the Raw Data Results table via the LabID field.

PRIMARY KEY: To ensure no duplicates within the data set the following fields will be used as a primary key to create unique records:

- LabID
- Instrument Type

Cost results table structure (primary key fields are indicated with bold text).

FIELD NAME	TYPE	REQUIRED	SIZE	DESCRIPTION
LabID	Text	Yes		This unique label for your lab is listed in Table A1.
InstrumentType	Text	Yes	25	e.g. Microscope, FTIR, Raman, Pyrolysis-GCMS. See InstrumentType lookup table.
InstrumentCost	Decimal	Yes		Upfront cost of instrument purchase, USD.
Consumables	Decimal	Yes		Approximate cost of consumables/maintenance for instrument per year, USD.
Personnel	Decimal	Yes		Hourly cost of the researcher carrying out the work, USD.

FIELD NAME	TYPE	REQUIRED	SIZE	DESCRIPTION
Position	Text	Yes		Position of researcher carrying out the work (e.g., Masters student, PhD, laboratory technician).
Comments	Text	No		Any comments relative to costs.

MICROSCOPY SETTINGS TABLE

The purpose of this table is to document everything associated with the stereoscope(s)/microscope(s) used to analyze microplastics samples. Each record in this table represents an instrument, its manufacturer, software and last calibration date. This table can be connected to the Raw Data Results table via the LabID, SampleID, and SizeFraction fields and to the Instrument Information table via the LabID field.

PRIMARY KEY: To ensure no duplicates within the data set the following fields will be used as a primary key to create unique records:

- LabID
- SampleID
- SizeFraction

Microscopy Settings table structure (primary key fields are indicated with bold text).

FIELD NAME	TYPE	REQUIRED	SIZE	DESCRIPTION
LabID	Text	Yes	25	This unique label for your lab is listed in Table A1.
SampleID	Text	Yes	25	The ID assigned to the sample (see Sample Receiving Table).
SizeFraction	Text	Yes		e.g. 1-20 um, 20-212 um, 212-500 um, >500 um. (see Size Fraction lookup list).
PickingPrep	Text	Yes		Wet (in a container with RO water) or Dry (on a filter). (see Wet/Dry lookup list).
MagnificationRange	Integer	Yes		Magnification used during assessment (e.g., 40 for 40× magnification).
Time	Decimal	Yes		Time taken to sort, pick and characterize (morphology/color) all particles for each size fraction per size fraction, in hours.

FIELD NAME	TYPE	REQUIRED	SIZE	DESCRIPTION
Comments	Text	No	255	Any comments relative to identification via microscopy.

NILE RED SETTINGS TABLE

The purpose of this table is to document everything associated with the use of Nile Red in microscopy, if that is being done.

This table can be connected to the Raw Data Results table via the LabID, SampleID, and SizeFraction fields, and to the Instrument Information table via the LabID field.

PRIMARY KEY: To ensure no duplicates within the data set the following fields will be used as a primary key to create unique records:

- LabID
- SampleID
- SizeFraction

Nile Red Settings table structure (primary key fields are indicated with bold text).

FIELD NAME	TYPE	REQUIRED	SIZE	DESCRIPTION
LabID	Text	Yes	25	This unique label for your lab is listed in Table A1.
SampleID	Text	Yes	25	The ID assigned to the sample (see Sample Receiving Table).
SizeFraction	Text	Yes		e.g. 1-20 um, 20-212 um, 212-500 um, >500 um. (see Size Fraction lookup list)
MagnificationRange	Integer	Yes		Magnification used during assessment.
EvaporationMethod	Text	Yes		Method used to allow dye to evaporate e.g. in petri dish or filtration unit.
FluorescenceApparatus	Text	Yes		CrimeLight, NightSea Adapter, or other.
FilterColor	Text	Yes		e.g. Orange.
FilterExcitation	Integer	Yes		e.g. 450–510 nm. (must be in nanometers)
FilterEmission	Integer	Yes		e.g. 529 nm. (must be in nanometers)
Time	Decimal	Yes		Time taken to sort, pick and visually characterize (morphology/color) all particles for each size fraction, in hours.

Comments	Text	No		Any comments related to Nile Red identification.

FTIR SETTINGS TABLE

The purpose of this table is to document everything associated with the FTIR(s) used to analyze microplastics samples.

This table can be connected to the Raw Data Results table via the LabID, SampleID,and SizeFraction fields and to the Instrument Information table via the LabID field.

PRIMARY KEY: To ensure no duplicates within the data set the following fields will be used as a primary key to create unique records:

- LabID
- SampleID
- SizeFraction

FTIR Settings table structure (primary key fields are indicated with bold text).

FIELD NAME	TYPE	REQUIRED	SIZE	DESCRIPTION
LabID	Text	Yes	25	This unique label for your lab is listed in Table A1.
SampleID	Text	Yes	25	The ID assigned to the sample (see Sample Receiving Table).
SizeFraction	Text	Yes		e.g. 1-20 um, 20-212 um, 212-500 um, >500 um. (see Size Fraction lookup list)
SpectraCollectionMode	Text	Yes	100	The method used to acquire spectra e.g. ATR, reflectance or transmission.
Accessories	Text	No	100	Describe any extra accessories, used, besides a filter, for sample presentation and analysis with the instrument. This can include a description of the type of detector used e.g., “room temperature”, “cooled”, “imaging”.
CrystalType	Text	Yes	100	Type of crystal used on the FTIR (e.g. diamond, germanium).
Background		Yes	100	Measurement of background spectra e.g. before spectrum, after spectrum, every 300

FIELD NAME	TYPE	REQUIRED	SIZE	DESCRIPTION
				minutes.
SpectralRange	Integer	Yes		Wavenumber range of the spectra, reported in cm^{-1}
SpectralResolution	Decimal	Yes		The maximum number of spectral peaks that the spectrometer can resolve, reported in cm^{-1}/pixel
NumberScans	Integer	Yes		Number of scans performed.
Smoothing	Text	Yes, if used	100	Pre-processing step to minimize background noise and interference.
BaselineCorrection	Text	Yes, if used	100	Pre-processing step to flatten baseline and minimize signal interference
DataTransformation	Text	Yes, if used	100	Spectral data processing technique, often used to normalize signal intensity values.
MatchThreshold	Integer	No	100	If used, minimum hit quality index (HQI) value for 'acceptable' matches, reported as a percentage.
SubsamplingMethod	Text	No		Method used for subsampling, e.g. novel or from literature. Cite all relevant literature.
Time	Decimal	Yes		Time taken to complete FTIR analysis on all subsampled particles, for each size fraction, in hours.
Comments	Text	No	255	Any comments related to the analysis of the microplastics via FTIR.

RAMAN SETTINGS TABLE

The purpose of this table is to document everything associated with the Raman(s) used to analyze microplastics samples. Each record in this table represents an instrument, its manufacturer, software and last calibration date.

This table can be connected to the Raw Data Results table via the LabID, SampleID, and SizeFraction fields and to the Instrument Information table via the LabID field.

PRIMARY KEY: To ensure no duplicates within the data set the following fields will be used

as a primary key to create unique records:

- LabID
- SampleID
- SizeFraction

Raman Settings table structure (primary key fields are indicated with bold text).

FIELD NAME	TYPE	REQUIRED	SIZE	DESCRIPTION
LabID	Text	Yes	25	This unique label for your lab is listed in Table A1.
SampleID	Text	Yes	25	The ID assigned to the sample (see Sample Receiving Table).
SizeFraction	Text	Yes		e.g. 1-20 um, 20-212 um, 212-500 um, >500 um. (see Size Fraction lookup list)
LaserPower	Integer	Yes		The laser power of the instrument should be reported (in mW).
LaserWavelength	Integer	Yes		e.g. 785nm, 532nm. (must be in nanometers)
LaserGrating	Integer	Yes		e.g. 1200nm, 600nm. (must be in nanometers)
SpectralResolution	Decimal	Yes		The maximum number of spectral peaks that the instrument can resolve, reported in nm or cm^{-1}/pixel
SpikeFilter	Text	Yes, if used	3	Correction of cosmic spikes within the spectra, ON or OFF.
ICSCorrection	Text	Yes, if used	3	Relative intensity correction of spectra setting, ON or OFF.
Smoothing	Text	Yes, if used	100	Pre-processing step to minimize background noise and interference
BaselineCorrection	Text	Yes, if used	100	Pre-processing step to flatten baseline and minimize signal interference (e.g. line, polynomial, manual).
DataTransformation	Text	Yes, if used	100	Spectral data processing technique, often used to normalize signal intensity values.
SpectralRange	Integer	Yes		Wavenumber range of the spectra, reported in cm^{-1}.
MatchThreshold	Integer	Yes, if used		Minimum hit quality index (HQI) value for 'acceptable' matches, reported as a percentage.

FIELD NAME	TYPE	REQUIRED	SIZE	DESCRIPTION
MatchingProcedure	Text	Yes	100	Software matching procedure (e.g. 'ID Expert' or 'Search It' when using Bio Rad 'KnowItAll' software). Where the top match was not chosen, what procedures were used to identify the most accurate match.
SubsamplingMethod	Text	No		Method used for subsampling, e.g. novel or from literature. Cite all relevant literature.
Time	Decimal	Yes		Time taken to complete Raman analysis on all subsampled particles, for each size fraction, in hours.
Comments	Text	No	255	Any comments related to the analysis of the microplastics by the Raman used.

PYRO-GCMS SETTINGS TABLE

The purpose of this table is to document everything associated with the pyrolysis-GCMS instrument(s) used to analyze microplastics samples. Each record in this table represents an instrument, its manufacturer, software and last calibration date.

This table can be connected to the Raw Data Results tables via the LabID, SampleID, and SizeFraction fields and to the Instrument Information table via the LabID field.

PRIMARY KEY: To ensure no duplicates within the data set the following fields will be used as a primary key to create unique records:

- LabID
- SampleID
- SizeFraction

Pyro-GCMS Settings table structure (primary key fields are indicated with bold text).

FIELD NAME	TYPE	REQUIRED	SIZE	DESCRIPTION
LabID	Text	Yes	25	This unique label for your lab is listed in Table A1.
SampleID	Text	Yes	25	The ID assigned to the sample (see Sample Receiving Table).

FIELD NAME	TYPE	REQUIRED	SIZE	DESCRIPTION
SizeFraction	Text	Yes		e.g. 1-20 um, 20-212 um, 212-500 um, >500 um. (see Size Fraction lookup list)
ReactingGasesConc	Text	Yes, if used	25	Concentration of reacting chemical used in samples in certain cases to realize thermochemolysis. Please note the units used e.g. %, ug/mL.
ReactingGasesAmount	Decimal	Yes, if used		Amount of reacting chemical used in samples in certain cases to realize thermochemolysis (µL or mL added to the cup).
Py-Temp	Decimal	Yes		Pyrolysis temperature (in °C).
Py-Duration	Decimal	Yes		Pyrolysis duration in minutes.
PyrolysisInterfaceTemp	Decimal	Yes		Temperature of the Pyrolysis interface at the Pyrolyzer and GC junction (in °C).
InjectionPortTemp	Decimal	Yes		Temperature of the GC injection port (in °C).
OvenProgram	Text	Yes	100	Temperature program (in °C) of the GC oven to seperate analytes on the GC column.
SplitRatio	Text	Yes	100	Amount loaded into the column.
CarrierGas	Text	Yes	100	Carrier gas used during experiments.
CarrierGasVelocity	Decimal	Yes, if Gas flow not stated	100	Carrier gas linear velocity (in cm/s).
CarrierGasFlow	Decimal	Yes, if Gas Velocity not stated		Carrier gas volumetric flow rate (in mL/min).
ColumnCharacteristics	Text	Yes	100	Column name, supplier, length (in m), diameter (in mm) and thickness (in µm) can have effect on retention times of the analytes.
TransferLineTemp	Decimal	Yes		Temperature of the transfer line between the GC and the MS (in °C).
IonizationVoltage	Decimal	Yes		Voltage to create ion in the MS (in eV).
MassRange	Decimal	Yes		Range of *m/z* analyzed.
ScanningFreq	Decimal	Yes		Frequency of scanning (in Hz or Scans/sec).

FIELD NAME	TYPE	REQUIRED	SIZE	DESCRIPTION
MSIonSourceTemp	Decimal	Yes		Temperature of the MS Source (in °C).
MatchThreshold	Integer	Yes, if software used		Percentage of adequation of the obtained pyrogram or mass spectrum against a library of references (in %).
LRIandKovatsIndex	Decimal	Yes, if manual peak identification is used		Retention indice taking into consideration the column phase used. Allows comparison between studies even if different parameters are used.
QuantificationCalibration Curve	Text	Yes, if done	100	Need to report all calibration curve realized wit slopes, R^2 and characteristic peaks or *m/z* used to realize the curves.
SubsamplingMethod	Text	No		Method used for subsampling, e.g. novel or from literature. Cite all relevant literature.
Time	Decimal	Yes		Time taken to complete Pyro-GCMS analysis on all subsampled particles, for each size fraction.
Comments	Text	No		Any comments related to Pyro-GCMS analysis.

RAW DATA RESULTS TABLE

The purpose of this table is to document everything associated with the sample results for all particles. Some fields will be left blank, and only filled in for subsampled particles which are run through a 'complete' analysis through to chemical identification. One row = one particle for each analysis technique (InstrumentType). In other words, up to 30 for each size fraction, color, and morphology will be subsampled (picked, as appropriate) for chemical identification, and the rest (if any) are left unidentified as noted. The total number of rows for each type of particle will be what you find as the particle count.

This table can be connected to the Sample Receiving, Sample Extraction and Raw Data Results tables via a combination of the SampleID and LabID fields.

PRIMARY KEY: To ensure no duplicates within the data set the following fields will be used as a primary key to create unique records:

- SampleID
- LabID
- SizeFraction
- ParticleID

Raw Data Results table structure (primary key fields are indicated with bold text).

FIELD NAME	TYPE	REQUIRED	SIZE	DESCRIPTION
LabID	Text	Yes	25	This unique label for your lab is listed in Table A1.
SampleID	Text	Yes	25	The ID assigned to the sample (see Sample Receiving Table).
SampleType	Text	Yes	2	Clean water (CW), Dirty water (DW), sediment (SD), fish tissue (FT). (See Matrix lookup list)
InstrumentType	Text	Yes	25	Type of instrumental analysis used for this data (see InstrumentType lookup list)
SizeFraction	Text	Yes	25	e.g. 1-20 um, 20-212 um, 212-500 um, >500 um. (see Size Fraction lookup list)
ParticleID	Text	Yes	25	The ID assigned to the particle "SampleID_above500_1", SampleID_,above500_2", "SampleID_212-500_4-9" for an image with particles 4-9 visible in size fraction 212-500 um, etc. Particle ID should be represented in the corresponding PhotoID (see below).
Morphology	Text	Yes	25	The shape of the particle (see Morphology Category Lookup List).
Color	Text	Yes	25	The color of the microplastic particle (see Color Lookup List).
PhotoID	Text	Yes	75	Same name as Particle ID.
Length(mm)	Decimal	Yes	-	Length of the particle along its longest axis in millimeters.
Width(mm)	Decimal	Yes	-	Length of the particle along its widest axis perpendicular to length in millimeters.
TimeImagesMeasurements	Decimal	Yes, for manually picked particles		Time taken to manually image and measure all subsampled particles per size fraction, in hours.
Chemical ID	Text	Yes, for FTIR, Raman and Py-GCMS		Result as shown via chemical ID matching software.
Comments	Text	No	255	Additional remarks relative to the sample preparation.

LOOKUP LISTS

Morphology Category Lookup List

MORPHOLOGY CATEGORY
Fragment
Fiber
Fiber bundle
Sphere
Foam
Film
Pellet

Color Lookup List

COLOR
Black
Blue
Brown
Clear
Gold
Green
Grey
Orange
Pink
Purple
Red
Silver
White
Yellow
Multicolor

Matrix Lookup List

MATRIX
CW (Clean water)
DW (Dirty water)
SD (Sediment)
FT (Fish tissue)

Sizefraction Lookup List

SIZEFRACTION
1-20 um
20-212 um
212-500 um
>500 um

Yes/No Lookup List

YESNO
Yes
No
NR

Wet/Dry Lookup List

WETDRY
Wet
Dry

InstrumentType Lookup List

INSTRUMENTTYPE CATEGORY
StereoScope
StereoScopewithFluorescenceStaining
FTIR
Raman
Pyro-GCMS
Other

Appendix K: Tally Sheet to Use for Microscopy Bench Work

The next four pages are tally sheets that can be printed and filled out when doing counts by microscopy. Please use these sheets to capture all requested visual microscopy (with or without Nile Red) information (color, morphology, total count). Please transfer the information of these sheets to the Excel spreadsheet template to submit your data.

Sample ID: _____ Size Fraction:_____ Page:____ of ____
Count Analyst: _____
Date:_____

	Fragment	TOTAL	Film	TOTAL	Fiber	TOTAL
Black						
White						
Clear						
Blue						
Red						
Green						
Pink						
Purple						
Yellow						
Orange						

Sample ID: _____ Size Fraction:_____ Page:____ of ____
Count Analyst: _____
Date:_____

	Fragment	TOTAL	Film	TOTAL	Fiber	TOTAL
Brown						
Grey						
Gold						
Silver						
Multicolor						

Sample ID: _____ Size Fraction:_____ Page:____ of ____
Count Analyst: _____
Date:_____

	Foam	TOTAL	Pellet	TOTAL	Sphere	TOTAL	Fiber Bundle	TOTAL
Black								
White								
Clear								
Blue								
Red								
Green								
Pink								
Purple								
Yellow								
Orange								

Sample ID: _____ Size Fraction:_____ Page:____ of ____
Count Analyst: _____
Date:_____

	Foam	TOTAL	**Pellet**	TOTAL	**Sphere**	TOTAL	**Fiber Bundle**	TOTAL
Brown								
Grey								
Gold								
Silver								
Multicolor								

Appendix L: Study Augmentations

Extraction method augmentations

Altering the concentration of KOH in the digestion process

Statement of the problem
Different strengths of KOH solutions are used across laboratories when digesting biological tissues. Changes across research laboratories could introduce variation and bias that are not accounted for. There could be differences in extraction efficiency, polymer preservation and overall particle counts even with small differences in the extraction method applied. Many researchers quote a single study dating back to the early years of microplastic research (Foekema et al. 2012). However, as the years have progressed studies have used different strengths of KOH due to concerns over particle destruction (Lusher et al., 2017). Comparing the efficiencies of KOH extraction using different strengths of digestion solution is paramount to identify the appropriate methods for future monitoring.

Approach
Participating laboratories will perform identical extraction methods with the only change being strength of the digestive agent (and temperature). Labs will use water and four incremental strengths of KOH: 5%, 10%, and 20%, each incubated at the same temperature for the same time period. No matter what stage of digestion has reached after the set time frame, participants will process the sample and record all possible data. Count, size and FT-IR data is important for all particles. The data obtained will be analyzed to determine differences between strength of digestive agent (and temperature).

Methods used: Raman and FTIR
Matrices used: Tissue
Lead scientist: Amy Lusher
Labs participating: NIVA, California Department of Public Health

Evaluating the effectiveness of two different digestion salts

Statement of the problem
Microplastics deposited into sediment need to be extracted before they can be counted. The higher the density of the material, the higher the density of salt solution required to remove them. Changes in the density of the salt solution between research teams could limit comparability of results. Many standardized protocols proposed by international organizations, including NOAA, GESAMP and OSPAR recommend the use of NaCl, or

seawater. This is not appropriate for marine sediments, as particles which settle in sediment must have a higher density than the over lying seawater (1.2 g/cm^{-3}). High density polymers such as PET and PVC also show low rates of recovery if a low-density solution is used. Understanding the percentage differences is extraction efficiencies with different salts tested against different polymers is therefore paramount.

Approach
Participating laboratories will perform extractions of microplastics from sediments using identical methods apart from the salt solutions. Labs will use two different salts: $CaCl_2$ and NaI (1.4 g/cm^{-3} and 1.7 g/cm^{-3} respectively). Participants will record data for all particles extracted, including particle count, color, morphology, size and chemical ID. The data obtained will be analyzed to determine differences between the two digestive agents.

Methods used: Raman and FTIR
Matrices used: Sediment
Lead scientist: Amy Lusher
Labs participating: NIVA, Chinese Academy of Sciences

Testing an alternative sediment extraction method

Statement of the problem
Extraction and isolation of microplastics from sediments is challenging due to the organic particulate nature of both the microplastic particles and the sediments. Plastic particles less than 150 µm are particularly difficult to visualize and even more difficult to manually transfer during the extraction and isolation process. Nevertheless, these smaller particles comprise a large proportion of plastics found in sediments (and other matrices) and are of the size that would interact with larval organisms, macrobenthos and meiobenthos. Previous research (Cashman et al., 2019) has shown that some commonly used sediment extraction methods generally do not extract greater than 70% of a mix of spiked microplastics in sediments. In contrast, a method based upon the SOP being used in this study with modifications below, had recoveries of greater than 70% for a mixture of microplastic shapes, sizes and densities in both sandy and muddy sediments. This procedure is currently being used to extract sediments from a survey of U.S. EPA Regional sediments.

Approach
Compare recoveries of a range of spiked microplastics in sediments using a method that differs from the core study as stated above. We anticipate extracting and isolating spiked microplastics from selected sediments with the two methods and comparing the results via Raman spectroscopy.

Between 100 and 150 g of wet sediment will be sequentially sieved through a 1 mm, 0.25 mm and 0.045 mm sieves. Sediment retained on the 0.25 and 0.045 mm sieves will be placed in separate separatory funnels and extracted with NaBr solution

(1.3g/cm^3). After a shaking and settling period, the dense portion of the sediments from each separatory funnel are collected into another separatory funnel. The floating portion of each sediment size class is collected onto a filter. The dense portion of each sediment is re-extracted with NaBr (1.5g/cm^3). After another shaking and settling period, the dense portion of the sediment is discarded and the floating particles are again collected onto a filter. This results in 4 different filters based on density and size. If necessary, an oxidation step using 15% H_2O_2 incubated at 60°C for 2 hours (Mladinich et al.) can be performed.

The initial sieving of sediments and subsequent loss of the fraction < 0.45 microns allows the removal of smaller organic and mineral fraction that cannot be identified and interferes with the identification step. NaBr was chosen as the salt due to its ease of purchase (used in hot tubs), low toxicity, high density, and low cost. Use of separatory funnels allows complete rinsing and transfer of different sediment portions. Avoiding manual transfer of particles more likely ensures that smaller particles to be retained and identified.

Methods used: Raman
Matrices used: Sediment
Lead scientist: Kay Ho
Labs participating: USEPA, SCCWRP

Measurement method augmentations

Automation of Raman spectroscopy

Statement of the problem

Raman spectroscopy achieves clear chemical identification of the smallest microplastics down to a size of 1 µm. Usually such small microplastics are not easily seen on a rough filter surface under white field illumination. Moreover, commercially available filters mostly show Raman and/or fluorescence background and/or get burned even under low laser illumination increasing measurement times. It has been shown that 100 nm aluminum-coated polycarbonate membrane filters under dark field illumination display ideal characteristics as a substrate for Raman spectroscopy in terms of particle visibility, no or minimal spectral interference with particle spectra, and acquisition (Oßmann et al. 2017).

Approach

Since in the present study, the number, size, and type of microplastics will be controlled, it will be possible to test how much volume and scanned area are needed to reach the known particle number. The automatic particle detection will be also tested in terms of measurement speed and relocalization accuracy. These tests will be used to validate Raman spectroscopy as a standard automated microplastics measurement method.

Methods used**:** Raman
Matrices used: Clean water and dirty water
Lead scientist: Silke Christiansen
Labs participating: Innovationsinstitut für Nanotechnologie und korrelative Mikroskopie (INAM), Germany; HORIBA Scientific

Chemical Identification of Microplastics Using a Raman Touch Probe

Statement of the problem

There have been various studies discussing methods for identification of microplastics including comparisons of different techniques' strengths and weaknesses. For example, one study compared results from relatively simple methods such as visual identification and Nile Red staining to more complex methods like ATR-FTIR and Raman spectroscopy (Shim et al. 2017). For definitive chemical identification, the study concluded that μ-ATR-FTIR is the recommended technique for routine analysis of environmental samples down to 20 μm in size. However, microspectroscopic systems (ATR-FTIR and Raman) tend to be complex to operate, large, and expensive, precluding their use in a wide variety of laboratories and environments.

Approach

In this study, a macroscopic Raman touch probe will be used to assess how a smaller, portable experimental setup performs in identifying polymers compared to a larger Raman microscope system. In the first part of the study, microplastic particulate of different polymer types in the 1 mm size range will be measured using the Raman touch probe and the results will be compared with those from a standard Raman microscope (Figure 1). In the second part of the study, a range of microplastics of varying size will be measured and the results compared to standard Raman microscopy to assess the lowest size limit identifiable using this technique (Figure 2).

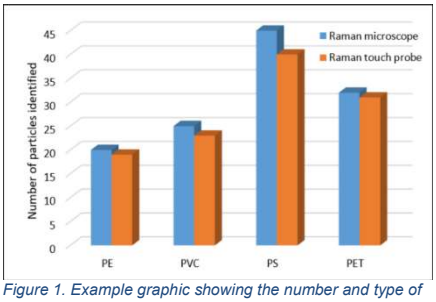


Figure 1. Example graphic showing the number and type of plastic particulate identified using Raman microscopy (blue) and Raman touch probe (orange).

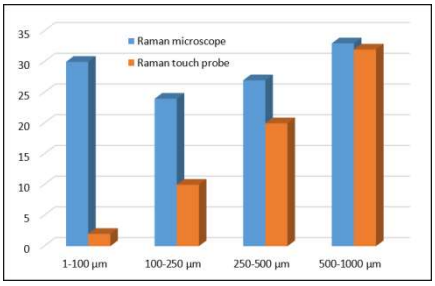


Figure 2. Example graphic showing the amount of particulate identified using Raman microscopy (blue) and Raman touch probe (orange) as a function of particle size.

Shim, W. J., Honga, S. H., Eoa, S., 2017. Identification methods in microplastic analysis: a review. Anal. Methods. 9, 1384-1391.

Methods used: Raman, Probe
Matrices used: Clean water
Lead scientist: Bridget O'Donnell
Labs participating: HORIBA Scientific

Macroscopic FTIR as a rapid screening technique

Statement of the problem
FTIR is well-known as a technique for the identification of plastics. Recently, the technique has been applied to the analysis of microplastics (e.g. Microbeads in toiletries: Method 445.0, Canadian Environmental Protection Act Registry). The identification of microplastics by FTIR relies on the analysis of a single particle at a time; a laborious and time-consuming process. This augmentation study will assess the potential of using macroscopic (unaided by microscopy) FTIR as a screening technique for microplastics in clean water, wherein the spectrum of a novel infrared-amenable microplastics extractor is collected in a single measurement and then analyzed to determine whether the total amount of microplastic falls above or below a certain threshold value.

Approach
In a field closely-related to microplastics, FTIR is used successfully to quantify the amount of oil and grease in water (internationally approved ASTM D7575). The water sample is filtered through a ClearShot™ infrared amenable extractor, which captures and homogenously distributes the oil-and-grease on an infrared transparent membrane. The extractor is then air dried to remove water content and then analyzed in an FTIR spectrometer. The intensity of the spectral peaks is correlated to the concentration of oil-and-grease in the sample and the spectral signature is characteristic of the oil-and-grease type(s). This field-portable, green-chemistry technique is simple to use and takes only minutes per sample. This augmentation will assess whether a similar methodology can be applied to the analysis of microplastics in clean water.

Four replicate samples from each of three 500ml clean water samples containing microplastics will be filtered through ClearShot™ extractors (Orono Spectral Solutions, ME, USA). The extractors will then be dried in an air stream. Four control samples containing no microplastics will be prepared using an identical procedure.

Each dried extractor will be placed in the sample compartment of an FTIR spectrometer. The infrared spectrum of the extractor will be obtained in transmission mode (the IR beam passing through the extractor).

Each spectrum will be compared to a database of infrared spectra to identify materials present on the extractor. Both single component and multiple-component searching algorithms will be used. This will assess the potential for identifying multiple polymer types collected by the extractor.

The spectral intensities, as measured by peak area or peak height, for the identified components will be measured and recorded. The optimum method for the measurement will be determined during the study. These measured intensities will be correlated with the known loadings of the plastics in the sample to assess the ability to quantify the amount of plastic present. In combination with the identification, both the types and quantities of plastics may be simultaneously determined from a single measurement.

Methods used: FTIR
Matrices used: Clean water

Lead scientist: Simon Nunn
Labs participating: Thermo Fisher Scientific

Evaluating the effect of filters for FTIR and Raman microscopy

Statement of the problem

FTIR and Raman microscopy have been identified as techniques for the analysis of microplastics. Filtration of the sample is required prior to analysis by FTIR or Raman. The microplastic particles may be physically removed from the filter prior to analysis by FTIR or Raman. However, for the sake of speed and simplicity it is desirable to analyze the particles in situ directly on the filter. This places additional requirements on the filter. Not only does it need to separate the particles from the matrix but also the filter material needs to be amenable to FTIR or Raman spectroscopy. The most commonly used filters are cellulose, nitrocellulose and PES based. While economical for analyzing large number of samples, these filters have significant IR and Raman peaks, or are not reflective / transmitting enough, resulting in lowquality IR and Raman spectra. Our preliminary study on microplastics in bottled water has indicated that the accuracy and ease of particle analysis is highly dependent on the filter type. Hence there is a need to standardize filter choices defined by type of sample matrix and analytical technique, which will allow direct and unbiased comparison of data from different laboratories. The choice of filter should offer favourable characteristics such a reasonable cost, efficient filtration times, a flat surface, minimize interference for FTIR and Raman spectroscopy and enable automated, high-throughput analyses.

Approach

This augmentation will evaluate at least five filter types; including gold-coated polycarbonate, polycarbonate, silicon and Teflon, and a proprietary filter type from SiMPore. Three blind samples from one matrix will be prepared and filtered through each filter type. Each filter will be measured directly by FTIR and Raman microscopy. The resulting data will be assessed for visual and spectral quality. Physical characteristics of the particles will be assessed from the visual information. Identity of the particles will be derived from the spectral information. Additions and modifications to the last step of filtration will be attempted to improve the outcome of spectroscopic analysis. A few economical recommendations for filters will be summarized.

Methods used: FTIR, Raman
Matrices used: Clean Water
Lead scientist: Suja Sukumaran
Labs participating: Thermo Fisher Scientific, SiMPore

Effects of subsampling and density of microplastic particles

The core study includes a certain number of microplastic particles of each polymer type and a determined subsampling strategy – e.g., pick 30 of each color/morphology category combination and chemically analyze, image and measure these. The rest are simply counted and visually characterized. This augmentation will determine best

practices for subsampling by simulating different subsampling procedures with various samples that are analyzed in full. Participants will determine the accuracy of subsampling results when the same number of particles, 2 ×, and 3 × the number are added to the sample matrix. We will also test out some of the methods we determine to be best practice on real samples. Additional factors to include are subsampling with and without size fractioning via sieving and to test subsampling directly from the spiked matrix to see if a representative sample can be analyzed to reduce sample volume and time.

Methods used: Raman
Matrices used: Clean water
Lead scientist: Chelsea Rochman
Labs participating: University of Toronto (Rochman)

Monitoring pressure changes during filtration as a rapid screening technique

Statement of the problem

Quantification of microplastics in water has typically relied on manual counting of individual particles on filters, including purification and isolation steps in processing such as digestion and density separation. These are laborious and time consuming. This augmentaiton study will assess the potential of using changes in pressure across a membrane during filtration to determine if this can reliably predict total numbers of particles, and by extension the mass concentration present if the particles are well-defined.

Approach

SiMPore has produced proprietary membrane filters with well-defined pores in various sizes, as well as filtration equipment that can measure the pressure changes across the membranes as particles are captured on them. This filtration equipment can be stacked, so that several units with membranes of different pore sizes can be stacked in series to filter simultaneously several different sizes of particles. In this augmentation, we will add known amounts of microplastic particle fragments to clean water, filter them through this equipment, and monitor pressure changes. The concentrations of the particles in samples will span the range needed to have at least a monolayer of coverage, as that would produce a detectable change in pressure. The actual amount of microplastics will be collected and quantified by visual microscopy to determine how well pressure changes reflect the amount of particles collected by the filters. Further experiments would evaluate mixtures of different size classes of particles, different compositions of polymers as appropriate, and utility of the system to monitor fibers. Confirmation of polymer identity would be done by Raman spectroscopy or FTIR/LDIR as appropriate.

Methods used: Proprietary transmembrane filtration equipment; visual microscopy
Matrices used: Clean water
Lead scientist: James Roussie
Labs participating: SiMPore

Evaluating laser direct infrared chemical imaging as a quantification and identification method

Statement of the problem

A number of methods have been used to date to quantify and identify microplastic particles by number count and polymer material, respectively. These methods, which are used in the Core Study, include FTIR spectroscopy, Raman spectroscopy and optical microscopy. A key disadvantage of these methods is long analysis times, especially over large areas (e.g., the size of a standard filter) on samples with many particles. Other disadvantages include limited automation (requiring time-consuming manual counting and chemical identification of particles), inability to reliably identify polymers visually, and sample fluorescence in the case of Raman spectroscopy. The LDIR technology has the potential to scan areas the size of a filter within a fraction of the time used in existing techniques to provide a map of particles, as well as their size and shape, and to identify the composition of individual particles automatically. This augmentation is intended to evaluate the advantages and limitations of LDIR, to determine if this method is a viable and time-saving alternate means to analyze microplastics in typical environmental matrices.

Approach

Participating laboratories will analyze extracted microplastic samples from the four Core Study matrices under LDIR. Particle count, size, and IR spectral data will be recorded, as well as the time and resources needed for analysis. The data obtained will be analyzed to determine the effectiveness of LDIR compared to other spectroscopic techniques.

Methods used: LDIR
Matrices used: Clean water, dirty water, sediment
Lead scientist: Charles Wong
Labs participating: Eurofins Australia, Agilent

Evaluating pyrolysis-GC/MS as a quantification tool for microplastics

Statement of the problem

A number of methods have been used to date to quantify and identify microplastic particles by number count and polymer material, respectively. These methods, which are used in the Core Study, include FTIR and Raman spectroscopy. However, these methods cannot measure mass concentrations of microplastics (e.g., ng polymer/L), and attempts to calculate such from number count and size estimations are difficult and subject to considerable uncertainty. Pyrolysis GC/MS provides information on chemical identity, and also provides mass concentrations, an important and complementary measure of microplastic abundance that may provide invaluable in exposure and risk assessment. This augmentation is intended to evaluate the capabilities and limitations of pyrolysis-GC/MS in quantifying microplastics in typical environmental matrices.

Approach
Participating laboratories will analyze custom blind samples that contain a spike of microplastic material for which mass has been measured gravimetrically (e.g., via microbalance). These samples would then be extracted and processed as described for the Core Study. Unlike the Core Study, in which a subset of extracted particles would undergo chemical confirmation via instrumental analysis, the entire collected mass of particles would be analyzed by GC/MS to provide a mass concentration for that matrix.

Methods used: Pyrolysis-GC/MS
Matrices used: Clean water, dirty water, sediment
Lead scientist: Charles Wong
Labs participating: Eurofins Norway, Penn State

Evaluating effects of new Raman imaging methods in microplastics measurement
Statement of the problem
It is common to use Raman spectroscopy in the analysis and identification of synthetic polymer particles from environmental samples. This is reflected in the Core Study, for which many participating labs are using this analytical approach. However, some of the disadvantages of Raman include long scan times, especially over large areas (e.g., the size of a standard filter) on samples with many particles. Other disadvantages include limited automation, requiring time-consuming manual counting and chemical identification of particles. Together, Lever Photonics and Professor Chan's lab at the University of California, Davis have been specializing in developing Raman instruments to provide more efficient operations that include new imaging approaches and automation of many tasks. This augmentation is intended to evaluate the advantages and limitations of such an approach, to determine how both technical performance and the effort expended to obtain results for such instrumentation compares to traditional equipment.

Approach
Core Study clean water samples will be processed as described, and analyzed both using unaltered Raman instrumentation, as well as equipment that has been designed and built by Professor Chan's lab at the University of California, Davis. Particle count, size, and Raman spectral data will be recorded, as well as the time and resources needed for analysis. The data obtained will be analyzed to determine the effectiveness of the modified Raman compared to unaltered Raman.

Methods used: Raman
Matrices used: Clean water
Lead scientist: Maria Navas-Moreno and James Chan
Labs participating: Lever Photonics, UC Davis, SCCWRP

Evaluating effects of tailoring Raman instrumentation specifically for microplastics measurement
Statement of the problem

It is common to use Raman spectroscopy in the analysis and identification of synthetic polymer particles from environmental samples. This is reflected in the Core Study, for which many participating labs are using this analytical approach. However, some of the disadvantages of Raman include potential large capital investment on equipment that has been designed to have optimal performance for a broad range of applications but often over-designed for specific applications. Lever Photonics has been specializing in customizing Raman instruments to provide more efficient and cost-effective operations by evaluating and selecting the hardware and software requirements for specific applications, such as the analysis of microplastics. This augmentation is intended to evaluate the advantages and limitations of such an approach, to determine how both technical performance and the effort expended to obtain results for such tailored instrumentation compares to unaltered equipment.

Approach

Core Study clean water samples will be processed as described, and analyzed both using unaltered Raman instrumentation, as well as equipment that has been tailored by Lever Photonics. Particle count, size, and Raman spectral data will be recorded, as well as the time and resources needed for analysis. The data obtained will be analyzed to determine the effectiveness of tailored Raman compared to unaltered Raman.

Methods used: Raman
Matrices used: Clean water
Lead scientist: Maria Navas-Moreno
Labs participating: Lever Photonics, SCCWRP

Evaluating efficacy of optical photothermal infrared spectroscopy for microplastics measurement

Statement of the problem

It is common to use FTIR and Raman spectroscopy to confirm the chemical identity of synthetic polymer particles in environmental analysis. This is reflected in the Core Study. However, both techniques have disadvantages. For FTIR, there are limitations in spatial resolution that preclude analysis below approximately 10 microns, as well as dispersion artifacts from traditional reflection FTIR, which limits chemical ID and spectral interpretation and issues with sample damage and loss with the use of a micro-ATR accessory. For Raman, long scan times can be an issue, as well as problems with sensitivity and fluorescence. Photothermal Spectroscopy has recently developed O-PTIR, that combines Raman and IR spectroscopy to address many of the limitations of both conventional techniques. This augmentation is intended to evaluate the advantages and limitations of O-PTIR, as a promising technique for environmental analysis of microplastics.

Approach

Core Study clean water samples will be processed as described, and analyzed using O-PTIR and simultaneous Raman spectroscopy. Particle count, size, and spectral data will be recorded, as well as the time and resources needed for analysis. The data

obtained will be analyzed to determine the effectiveness, advantages, and limitations of O-PTIR for environmental microplastics analysis compared to FTIR and Raman.

Methods used: O-PTIR and simultaneous Raman spectroscopy
Matrices used: Clean water
Lead scientist: Eoghan Dillon
Labs participating: Photothermal Spectroscopy Corp., SCCWRP

LITERATURE CITED

Besley, A., Vijver, M.G., Behrens, P., Bosker, T., 2017. A standardized method for sampling and extraction methods for quantifying microplastics in beach sand. Mar. Pollut. Bull. 114:77–83.

Foekema, E.M., De Gruijter, C., Mergia, M.T., van Franeker, J.A., Murk, A.J. and Koelmans, A.A., 2013. Plastic in North Sea fish. Environmental Science & Technology, 47:8818-8824.

Hanvey, J.S., Lewis, P.J., Lavers, J.L., Crosbie, N.D., Pozo, K., Clarke, B.O., 2017. A review of analytical techniques for quantifying microplastics in sediments. Anal. Methods 9:1369–1383.

Herrera, A., Garrido-Amador, P., Martinez, I., Samper, M.D., Lopez-Martinez, J., Gomez, M., Packard, T.T, 2018. Novel methodology to isolate microplastics from vegetal-rich samples. Mar. Pollut. Bull. 129, 61.69.

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification.

Lusher, A.L., Welden, N.A., Sobral, P. and Cole, M., 2017. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Analytical Methods 9:1346-1360.

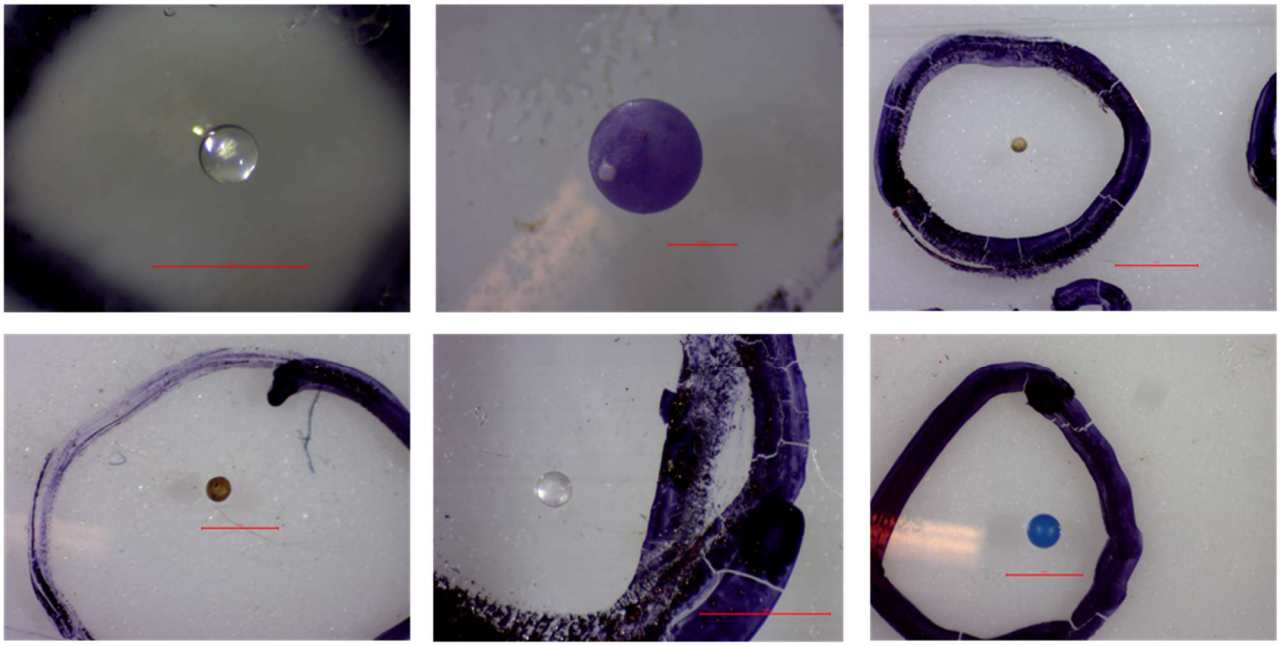
Michaela Cashman, Kay Ho, Stephen Russo, Thomas Boving, Robert Burgess (2019) Microplastics in marine sediments: An assessment of current extraction and isolation methods. Poster Presentation. Society of Environmental Toxicology and Chemistry 39th Annual meeting Sacramento CA. Nov 4-8, 2018

Mintenig, S.M., Int-Veen, I., Loder, M.G., Primpke, S., Gerdts, G., 2017. Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging. Water Res. 108:365-372.

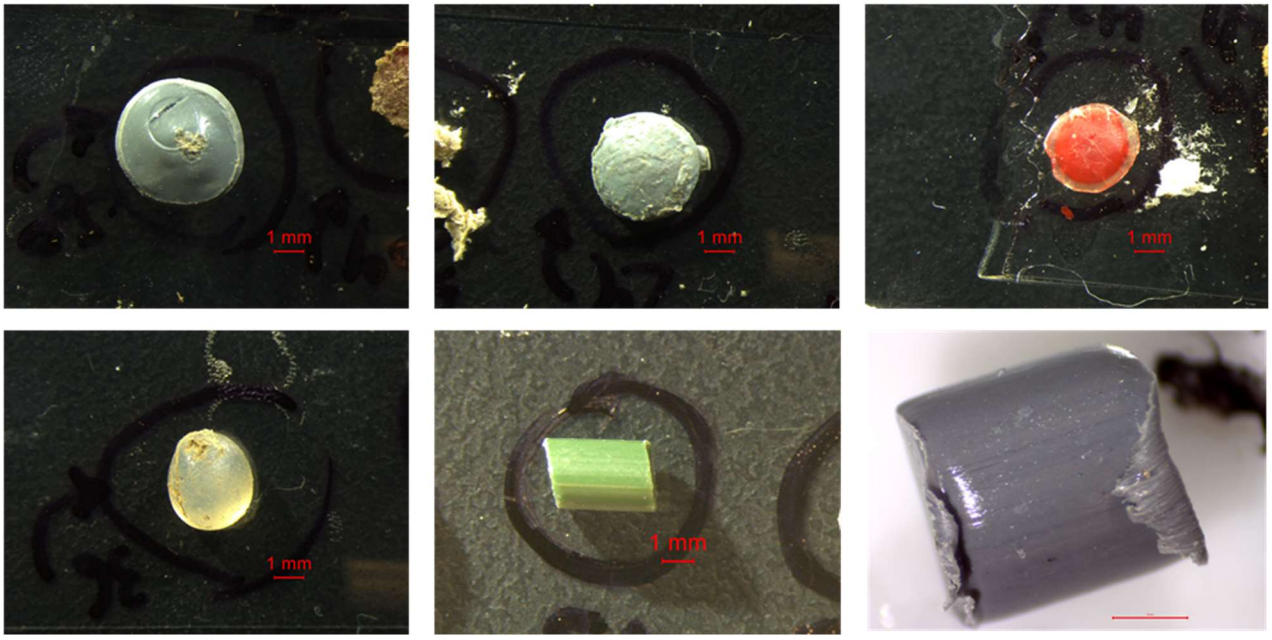
Appendix M: Microplastics Morphology Key

FRAGMENT
Rigid edges
Irregular shapes (angular, subangular, rounded, sub-rounded)
Hard
Do not break easily when compressed

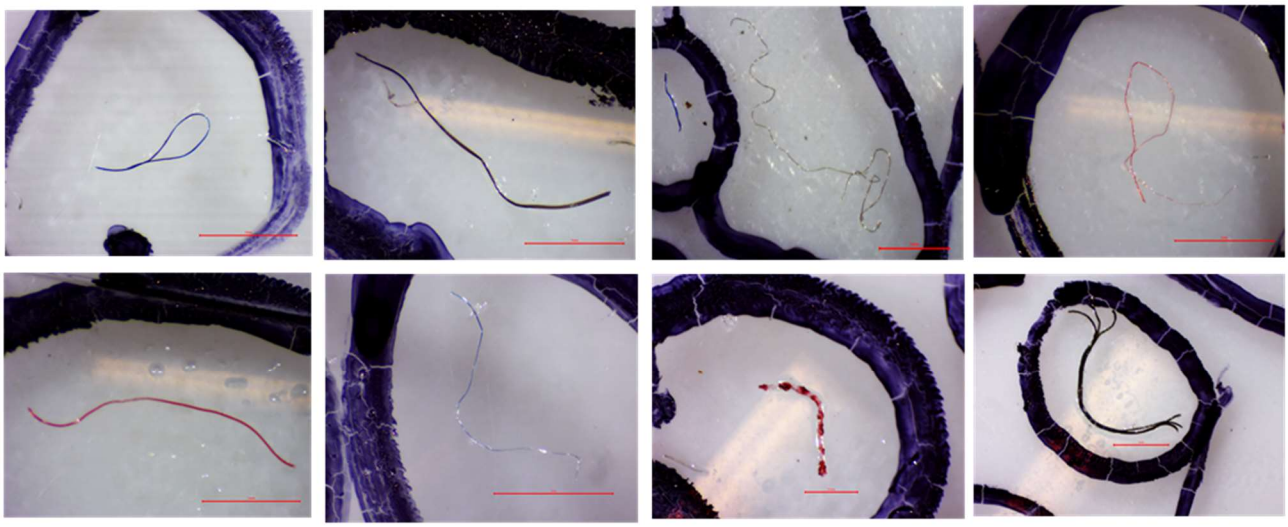
SPHERE
Perfectly spherical
Can be hemispheres (broken spheres)
No irregularities
Smooth, often shiny surface



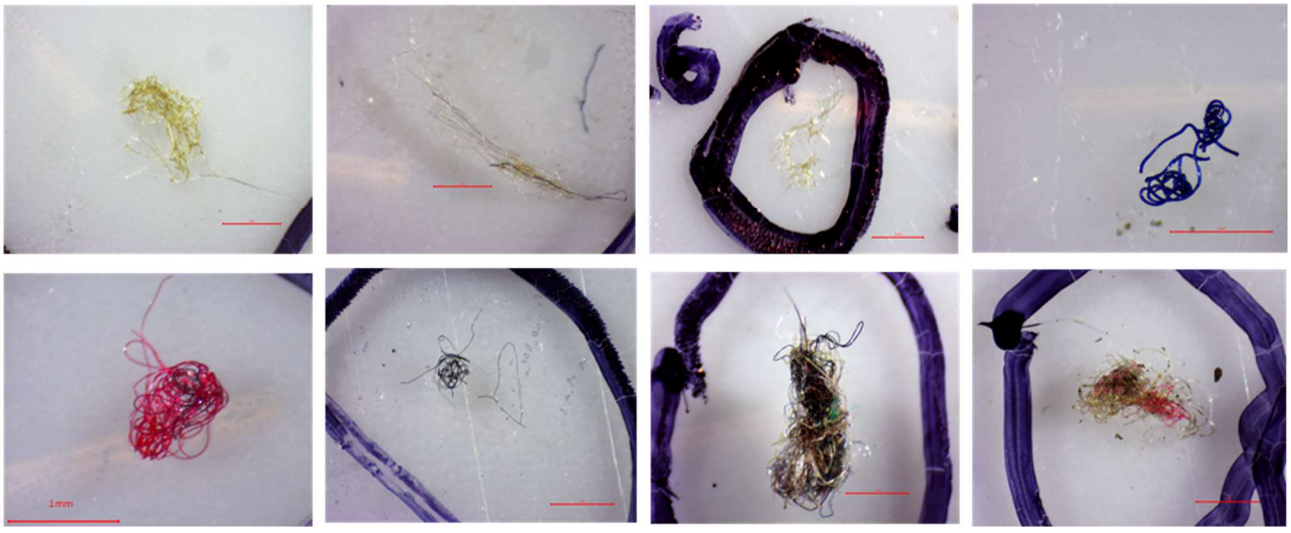
PELLET
Flattened oval shape
Sometimes rectangular or cylindrical with a clear 'machine cut' edge
Larger in size than spheres (typically 3-5 mm)
Often clear, opaque or white in color



FIBER
Strand or string-like
Often equally thick throughout
Can change colour due to bleaching
Can be easily bent and twisted
Ends can be flat, pointed, or fraying

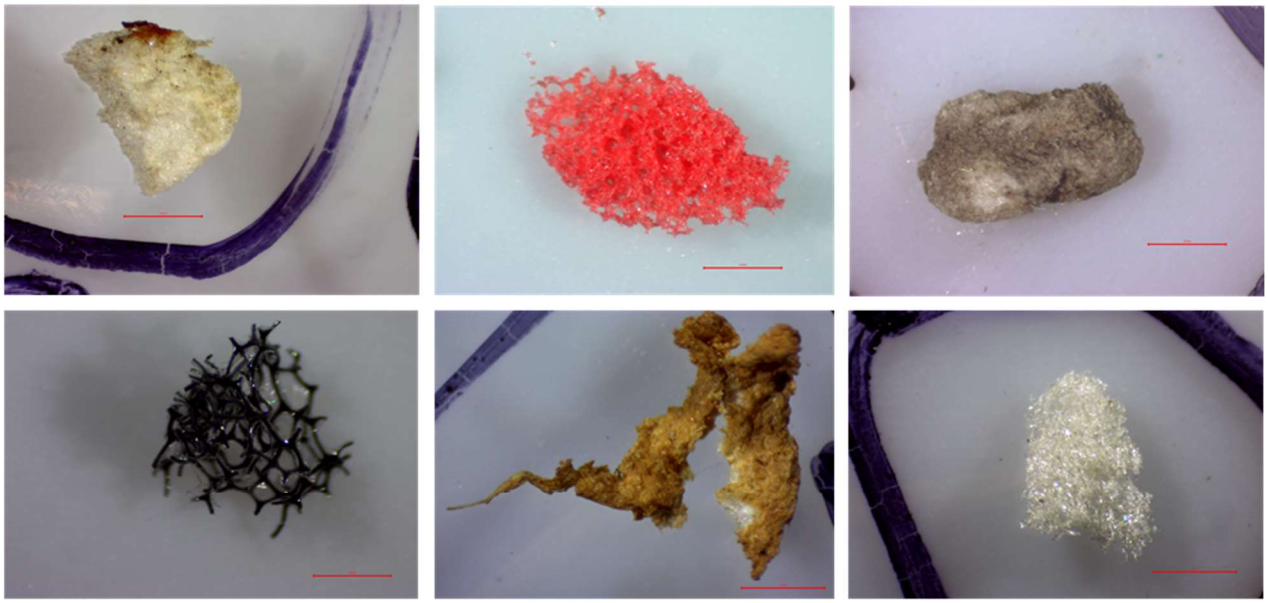


FIBER BUNDLE
>20 tightly wound individual fibers
Cannot be teased apart
Separation would cause breakage
Can consist of fibers of different colors



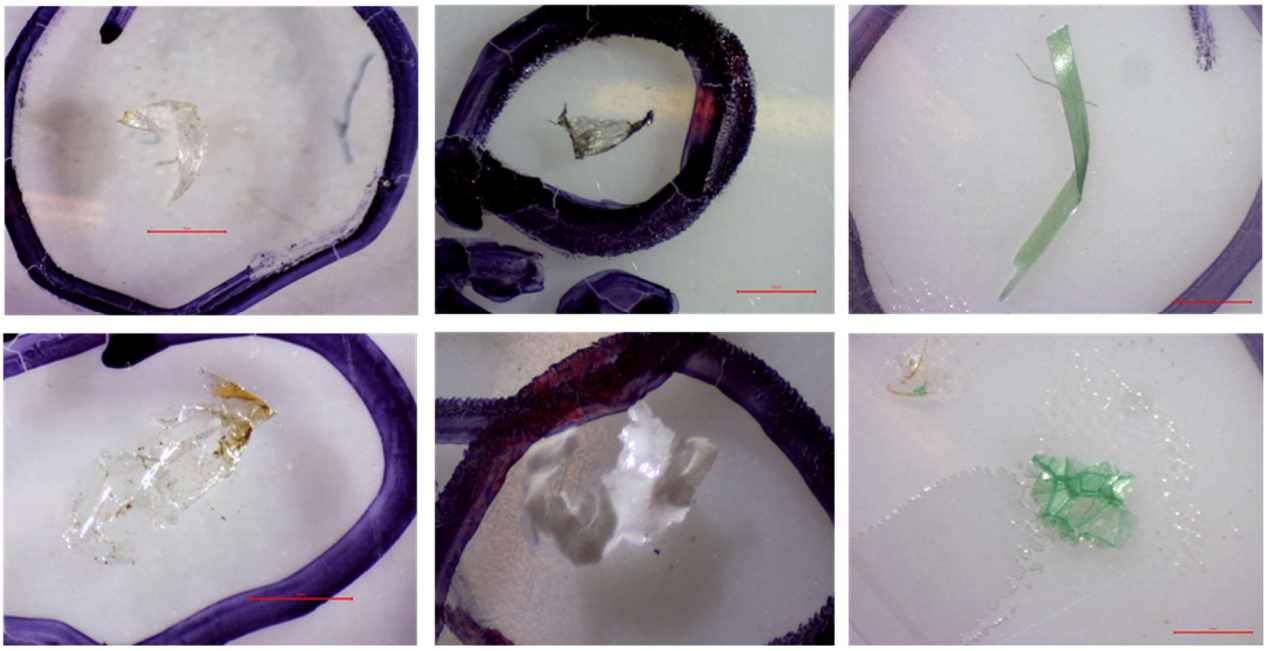
FOAM

Holes within the particle structure
Will bounce back when compressed
'Cloud like' appearance



FILM

Thin, flat, flexible sheets
Can fold or crease
Partially or totally transparent
Does not break easily



Appendix N: Microplastics Color Key

No subcategorization, e.g. all shades of blue are within one color category.

Clear: Transparent with no color

White: Solid color i.e., not transparent

Multicolor: Only for use if the particle consists of multiple colors, where one color does not appear to be dominant.

Silver: shiny, reflective, metallic surface. If the surface is matte in appearance the particle should be characterized as grey.

Gold: Same applies as for silver. If the surface is matte the particle should be characterized as yellow or orange.

Analytical and Bioanalytical Chemistry (2023) 415:2873–2890
https://doi.org/10.1007/s00216-023-04671-1

CRITICAL REVIEW

Previous successes and untapped potential of pyrolysis–GC/MS for the analysis of plastic pollution

Meredith Evans Seeley[1,2] · **Jennifer M. Lynch**[1,2]

Received: 23 February 2023 / Revised: 23 March 2023 / Accepted: 24 March 2023 / Published online: 10 April 2023

Abstract

There is growing concern from scientists, policy makers, and the public about the contamination of natural and indoor environments with plastics, particularly micro/nanoplastics. Typically, characterizing microplastics in environmental samples requires extensive sample processing to isolate particles, followed by spectroscopic methodologies to identify particle polymer composition. Spectroscopic techniques are limited in their ability to provide polymer mass or advanced chemical composition (e.g., chemical additive content), which are important for toxicological assessments. To achieve mass fraction quantification and chemical characterization of plastics in environmental samples, many researchers have turned to thermoanalytical spectrometric approaches, particularly pyrolysis–gas chromatography/mass spectrometry (Py–GC/MS). Sample preparation for Py–GC/MS may be approached similarly to techniques needed for spectroscopic approaches (e.g., isolate particles on a filter), employ pressurized solvent extraction, or use ultrafiltration techniques to concentrate nanoplastics. Great strides have been made in using calibration curves to quantify plastics in complex matrices. However, the approaches to the pyrolysis thermal program, as well as calibrant and sample preparation, are inconsistent, requiring refinement and harmonization. This review provides a critical synthesis of previous Py–GC/MS work and highlights opportunities for novel and improved Py–GC/MS analysis of plastics in the future.

Keywords Pyrolysis · Microplastics · Nanoplastics · Thermal desorption · Plastic pollution · Marine debris

Introduction

Plastic pollution is omnipresent across natural and indoor environments. This is particularly true of microplastics (1 μm–5 mm) and nanoplastics (< 1 μm), which are formed as larger plastics wear, weather, and fragment [1, 2]. There is a demand for analytical techniques to measure plastic pollution in a variety of matrices, which is a critical need for regulatory actions. Quantifying plastic in environmental samples requires polymer identification and the ability to count particles or measure the mass of each polymer type. Currently available methods are challenged by the fact that no two pieces of plastic in the environment are alike. Plastics vary by polymer type(s), chemical additive constituents, size, shape, color, density, molecular weight distribution, crystallinity, and more [3, 4]. Furthermore, despite their seemingly ubiquitous distribution, sample processing and analytical instrumentation capacities are likely leading to an underestimation of plastics in the environment, particularly in the smallest size ranges [5].

The analysis of plastics in any environmental sample hinges upon confirmation that a suspected plastic is a synthetic polymer (and not natural particulate), which is usually accomplished using chemical identification methods. Polymer identification can also be important in sourcing the debris by original product type. Commonly, spectroscopy (e.g., Raman and Fourier transform infrared (FT-IR)) is employed, providing a count of plastic particles by polymer type [6]. These data may be difficult to translate into risk assessments or policy, as they do not measure particle mass toward dose estimates [7]. Complementary mass-based

Published in the topical collection *Advances in Chemical Analysis of Micro- and Nanoplastics* with guest editors Natalia P. Ivleva, Jennifer M. Lynch, and Sebastian Primpke.

✉ Meredith Evans Seeley
Meredith.Seeley@nist.gov

[1] Chemical Sciences Division, National Institute of Standards and Technology, Waimanalo, HI 96795, USA

[2] Center for Marine Debris Research, Hawaii Pacific University, Waimanalo, HI 96795, USA

approaches have not been as commonly used. Pyrolysis–gas chromatography/mass spectrometry (Py–GC/MS) has grown in popularity for the analysis of plastic debris in diverse environmental matrices over the past decade [8]. The analytics possible for plastics using Py–GC/MS, with associated sample types and preparation, is provided in Fig. 1. Considering its growing utility, this is a critical time to consider best practices for Py–GC/MS and harmonize approaches moving forward [9–11]. This review provides a synthesis of previous Py–GC/MS research for the analysis of plastic in the environment and recommendations for future work.

Approaches to pyrolysis–GC/MS

Pyrolysis instrumentation

In general, Py–GC/MS is made possible by a sample furnace attached to a GC/MS inlet [12]. The pyrolysis mechanism can be categorized as pulse mode (a sample is introduced cold and then flashed at pyrolysis temperature) or continuous mode. Pulse mode systems use a heated filament or Curie-point pyrolysis, while continuous systems use furnaces or microfurnaces [13]. For either system, a small sample size and a heating area are required to ensure rapid, homogeneous pyrolysis and successful purging. The analysis of environmental plastics has been carried out with filament [14, 15], Curie point [16, 17], and microfurnace [18–34] pyrolysis (Fig. 2). Microfurnace pyrolysis (specifically vertical microfurnace) is the most common due to its ability to rapidly heat a sample (improving transfer onto the column and peak resolution) and allow different thermal schedules. Fischer and Scholz-Böttcher [19] illustrated the advantages of microfurnace over Curie-point pyrolysis, including a large sample volume capacity. Nonetheless, the overall sample size capacity for pyrolysis is generally small; for example, in microfurnace pyrolysis, only 0.1–0.5 mg is recommended, and a common sample cup volume is 80 µL. Sample overloading can lead to incomplete purging of the sample onto the column, yielding ghost peaks in subsequent runs [19, 33].

Commonly, the pyrolysis unit is directly attached to the GC/MS for rapid and effective transfer. Sample heating and column evolution occurs via an inert carrier gas (commonly, helium) in the absence of oxygen. Rarely, off-line pyrolysis may be employed, which condenses pyrolyzates onto a solid-phase capture device which are resuspended in solvent prior to GC/MS [35, 36]. This technique can generate

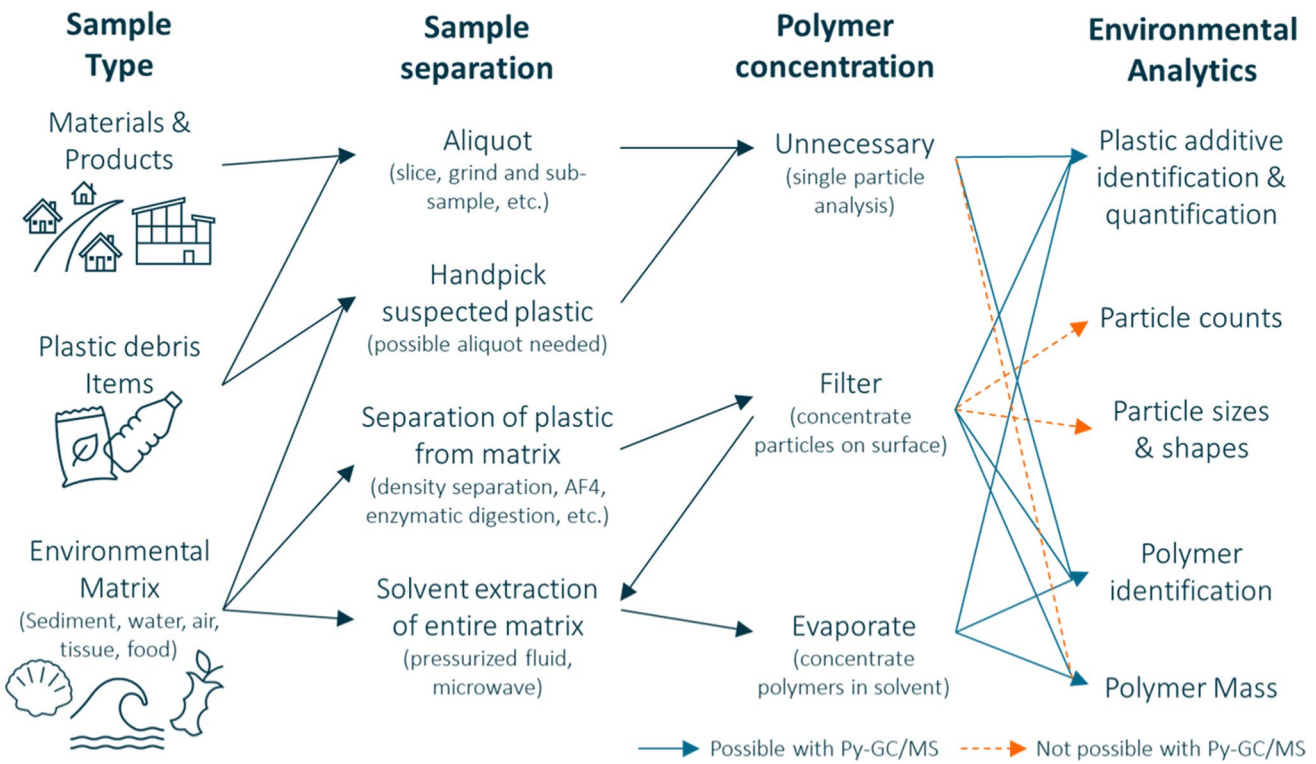


Fig. 1 Schematic illustrating the utility of Py–GC/MS for different environmental analytics. The sample type, preparation, and necessity of polymer concentration leading to analysis detailed. Connecting polymer concentration and environmental analytics, solid blue lines indicate that the analysis is possible with Py–GC/MS while dashed orange lines are not. Of note, while Py–GC/MS does not measure particle size or shape, some size information is possible with sample sieving or sequential filtration in sample preparation

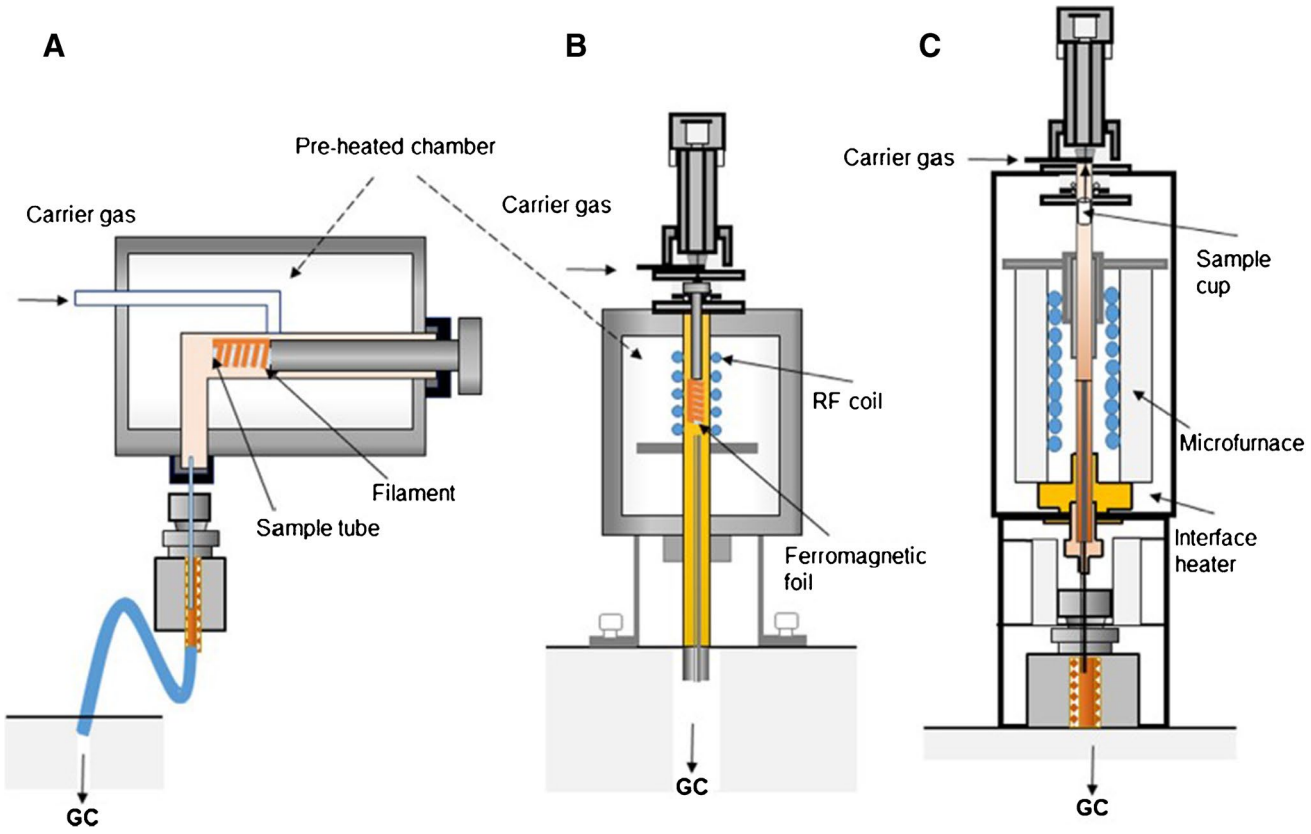


Fig. 2 Common types of pyrolysis instrumentation, including filament (A), Curie point (B), and vertical microfurnace (C). Reproduced from Pico and Barcelo [13] with permission from the publisher (license number: 5461480936973)

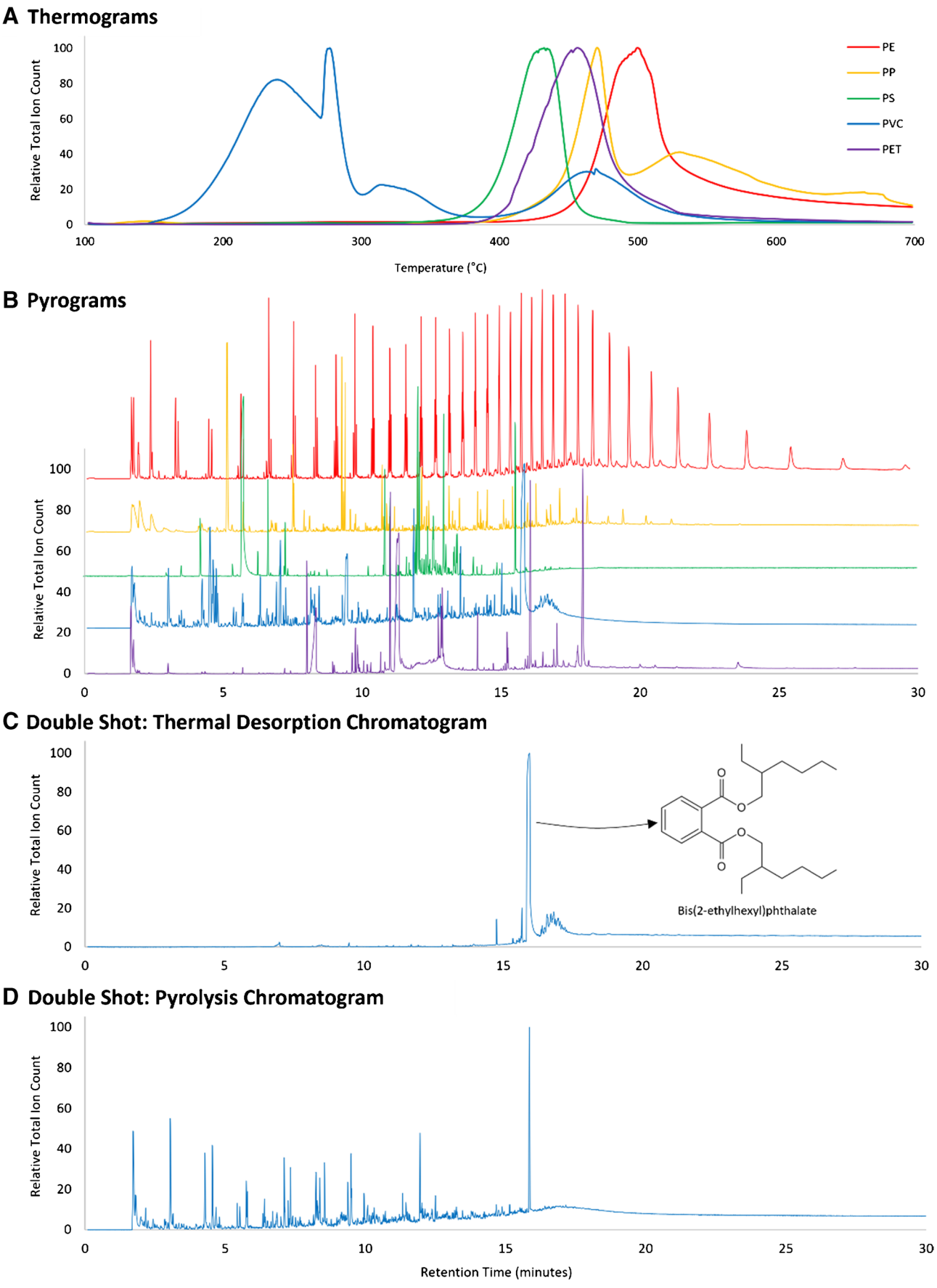
considerable variability, resulting from different behaviors between pyrolyzates and solid-phase capture devices [36]. An advantage, however, is that the solvent-suspended pyrolyzate can be retained for repeat or different analyses, possibly expanding beyond the GC-amenable window [37]. Online pyrolysis units (e.g., microfurnace) can also be attached to different analytical suites, such as time-of-flight MS [38] or tandem MS [39], but these can require increased data-processing time and training.

Evolved gas analysis Py–GC/MS

Evolved gas analysis (EGA) is the simplest of the pyrolysis approaches. EGA is commonly utilized to gain insight into the thermal deconstruction profile of a sample. During this analysis, the sample is slowly heated (e.g., 50 to 700 °C at 20 °C min^{-1}) and volatilized material is simultaneously eluted through a short and narrow (e.g., 2.5 m, 0.15 mm) capillary tube without stationary phase. As the goal is to understand total thermal characteristics (i.e., and not presence/absence of specific compounds), the MS is run with a slower scan speed, yielding a smooth thermogram (temperature versus total ion count). Figure 3(A) shows EGA thermograms of five polymers from Hawaii Pacific University Center for Marine Debris Research (HPU CMDR) Polymer Kit 1.0 [40]. EGA thermograms are complimentary to other thermal analytical techniques, but cover a wider temperature range and provide mass spectra (as opposed to melt characteristics provided by differential scanning calorimetry (DSC), for example). EGA is particularly useful for individual samples, not complex mixtures. As such, EGA can be a valuable first step in determining temperature programs for double-shot and thermal slicing analysis.

Single-shot pyrolysis–GC/MS

In single-shot pyrolysis, a sample is flash pyrolyzed at a high temperature, ≥ 500 °C. This heating occurs as rapid as possible, inducing quasi-instantaneous and homogenous pyrolysis. The resulting gaseous products, or pyrolyzates, are formed via random chain scission, end-chain scission, and side-chain cleavage reactions. Pyrolyzates are typically deposited onto a separatory column (commonly 30 m, 0.25 mm internal diameter, 0.25 µm film thickness) connected to a quadrupole MS, although tandem MS has recently been used [39]. The resulting "pyrogram" details


A Thermograms
Relative Total Ion Count
Temperature (°C)
PE
PP
PS
PVC
PET
B Pyrograms
Relative Total Ion Count
C Double Shot: Thermal Desorption Chromatogram
Relative Total Ion Count
Bis(2-ethylhexyl)phthalate
D Double Shot: Pyrolysis Chromatogram
Relative Total Ion Count
Retention Time (minutes)

◂**Fig. 3** Examples of Py–GC/MS analyses of five plastics from the HPU CMDR Polymer Kit 1.0, including polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), and polyethylene phthalate (PET). The figure includes evolved gas analysis thermograms (A), flash pyrolysis pyrograms (B; offset to improve readability), and double-shot PVC chromatograms from thermal desorption (C) and pyrolysis (D) zones, illustrating the separation of phthalate additive bis-2ethylhexyl phthalate in the thermal desorption zone. Analysis parameters are detailed in Tables S1–S3

pyrolyzate concentration (i.e., total ion count) as a function of retention time (Fig. 3(B)). This pyrogram is like traditional GC/MS chromatograms but is specific to the flash pyrolysis products. Single-shot Py–GC/MS has been applied to the analysis of discrete plastic particles [18, 41–43] as well as complex environmental samples [14–16, 19–27, 44, 45].

Single-shot Py–GC/MS can vary by temperature. Generally, the aim is to pyrolyze a sample at a temperature that fully fragments the polymer, but does not degrade pyrolyzates [12]. Hermabessiere et al. [31] explored pyrolysis temperatures for several common plastic polymers. These authors found that PE, for example, had a maximum pyrolysis yield at 700 °C, above which detectability decreased. Recently, Okoffo et al. [29] found that many detector responses for some polymers reached their peak at 650 °C, after which the signal decreased. This is true for styrene as a pyrolyzate of PS, for example, which degrades above 650 °C [29, 31]. Most researchers employ moderate pyrolysis temperatures to avoid degrading pyrolyzates. Indeed, pyrolysis temperatures reported in the literature for the analysis of environmental plastics include 450 °C [46], 500 °C [30, 35, 36, 47, 48], 550 °C [33, 46, 49], 590 °C [16, 20, 20–22, 24–26, 36], 600 °C [14, 23, 28, 28, 32, 38, 42, 50], 650 °C [27, 29, 41, 45, 51], 700 °C [42, 52, 53], or 750 °C [15]. While the sensitivity of the results may vary for different polymers by temperature, this is a minimally consequential decision if a consistent temperature is used.

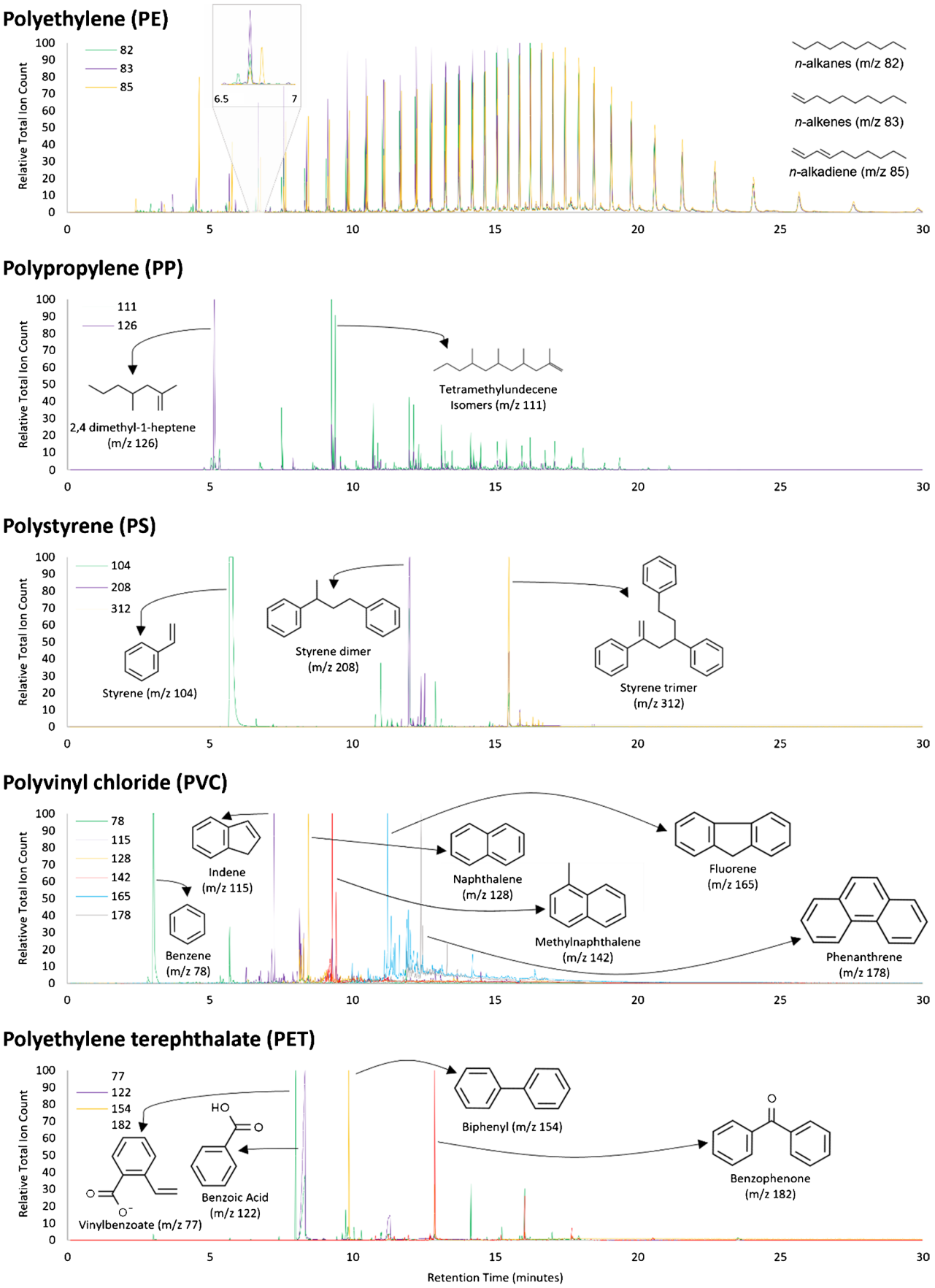
Double-shot pyrolysis–GC/MS

In a single-shot pyrolysis isothermal program, mobile (labile) components of a sample (i.e., plastic additives) are not separated from the more recalcitrant components (i.e., polymers). Double-shot Py–GC/MS (or TD–Py–GC/MS) employs two temperature programs. The first is thermal desorption (TD), where a sample is heated over a low temperature ramp (e.g., 100–300 °C at 20 °C min^{-1} and held for 1 min) and analyzed via GC/MS on a separatory column, yielding a TD chromatogram (Fig. 3(C)). Following TD, pyrolysis occurs according to single-shot parameters (e.g., flash pyrolysis at 550 °C; Fig. 3(D)). A growing number of studies have used TD–Py–GC/MS for the analysis of plastic debris, as there are multiple advantages [28, 29, 52–55].For individual plastic particles, double-shot can separate potential additives in the TD zone from polymeric pyrolyzates in the Py zone. This was demonstrated in 2013 by Fries et al. [52], and even earlier for presence of phthalates in recycled plastics [47] and other additives of plastics in environmental samples [54]. A recent study quantified phthalate additives (mass fraction) via thermal desorption of solvent-extracted beach sand [55], demonstrating the quantitative capacities beyond plastic polymers. A barrier to expanded additive identification in TD–Py–GC/MS is that thousands of plastic additives exist in commerce, many of which are not currently available in reference mass spectral libraries [56]. Further, GC/MS methods for the identification of additives are often directed toward one additive class (e.g., phthalates, antioxidants), and not several types of plastic additives simultaneously.

Beyond separating plastic additives, double-shot Py–GC/MS can be used to separate natural organic materials from polymer pyrolyzates, improving quantification. For example, Okoffo et al. [29] used TD–Py–GC/MS for the analysis of solvent-extracted sewage sludge samples. The authors report that adding TD reduced matrix interference from the natural organic matter present in the sample, but they did not analyze the TD chromatogram for additives. Similarly, analysis of microplastics in human blood by Leslie et al. employed TD–Py–GC/MS to reduce interference of unpolymerized monomers, additives, and adsorbed compounds, but the TD chromatogram was not analyzed [28]. Finally, double-shot analysis has been employed for analysis of changes in oxidation and pyrolysis cracking patterns in artificially weathered plastics [46]. A drawback of double-shot Py–GC/MS is that it is more time and resource intensive, taking twice as long to analyze a sample as single shot. In addition, while most polymers do not break down below 300 °C, there may be exceptions (e.g., PET [28]). Polymer fragmentation during TD could result in underestimated polymer concentrations, if sample and calibrant polymer thermal properties vary.

Thermal slicing pyrolysis–GC/MS

Some pyrolysis instruments are capable of advanced thermal programing, facilitating deeper exploration of the thermal properties and products of different materials. Thermal slicing Py–GC/MS analyzes a sample over more than two thermal ranges. This can be interchangeable with heart-cut Py–GC/MS, although heart-cut analysis may specifically refer to when a thermal range is not analyzed, accomplished by selective sampling [57]. Thermal slicing can be useful for samples where finer thermal resolution than TD–Py–GC/MS is informative. However, thermal slicing Py–GC/MS has not yet been employed, to our knowledge, for the analysis of plastics in environmental samples. Thermal slicing offers some potential benefits for advanced analysis of plastic


Polyethylene (PE)
Relative Total Ion Count
82
83
85
6.5
7
n-alkanes (m/z 82)
n-alkenes (m/z 83)
n-alkadiene (m/z 85)
Polypropylene (PP)
111
126
2,4 dimethyl-1-heptene (m/z 126)
Tetramethylundecene Isomers (m/z 111)
Polystyrene (PS)
104
208
312
Styrene (m/z 104)
Styrene dimer (m/z 208)
Styrene trimer (m/z 312)
Polyvinyl chloride (PVC)
Relative Total Ion Count
78
115
128
142
165
178
Indene (m/z 115)
Benzene (m/z 78)
Naphthalene (m/z 128)
Methylnaphthalene (m/z 142)
Fluorene (m/z 165)
Phenanthrene (m/z 178)
Polyethylene terephthalate (PET)
Relative Total Ion Count
77
122
154
182
Vinylbenzoate (m/z 77)
Benzoic Acid (m/z 122)
Biphenyl (m/z 154)
Benzophenone (m/z 182)
Retention Time (minutes)

◂**Fig. 4** Extracted ion chromatograms of marker pyrolyzates for five common plastic polymers from HPU CMDR Polymer Kit 1.0. A single chromatogram is present for each marker and compound peak labeled with molecular structure. Pyrolysis conducted at 650 °C (analysis parameters detailed in Table S2)

materials, including information on structural changes and oxidation following weathering (as demonstrated in the analysis of weathered oil spill residues [58]) and distinction between labile adsorbed versus additive components.

Other Py–GC/MS adaptations

Thermochemolytic (or reactive) Py–GC/MS has been employed to improve the analysis of polymers with non-GC-amenable or polar pyrolyzates. When a thermochemolytic agent is added to a sample, esterification, transesterification, and methylation occurs, improving sensitivity for polyamides (PA; nylon), PET, polyurethane (PU), poly(methyl methacrylate) (PMMA), and others. This does not typically interfere with pyrolyzates of other polymers [16]. The most common derivatizing agent is tetramethylammonium hydroxide (TMAH) [16, 18–22, 24, 26–28, 30, 33, 36, 49]. TMAH is a very toxic and dangerous compound as it can cause chemical burns, respiratory failure, organ and central nervous system disruption, and even death; safer alternatives should be explored [59].

A less-often employed pyrolysis adaptation is cryogenic trapping Py–GC/MS, made possible with a micro-jet cryo trap accessory [58]. This cryo-focuses analytes at the start of the column, as opposed to direct elution as a sample is pyrolyzed. This can improve separation of compounds following an extended heating ramp, for example, the temperature ramp during the TD portion of double-shot Py–GC/MS or during thermal slicing Py–GC/MS. It can also improve capture of low molecular weight, volatile compounds. This has not been utilized for the analysis of environmentally sourced plastics, but has proven useful in the analysis of oil spill residues [58].

Pyrolysis GC/MS for environmental plastics analysis

Polymer identification

The pyrolysis behavior of most polymers is predictable, leading to the creation of specific characteristic pyrolyzate(s) that are identifiable by their mass spectra. These pyrolyzates are termed "marker compound(s)" when used to identify the polymer type of unknown plastics. In many cases, marker compounds are mono- or oligomeric components of the polymer. For example, polystyrene (PS) is identifiable by styrene monomer, dimer, and trimer. The PA marker compound is the monomer (N-methyl)-E-caprolactam for polyamide-6 and 1,8-diazacyclotetradecane-2,7-dione for polyamide-6,6. Common long-chain thermoplastics, polyethylene (PE) and polypropylene (PP), are characterized by a series of hydrocarbons from polymer decomposition. For PE, pyrolysis yields a series of n-alkane, alkene, and alkadiene triplets; for PP, 2,4-dimethyl hept-1-ene and a series of tetramethylundecene isomers. There is a growing interest in using Py–GC/MS to identify tire and road wear particles, as spectroscopic techniques are not robust enough for rubber analysis [60, 61]. Markers of tire-derived rubbers, including styrene butadiene rubber (SBR) and butadiene rubber (BR), are often small aromatic hydrocarbons such as benzene, ethylstyrene, styrene, butadiene, and vinylcyclohexene [17, 60, 61]. Natural rubber (NR) may also be distinguished via dipentene and isoprene markers [17]. Often, but not always, the best marker compound is the most abundant pyrolyzate of a polymer (see "Matrix interferences").The extracted ion chromatograms of common marker compounds for five polymers are provided in Fig. 4. Details of pyrograms with marker compounds from a variety of additional polymers can be found in at least one reference textbook [37].

Occasionally, the mass spectra of an entire thermogram or pyrogram (or an area of interest within a pyrogram) can be compared to reference polymers for identification. This is an option provided by the F-Search library (Frontier Labs, Koriyama, Fukushima, Japan). This approach, however, is not possible for complex mixtures and can be complicated by varying additive components between reference and sample polymers.

Polymer identification is simplest for a discrete piece of plastic but increases in complexity for mixtures. For individual particles, an advantage over spectroscopic methods is that copolymers can be identified more easily. However, the small sample size for pyrolysis could lead to only characterizing one component of a multilayer composite (a similar pitfall of surface-only spectroscopy). Taking multiple samples within a plastic item (e.g., outer and inner core) or carefully sampling across the entire composite can remedy this. In environmental samples with a mixture of polymers, manual inspection of the total pyrogram for a set of pyrolyzates is necessary to confirm polymer presence, even though only one marker compound may be used for quantification. For example, the presence of PE should be validated by confirming the presence of at least five of these homologous series of triplets in the C_7–C_{41} range, even though one or two compounds may be selected for quantification [29].

Quantifying polymer mass–calibrant preparation

A unique capability of Py–GC/MS, in comparison to spectroscopic techniques, is quantifying the mass of a polymer.

The quantification approach is based on external calibration curves of reference polymers using pyrolysis indicator compound(s). An indicator ion of the marker compound is extracted, and the peak area of that ion is used for calibration. This approach is akin to calibration curves for GC/MS quantification of organic compounds, including environmental pollutants. Unlike a single-compound analyte, however, it is best practice to confirm the presence of a polymer in a sample by identifying multiple marker compounds of that polymer. For example, the presence of PE should be validated by confirming the presence of at least five of these homologous series of triplets in the C_7–C_{41} range, even though one or two compounds may be selected for quantification [29].

An important assumption behind quantifying polymer content with Py–GC/MS is that the marker compound yield is consistent between plastic varieties of the same bulk polymer type. While this is typically an acceptable assumption, recent findings have illustrated that tires are highly variable in SBR and BR content, meaning quantification of tire wear particle concentrations using a marker of SBR or BR may be inconsistent [61]. In some cases, multiple marker compound peak(s) may be integrated for improved tire wear quantification [60], a concept that can be applied to other plastics/markers.

External calibration curves can be created by weighing particles of a reference polymer for pyrolysis [16, 19–22, 24, 26, 30, 36]. Using this approach, linear calibration curves with coefficients of determination (i.e., R^2) generally > 0.9, and limits of quantification as low as 0.3 µg per injection, have been obtained [16, 19]. This is constrained by the minimum weight limit and errors of the analytical balance, which often does not reach the limit of detection (based on peak signal-to-noise ratio parameters) and is a time-consuming process [19]. The upper calibration limit is constrained by the mass limit for the instrument (i.e., avoiding overloading the column or detector). To overcome these constraints, some have weighed polymers in an inert solid matrix and subsampled, achieving lower limits of detection [17, 32, 34, 50]. However, the heterogeneity of microplastics within a matrix may cause inaccuracies [19]. Similarly, Funck et al. [14] dispersed PS and PE microplastic standards in ethanol, achieving lower limits of quantification and detection for PS, which is soluble therein.

Indeed, dissolving calibrant standards in solvent is also widely used. Fisher and Scholz-Böttcher [16, 19] dissolved PS in dichloromethane (DCM) to lower the LOD an order of magnitude, from 0.3 to 0.03 µg, while continuing to weigh other calibrant polymers. Other common polymers are poorly soluble at room temperature. Accordingly, pressurized liquid extraction may be used to increase solubility. Okoffo et al. [29], Ribeiro et al. [27], and Leslie et al. [28] used pressurized fluid extraction (PFE) to dissolve polymer calibrants in DCM. This facilitated calibration curves for PE, PMMA, PS, PET, PC, polypropylene (PP), and polyvinyl chloride (PVC) by Okoffo et al.; Leslie et al. did not report using PC or PVC; PC was not tested by Ribeiro, and PET was not used as recoveries were too low (mean mass recovery: 32%). Hermabessiere and Rochman [25] reported that microwave-assisted extraction (MAE) in DCM facilitated extraction of PE, PP, PS, PMMA, PVC, and PC with gravimetric calibrant recoveries ranging from 93 to 120%, while PET was insoluble. Indeed, reporting calibrant recovery (i.e., weight of solid polymer retained in final solution) is recommended, as this varies between solvents. Krauskopf et al. [36] used tetrahydrofuran to dissolve PP, PS, and PVC for analysis, but weighed PE and PET calibrants citing these were insoluble at room temperature. However, they do not report the degree of solubility (i.e., a polymer calibrant percent recovery in solution). Steinmetz et al. [15] dissolved PE, PP, and PS in 1,2,4-trichlorobenzene (TCB), heated to 120 °C to facilitate dissolution. They reported that the plastics formed a solution phase that could be dispersed upon mixing. Efforts to use tailored solvents for different polymers for calibration have been carried out [32, 34]. For example, Matsueda et al. [34] used a 1:1 DCM:tetrahydrofuran (THF) mixture for PS, PVC, PMMA, acrylonitrile butadiene styrene (ABS), PC, and PUR, but hexafluoroisopropanol (HFIP) for nylon-6, nylon-6,6, and PET, while PE and PP were retained in solid suspension with deactivated silica. Once a polymer calibrant or standard has been brought into solution, in most cases, the extracted calibrants are diluted to create calibrant curves [15, 27–29, 34, 36]. In other cases, the calibrants may be concentrated, for example, under an inert gas stream such as N_2 [25].

When using solvent-suspended polymer standards, solution stability is an important consideration. Some authors note that analysis should take place within a 3-h window post-extraction, so that the polymer does not precipitate [27, 29]. Alternatively, inter- and intra-day variability can be tested. Hermabessiere and Rochman [25] documented these values for PE, PP, PS, PMMA, PVC and PC following microwave extraction, reporting relative standard deviations of marker peak areas among sample runs (five replicates in one day, or runs over five consecutive days). The inter-day and intra-day relative standard deviations of selected markers ranged from 9.5% to 23.6% and 12.4% to 21.1%, respectively [25]. Other groups reported similar inter- and intra-day variabilities, generally under 20% and higher for inter-day than intra-day, when both are reported [14, 15, 23, 27, 29, 55]. These variabilities should be reported and used to validate marker choice when calibrants are generated in solution. For example, Hermabessiere and Rochman [25] found that the inter and intra-day variability for bisphenol A as a marker for PC were 42% and 81.7%, respectively, leading these authors to use a different marker pyrolyzate

for quantification. This variability could be attributed to the precarious suspension of polymers in solvent, meaning calibrants of solid polymers would produce more reliable calibrations. In general, the calibrant preparation approach should be decided based on the sample type, recognizing the tradeoffs between variability involved in solvent dissolution compared to sensitivity. A summary of the limits of quantification reported in literature for different polymers/marker compounds is provided (Table 1).

An additional calibrant preparation consideration is whether to run polymer standards individually or in combination. When calibrants are weighed, the typical approach is to run calibrants individually, to obtain the lower and higher limits of calibration for each polymer while staying within instrumental loading recommendations [19]. This approach is also common for solvent-dissolved polymer standards, as the volume of the sample container (e.g., 80 µL) can be limiting. (Note: solvents are generally evaporated in a controlled manner prior to loading.) While individual calibration curves expand the calibration range, a drawback is that changes in relative signal intensity resulting from polymer interactions are not captured. Matsueda et al. [34] explored this with their solvent and inert solid matrix of 11 mixed polymer standards. They hypothesized that polymer interactions caused PE and PP to fit a quadradic calibration curve (as opposed to linear). In addition, they suggest that pyrolyzates of PUR and PET interacted, reducing the calibration quality (partially a caveat of secondary reactions between the PUR pyrolyzate, 4,4′-diphenylmethane diisocyanate, with the deactivated silica matrix used to dilute PE and PP, an issue that is unique to their sample preparations). Similarly, Steinmetz et al. [15] investigated the suitability of marker compounds based on potential interferences and found that PP may be overestimated (using 2,4-dimethyl-1-heptene as marker) when PE is present, but that interference among all polymers tested (PE, PP, PS) was generally under 10%. As such, individual calibration curves for polymers may result in inaccuracies in final calibration of samples with complex combinations of polymers. In reality, however, not all polymer interactions would be relevant for any given sample.

Regardless of sample preparation approach, careful consideration should be made on how often calibration is essential. A combination of charring, secondary reactions, and condensation of pyrolyzates can lead to residual organic material within the pyrolysis chamber [19]. Ghost peaks confounding subsequent analyses may be observed as a consequence [33]. In this case, running calibrant curves for each sample batch (e.g., group of < 20 samples) can improve calibration [19], as can running blanks (instrumental: no sample container or cup; procedural: empty sample cup) periodically [29, 32]. Notably, routine maintenance operations and reactive internal surfaces can also cause secondary reactions, resulting in calibrant variability [19].

Quantifying polymer type—internal standards

The use of internal standards can help to overcome many of the challenges associated with calibrant/sample preparation and instrument variability. Fischer and Scholz-Böttcher [19] reported improved calibration using a combination of internal standards: of 9-dodecyl-1,2,3,4,5,6,7,8-octahydro anthracene, anthracene-d^{10}, androstane, and cholanic acid methyl ester (each 0.02 mg mL^{-1} in n-hexane), collectively termed $ISTD_{PY}$. These represent aliphatic, planar aromatic, and nonpolar aromatic compounds, respectively, as analogs of the marker compounds and not parent polymers. In some cases, 9-tetradecyl-1,2,3,4,5,6,7,8-octahydro anthracene was added to mimic methylation of acid groups during thermochemolysis. These authors determined the best internal standard for each polymer based on calibration curve fit, finding that standard deviation reduced from 100 to 6 µg with internal standards. Others have successfully used this $ISTD_{PY}$ solution [20–22], or just anthracene [50]. Deuterated PS (dPS; d5 or d8) can also be used as an internal standard, often in solution [14, 25], but solid powder is also available [23]. The importance of internal standards was highlighted in a 2013 study of tire wear particles, in which deuterated polyisoprene (d8), polybutadiene (d6), and dPS (d8) were used [17]. Nonetheless, not all Py–GC/MS studies use internal standards.

Advancing internal standards for plastic analysis is limited by the commercial availability of isotopically labeled plastics. While dPS is widely available, it is often in suspension and thus not analogous to microplastics. In addition, there is increased potential for hydrogen–deuterium ion exchange during pyrolysis, meaning the marker ions will not reliably identify the portion of dPS [62]. Lauschke et al. [62] illustrated that this H–D exchange reaction is heavily dependent upon the inorganic matrix and is less reliable when substrates such as aluminum oxide filters are used. Unfortunately, ^{13}C-labeled polymers that would be better suited for these analyses have limited availability and are costly. To overcome this, Lauschke et al. [62] demonstrated that poly(4-fluorostyrene) (PRS) may be a better internal standard than dPS for PS, PP, and PE. Further work is needed to determine the best internal standards for plastic Py–GC/MS analysis. Ideally, however, these should be particles of similar size as calibration standards or plastics expected in the sample, and samples should be processed through all extraction/purification procedures with the internal standard present. This can help capture variability created by sample processing and matrix interferences. Additionally, multiple internal standards of different plastics (e.g., amorphous and semi-crystalline polymers) are necessary.

Table 1 Indicator compound and ions used for the analysis of different polymers with Py–GC/MS. The limits of quantitation (LOQ) are provided for each reference, categorized by calibrant preparation as a solid or in solution

Polymer	Indicator compound	Ion(s)	LOQ solid (μg)	LOQ in solution (μg)
PE	Alkane(s)	57, 71, 85, 99	1.0 [18], 4.0 [16], 2.3 [25]	0.02 [27][a], 12.0 [36]
	Alkene(s)	56, 70, 83, 97	0.7 [24][a]	1.0 [18], 3.2 [25], 1.8 [26], 0.02 [27][a], 9.8 [36], 0.02 [28], 0.03 [29], 0.02 [27][a]
	Alkadiene(s)	55, 67, 81, 82, 95	0.44 [55], 0.5 [19], 0.5 [20], 0.7 [22], 9.2 [21], 4.0 [16], 3.6 [32][c], 32.0 [34][c], 0.13 [39]	1.0–1.2 [14], 62.0 [36]
PP	2,4 Dimethyl-1-heptene	70, 83, 126	0.03 [39][c], 0.85 [55], 8.0 [34][c], 0.77 [32][c], 0.3 [19, 20], 0.6 [20], 0.8 [21, 22], 0.9 [18]	2.3 [28], 0.02 [27][a], 0.03 [29], 1.2 [25]
	Tetramethylundecene isomers	69	0.5 [24][a], 1.9 [26]	1.4 [36], 3.0 [36], 1.7 [36]
PS	Styrene	104, 78	1.2 [26], 1.5 [35], 0.005 [50]	0.001 [14], 1.1 [28]
	Styrene dimer	91, 130, 208	0.1 [24][a], 1.2 [26]	0.02 [27][a], 0.03 [14], 0.35 [55], 0.9 [36]
	Styrene trimer	91, 207, 312	0.005 [50], 0.2 [55], 0.8 [19, 20, 34], 0.9 [21, 22], 1.2 [16], 0.385 [39][c]	0.01 [29], 0.03 [14], 0.282 [19], 0.53 [32], 1.2 [25], 2.4 [36]
PVC	Methylnaphthalene	142	0.5 [24][a], 2.4 [26]	5.7–6.2 [36]
	Benzene	178	0.3 [19], 0.7 [21], 0.8 [22], 2.9 [18], 3.0 [16]	0.02 [27][a], 0.03 [29], 0.3 [20]
	Fluorene	165		13.0 [36]
	Indene	115		2.3 [25]
	Naphthalene	128		0.54 [32], 1.1 [36], 4.0 [34]
	Phenanthrene	178		5.5 [36]
PET	Dimethyl terephthalate[b]	163, 194	0.8 [18], 3.5 [26], 0.5 [24][a], 1.3 [36], 0.6 [19, 20], 0.7 [22], 0.9 [21], 5.0 [16], 0.025 [39][c]	0.43 [28]
	Benzoic acid	122		1.6 [34]
	Benzophenone	182		1.1 [32]
	Ethyl or vinyl benzoate[b]	105	25.0 [36]	0.03 [29]
PC	Dimethyl bisphenol A	241	0.5 [16], 0.9 [19–22]	
	4-Isopropenylphenol	134	0.1 [32]	5.8 [25]
	Bisphenol A	213, 288	0.2 [34]	0.03 [29]
	p-Methoxy-tert-butylbenzene[b]	149	3.2 [26]	
	2,2-Bis(4′methoxyphenyl) propane[b]		0.027 [39][c]	
PA	ε-Caprolactam	113, 84/85	0.5 [19, 20], 1.0 [21, 22], 9.0 [16]	0.1 [32], 0.28 [34]
	N-Methyl caprolactum[b]	127, 70	0.5 [19, 20], 1 [21, 22], 9.0 [16]	
	1,8-Diazacyclotetradecane-2,7-dione	226	1.2 [21]	
	Cyclopentanone	84	1.3 [26]	0.57 [32], 1.8 [34]
	Hexane	84	0.5 [24][a]	
PU	4,4′-Diphenylmethane diisocyanate (MDI)	250	3.0 [34]	
	4,4′-Methylenbis(N,N-dimethylaniline)	254	0.9 [22], 1.2 [21], 1.4 [19, 20]	
	4,4′-Methylenedianiline	198		1.1 [32]
SBR &	Vinylcyclohexene (butadiene dimer)	54, 108	0.1 [17][a]	0.5 [32]
BR	Styrene	103, 78	0.13 [17][a]	
	Butadiene	39, 54	0.65 [17][a]	
	Benzene	78	1.0 [60]	
	α-Methylstyrene	118	5.0 [60]	
	Ethylstyrene	117	5.0 [60]	
	Butadiene trimer	91	5.0 [60]	

Table 1 (continued)

Polymer	Indicator compound	Ion(s)	LOQ solid (μg)	LOQ in solution (μg)
NR	Dipentene	68, 136	0.03 [17][a]	
	Isoprene	39, 68	0.04 [17][a]	
PMMA	Methyl methacrylate	69, 100	0.4 [16], 0.5 [24][a], 0.8 [19–22], 3.3 [26], 0.035 [39][c]	0.02 [27][a], 0.09 [29], 0.26 [32], 0.33 [28], 0.8 [34], 1.6 [25]
ABS	2-Phenethyl-4-phenylpent	170		0.42 [32], 16 [34]

[a]Limit of detection; limit of quantitation not reported

[b]Improved with thermochemolysis

[c]Polymer diluted in solid matrix (e.g., glass fiber, deactivated silica, etc.)

Sample preparation

Py–GC/MS is often known for requiring minimal sample preparation. While this is true for discrete pieces of plastics, from which an aliquot can be sliced and directly pyrolyzed [42, 43, 52, 53], most environmental samples require preparation. Sample preparation for Py–GC/MS is necessary to (1) concentrate the plastics within a sample for analysis and (2) minimize interference of the sample matrix.

Isolation of microplastics

Often, sample preparation for Py–GC/MS is similar to preparation for spectroscopic techniques. This includes physical separation of plastics from the matrix (e.g., density separation) and isolation from the matrix (e.g., chemical or enzymatic digestion; Fig. 1) [6]. In many workflows, samples will be concentrated on a filter which may be folded, cut, or crushed to fit into the pyrolysis chamber [15, 17, 20–22, 24, 26, 28, 39]. For example, Albignac et al. [39] digested marine benthic organisms in a potassium hydroxide solution, coarse filtered to remove any undigested material (> 500 μm), and concentrated the microplastics on a 20-μm filter, which was cryoground and aliquoted for Py–GC/MS. This split ratio can be lowered to overcome low analyte concentration.

Due to the minimal sample-processing requirements, unique approaches to Py–GC/MS analysis are also possible. Nakano et al. [50] quantified the PS concentration in individual daphnia fed PS microplastics in experimental conditions, demonstrating the possibility for Py–GC/MS to analyze discrete organismal samples [50]. In general, this split ratio can often be lowered to overcome low analyte concentration, but in the case of environmental or animal samples, this may overload the detector with matrix material.

Isolation of nanoplastics

Unlike spectroscopic approaches, there is no lower size limit of detection for Py–GC/MS, making it well poised to quantify nanoplastics [63–65]. For nanoplastics, the limiting factor is sample preparation to extract and concentrate nanoplastics above the instrument LOD. In one of the first publications of nanoplastics in oceanic waters, ter Halle et al. [66] concentrated the samples with ultrafiltration and analyzed this colloidal fraction with Py–GC/MS. In this study, the authors determined the relative abundance of the three most abundant polymers using chemometric principal component analysis of aromatic pyrolyzates but did not quantify polymer content by weight. They also used dynamic light scattering to confirm the presence of nanoparticulate prior to Py–GC/MS analysis [66]. Similarly, Mintenig et al. [64] proposed using crossflow-ultrafiltration to concentrate small micro- and nanoplastics, followed by asymmetrical field-flow fractionation (AF4) to size the particles. They demonstrated that Py–GC/MS is a viable technique for analysis of polymer type and concentration, with a lower limit of detection of 100 ng for PS nanoplastics [64]. This AF4 fractionation approach has been used to isolate nanoplastics in soil and identify them with Py–GC/MS [65].

Other mechanisms to concentrate nanoplastics for Py–GC/MS have been proposed. Zhou et al. [67] used cloud point extraction with a TritonX-45 (TX-45) surfactant to concentrate nanoplastics in water. They demonstrate a workflow in which micelles surrounding the nanoplastics are created and concentrated via centrifugation. To reduce the TX-45 interference, the surfactant was thermally desorbed at 190 °C prior to Py–GC/MS analysis. Theoretically, this could be accomplished with a double-shot Py–GC/MS. These authors successfully quantified PMMA- and PS-spiked nanoplastics in riverine water samples using this technique, but the nanoplastics in the environmental water samples were below the LOD. Zhou et al. [67] adapted a similar centrifugation approach to analyze nanoplastics in biota. Animal tissue was digested with TMAH and ethanol, filtered to exclude microplastics (> 1 μm) and centrifuged to create a pellet of nanoplastics and residual protein for Py–GC/MS analysis. This resulted in recoveries of ~ 80–90% of spiked PS and PMMA nanoplastics; PS nanoplastics were detected in tissue samples ranging from 0.8 to 2.7 μg g^{-1}, but

no PMMA was found. Sullivan et al. [68] proposed a technique to semi-quantify nanoplastics that were retained on 0.45-µm and 0.1-µm polytetrafluoroethylene (PTFE) filters. These authors used a slow temperature ramp up to 500 °C, followed by GC–TOF MS to increase detection capabilities. A laser cutter was used to subsample portions of the PTFE filter with cryomilled polymer standards or environmental samples. They demonstrated that the PS and PVC signals were above filter background, with relative standard deviation below 20% when using an internal standard. They provide an example of a river sample containing 241.8 mg L^{-1} PS nanoplastics [68].

A limitation on nanoplastics analysis is the availability of reference materials. While PS, PET, and PMMA spheres of nominal nanoplastic sizes are commercially available, this does not represent the variety of polymers or complexity of shapes/sizes in the environment [63]. As such, some laboratories have generated nanoplastics for experiments in the laboratory [69–71]. Although researchers may reach limits of detection low enough for nanoplastics by diluting standards of larger plastics (e.g., solvent-extracted microplastics), sample processing should be consistent to avoid dissimilarities in pyrolysis behavior. Moreover, methods to extract and concentrate nanoplastics require standards to test recovery; consequently, methodologies may be biased toward the polymers/shapes of nanoplastic standards and against environmentally relevant nanoplastics. Moving forward, the availability of nanoplastic standards of different polymers, shapes, and sizes, and/or techniques to generate nanoplastic reference materials (e.g., cryomilling) would improve measurements.

Solvent extraction

The same solvent extraction approaches used for calibrants can be used for environmental samples, but their analysis approach varies [15, 25, 27, 29, 36]. Okoffo et al. [29] directly aliquoted solvent-extracted biosolid samples and evaporated the solvent in the sample container, without pre-concentrating the extracted samples (biosolids were presumed homogeneous via pre-extraction freeze-drying, milling, and shaking). Ribeiro et al. [27] used the same pressurized extraction approach as Okoffo et al. [29], but pre-concentrated microplastics from seafood samples with matrix digestion before solvent extraction. Steinmetz et al. [15] applied an aliquot of solvent-extracted soil samples to a small filter for pyrolysis, without evaporating the solvent. Hermabessiere and Rochman [25] concentrated both sample and standard solvent extracts under N_2. In some cases, only calibrants or samples, not both, have been prepared with solvent extraction. For example, Leslie et al. [28] concentrated the plastics on a filter for pyrolysis but extracted calibrants in DCM. Dierkes et al. [23] diluted calibrants for analysis in a solid matrix (silica), but solvent-extracted samples which were subsequently concentrated to dryness on silica, ground for homogenization, and subsampled for pyrolysis. Importantly, these authors noted that during concentration any polymers that suspended on vial walls were rinsed with DCM onto the silica gel [23]. As discussed with calibrants, the propensity for polymers to be resuspended in solvents is an important consideration, especially if samples are concentrated post-extraction.

Matrix interferences

A major consideration in the selection of indicator compounds is specificity to the polymer of interest. While some pyrolyzates are highly specific to a polymer type, others are common pyrolyzates of natural organic matter. For example, alkanes are a common pyrolyzate of fats and petroleum hydrocarbons, among others. Likewise, styrene (a PS marker) and benzene (a PVC, BR, and SBR marker) may condense during pyrolysis of aliphatic hydrocarbons (e.g., Diels–Alder reaction) [68]. This is why a styrene trimer is recommended over styrene as a PS marker, despite the relatively higher signal of styrene. Alternatively, the ratio of styrene to toluene can be used to confirm the presence of PS versus styrene monomer from natural organic matter [72].

It is recommended to test the interference of relevant organic matrices for proposed indicator compounds [15, 23, 29]. Dierkes et al. [23] found little interference from wood, leaf litter, humic acids, fir needles, fish filet, crayfish, engine oil, or filter paper for PS and PP, but that several matrix materials interfered with detection of PE (most specifically engine oil); however, sample pretreatment (methanol pre-extraction, THF solvent extraction) adequately reduced interference [23]. Likewise, Steinmetz et al. [15] tested their extraction procedure for removing interferences from soils. Okoffo et al. [29] tested the interference of fish filet, fir needles, humic acids, prawns, wood, engine oil, leaf litter, filter paper, and rice, and found that most polymers of interest (PE, PP, PS, PVC, PET, PC, PMMA) were not confounded by the matrix presence, notably due to the double-shot pyrolysis approach which could presumably devolve more volatile natural organic material in the thermal desorption step. They still reported interference from several materials for PE, however [29].

It is important to note that matrices can also interfere with the pyrolysis behavior of analytes. An extensive organic matrix can lead to different pyrolysis rates/products, cause ghost peaks, or create a variety of non-volatile products confounding polymer identification. Inclusion of an internal standard to mimic the pyrolysis reactions of the target analyte can directly help with this issue. Further, the composition of some non-petroleum-based plastics may generate pyrolyzates similar to natural organic

matter. For example, Käppler et al. [18] found it difficult to identify cellulose-based fibers using Py–GC/MS, as the signals were similar to those of natural plant matter. With these considerations in mind, reducing matrix is recommended wherever possible. Further, even when an inert matrix (e.g., glass fiber filter) is used for samples, the same material should be included for calibration standards so that any potential differences in pyrolysis behaviors with/without matrix are captured.

Py–GC/MS vs. other techniques

Other thermoanalytical approaches

Py–GC/MS is just one of several thermoanalytical techniques used for the characterization of organic matter. The simplest of these is thermogravimetry (TGA), which tracks the weight differential of a sample over a heating program. TGA alone provides no chemical information; therefore, it is often combined with chemical analysis via methods such as MS, GC/MS, or FT-IR [8]. TGA on solid-phase adsorbers followed by (TD)–GC/MS combined is TED–GC/MS. This has been used for the analysis of plastics in environmental matrices, including tire wear particles [73, 74]. Another thermoanalytical method that is used for the analysis of plastics is DSC, which measures phase transitions [44, 46, 75]. In TED–GC/MS and DSC, the sample size can be one to two orders of magnitude greater than that in Py–GC/MS (1 to 10 mg versus 0.1 mg). However, condensation of the evolved gas in transfer lines may reduce transfer efficiencies to detectors [8, 44].

Analytical spectroscopy

Spectroscopic techniques such as FT-IR and Raman are less complimentary to Py–GC/MS, but widely used for the analysis of plastics. While Py–GC/MS measures the mass, FT-IR and Raman use vibrational chemistry to identify the polymeric composition of individual particles, which may be counted. A variety of the differences and similarities between these approaches are summarized in Fig. 5.

Three studies have compared datasets provided on the same sample from spectroscopic approaches and Py–GC/MS. In 2018, Käppler et al. provided a comparison between µ-attenuated total reflectance (ATR)-FT-IR and Py–GC/MS of 27 individual particles [18]. They found that both approaches were able to distinguish between plastic and non-plastic particles and identify polyolefins correctly. They highlighted the ability of Py–GC/MS to correctly identify additives in a PVC polymer, for which the polymer identification via FT-IR was confounded by additives. They also show that natural polymers as well as paint particles were more easily identified by FT-IR than Py–GC/MS; however, thermochemolysis with TMAH improved paint particle identification using Py–GC/MS. In addition, a sample was identified as ethylene vinyl–acetate by FT-IR but as PE by Py–GC/MS. The reference libraries available for spectroscopic data of environmental plastics are larger and more widely accessible [76] than libraries for Py–GC/MS, possibly biasing this result. Primpke et al. [22] undertook a similar comparison for a complex sample containing multiple particles on a filter, finding relatively similar polymer compositions between techniques. Firstly, the authors recognized that the PS pyrolyzate styrene could also derive from styrene acrylate used in paints, which would be considered a paint particle via

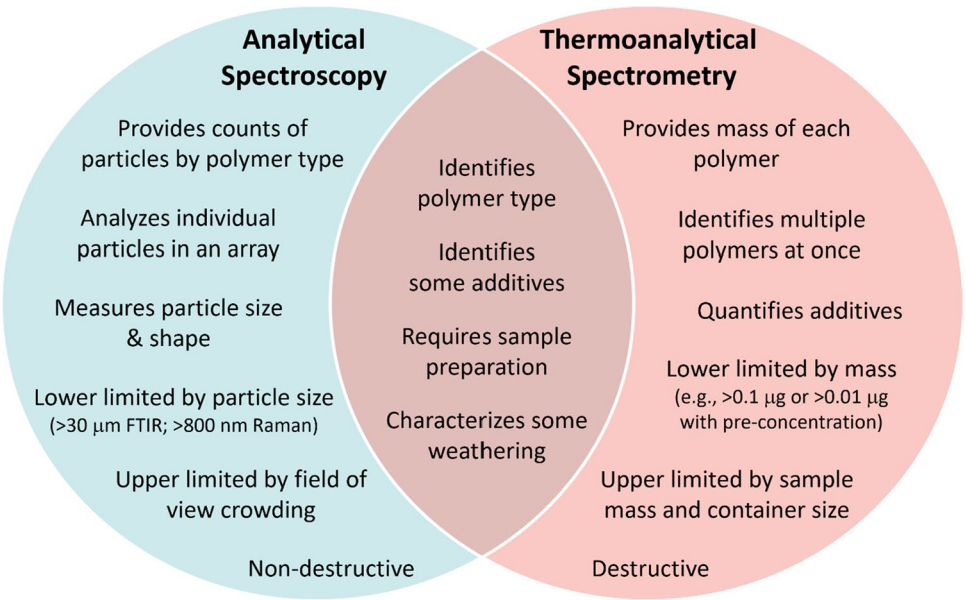


Fig. 5 Similarities and differences between common analytical spectroscopy techniques (i.e., FT-IR and Raman) and thermoanalytical spectrometry (e.g., Py–GC/MS) for the analysis of plastic in the environment

FT-IR. Likewise, PS is likely a common co-polymer in samples, leading to its underestimation in FT-IR. These authors also used polymer density and particle size to estimate the mass of plastics identified by FT-IR. While these data were similar to mass estimates derived from Py–GC/MS, they were biased from the presence of a small number of large particles [22]. In an interlaboratory study, authors report that μRaman and Py–GC/MS were most accurate for polymer identification, but they did not attempt to normalize quantification (count vs. mass) for comparison [77].

Interestingly, several Py–GC/MS studies report larger estimates of PVC content than other techniques [22, 43, 78]. Primpke et al. [22] suggested that matrix material such as plant matter may also contribute benzene pyrolyzates, leading to the overestimation. However, in studies that investigate matrix interference in a variety of natural matrices, including plant matter, there were no observed interferences for PVC quantification. In fact, one group observed matrix interference may lower the PVC pyrolyzate signal [72]. It is possible that secondary reactions of multiple polymers in a sample can lead to benzene, or perhaps PVC is underestimated spectroscopically due to high additive (i.e., phthalate) content. Alternatively, Hendrickson et al. [43] found that chlorinated PE was identified as PVC via Py–GC/MS and PE via FT-IR. This bias of Py–GC/MS toward PVC has otherwise not been addressed in the literature. As such, the question remains—is the environment truly more polluted with PVC micro/nanoplastics than we realize, or is there an analytical bias created by Py–GC/MS?

Conclusions

In the context of this review, future opportunities to improve or expand upon Py–GC/MS analyses of plastics emerge. Suggested best practices include:

1. *Sample and calibrant preparation procedures should be identical.* Due to the complex pyrolytic nature of plastics and organic matter, secondary reactions that can magnify or reduce the production of a given pyrolyzate are possible. As such, samples and calibrants should be prepared similarly. If a sample is suspected to contain multiple polymers, calibrants of those polymers should be mixed. Similarly, if a sample is collected and analyzed on a filter, the same filter type should be used for calibrants.
2. *Size-sort samples prior to analysis.* A limitation of Py–GC/MS is the inability to quantify particle size. Toward rectifying this gap, sequential sieving or filtering of samples prior to Py–GC/MS could help determine the mass of polymers in a given particle size range. This, however, would not inform on particle form (i.e., fragment, fiber, etc.).
3. *Use internal standards, preferably solid, carbon-labeled polymers, for quantification.* Studies have demonstrated that internal standards improve Py–GC/MS polymer quantification. The most common approaches are a mix of deuterated organic compounds, anthracene [19–22, 50], or solvent-suspended dPS [14, 17, 23, 25]. While these improve quantification, Lauschke et al. [62] demonstrated that deuterated standards are subject to hydrogen–deuterium ion exchange in pyrolysis when some matrices are present [62]. Although expensive, carbon-labeled polymers of a similar state, size, and shape as calibrants/samples are ideal.
4. *Use thermal desorption to identify or quantify additives.* While some of the earliest work with Py–GC/MS demonstrated additive identification [52], little work since has been done to simultaneously quantify additives and polymers [55], or even identify additives within a sample. As mass spectral libraries of plastic additives expand, further work should use Py–GC/MS to better characterize additives in plastics and environmental samples, especially considering that additives may be an important component of toxicity [5].
5. *Improve our understanding of the sensitivity of Py–GC/MS in scan versus selected ion monitoring (SIM) modes.* While Py–GC/MS is destructive, efforts to preserve the data for later analyses are possible when samples are processed in full-scan mode with a wide ion range. This can facilitate future data mining as GC/MS reference libraries expand. The benefits of selected ion monitoring cannot be ignored, however; these include (a) improving calibration and lowering limits of detection, particularly for nanoplastics, and (b) increasing detection of low-concentration plastic additives [79]. When possible, duplicating sample analysis in scan and SIM modes could be considered a cautionary approach, as our understanding of pyrolyzate compound sensitivity increases.
6. *Facilitate Py–GC/MS data sharing between users to improve sample characterization.* By comparing data between labs working with Py–GC/MS, as has been developed for spectroscopic techniques [76], identification of both complex polymers and additives, as well as weathering patterns, could be improved.
7. *Characterize extent of plastic weathering.* A major data gap in plastics fate models is environmental residence time, which may be demystified by quantifying weathering severity. Research has illustrated that Py–GC/MS can detect polymeric changes due to photooxidation [46]. Likewise, the relative photooxidation of polluted petroleum has been characterized with Py–GC/MS [58]. Future investigations may expand this realm of research to help fill the "age" gap of polluted plastics.
8. *Continue and improve quality assurance and quality control (QA/QC) measures.* Recently, the plastic pollution field has made great strides in refining QA/QC protocols

[80, 81]. Best practices include limiting contamination by plastics (particularly from clothing, supplies/instrumentation, and dust) in field and laboratory processes, as well as conducting field and laboratory blanks to characterize unavoidable contamination. Consistent with previous discussion, additional QA/QC requirements for Py–GC/MS should include furnace and inlet cleaning, as well as running calibrants for each sample batch. In addition, spike recovery experiments are recommended for all environmental matrices analyzed. This is particularly important as calibrant and sample preparation techniques evolve for Py–GC/MS. Strategic use of existing reference materials (such as NIST polystyrene nanosphere Standard Reference Materials (SRMs 1691 and 1964)) are invaluable in QA/QC, particularly for nanoplastics [67]. The field would benefit from development of environmental matrix reference materials that are certified for masses of micro- and nanoplastics, and additives therein.

In conclusion, Py–GC/MS is an advantageous technique for the analysis of plastics. This review highlights the approaches, benefits, and caveats of Py–GC/MS for the analysis of plastics, pointing toward the potential benefits of Py–GC/MS for future use.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00216-023-04671-1.

Acknowledgements The authors appreciate manuscript revision provided by Drs. John Kucklick, Katherine Shaw, Robert C. Hale, and Carlos Gonzalez. Graphical abstract created with biorender.com.

Author contribution MES: conceptualization, investigation, data curation, writing—original draft, writing—review and editing, and visualization; JML: conceptualization, writing—review and editing, and visualization.

Funding Funding for Dr. Seeley is provided by the National Research Council through the Research Associateship Program.

Declarations

Competing interests The authors declare no competing interests.

NIST disclaimer Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified is necessarily the best available for the purpose.

References

1. Hartmann NB, Hüffer T, Thompson RC, Hassellöv M, Verschoor A, Daugaard AE, Rist S, Karlsson T, Brennholt N, Cole M, Herrling MP, Hess MC, Ivleva NP, Lusher AL, Wagner M. Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. Environ Sci Technol. 2019;53:1039–47. https://doi.org/10.1021/acs.est.8b05297.
2. Arthur C, Baker J, Bamford H (2008) Proceedings of the international research workshop on the occurrence, effects and fate of microplastic marine debris. NOAA Technical Memorandum NOS-OR&R-30. https://repository.library.noaa.gov/view/noaa/2509/noaa_2509_DS1.pdf
3. Rochman CM, Brookson C, Bikker J, Djuric N, Earn A, Bucci K, Athey S, Huntington A, McIlwraith H, Munno K, De Frond H, Kolomijeca A, Erdle L, Grbic J, Bayoumi M, Borrelle SB, Wu T, Santoro S, Werbowski LM, Zhu X, Giles RK, Hamilton BM, Thaysen C, Kaura A, Klasios N, Ead L, Kim J, Sherlock C, Ho A, Hung C. Rethinking microplastics as a diverse contaminant suite. Environ Toxicol Chem. 2019;38:703–11. https://doi.org/10.1002/etc.4371.
4. Bucci K, Rochman CM. Microplastics: a multidimensional contaminant requires a multidimensional framework for assessing risk. Micropl&Nanopl. 2022;2:7. https://doi.org/10.1186/s43591-022-00028-0.
5. Hale RC, Seeley ME, La Guardia MJ, Mai L, Zeng EY. A global perspective on microplastics. J Geophys Res Oceans. 2020;125:e2018JC014719. https://doi.org/10.1029/2018JC014719.
6. Lusher AL, Munno K, Hermabessiere L, Carr S. Isolation and extraction of microplastics from environmental samples: an evaluation of practical approaches and recommendations for further harmonization. Appl Spectrosc. 2020;74:1049–65. https://doi.org/10.1177/0003702820938993.
7. Gouin T, Becker RA, Collot A, Davis JW, Howard B, Inawaka K, Lampi M, Ramon BS, Shi J, Hopp PW. Toward the development and application of an environmental risk assessment framework for microplastic. Environ Toxic Chem. 2019;38:2087–100. https://doi.org/10.1002/etc.4529.
8. Peñalver R, Arroyo-Manzanares N, López-García I, Hernández-Córdoba M. An overview of microplastics characterization by thermal analysis. Chemosphere. 2020;242:125170. https://doi.org/10.1016/j.chemosphere.2019.125170.
9. Primpke S, Christiansen SH, Cowger W, De Frond H, Deshpande A, Fischer M, Holland EB, Meyns M, O'Donnell BA, Ossmann BE, Pittroff M, Sarau G, Scholz-Böttcher BM, Wiggin KJ. Critical assessment of analytical methods for the harmonized and cost-efficient analysis of microplastics. Appl Spectrosc. 2020;74:1012–47. https://doi.org/10.1177/0003702820921465.
10. Rochman CM, Regan F, Thompson RC. On the harmonization of methods for measuring the occurrence, fate and effects of microplastics. Anal Methods. 2017;9:1324–5. https://doi.org/10.1039/C7AY90014G.
11. Provencher JF, Covernton GA, Moore RC, Horn DA, Conkle JL, Lusher AL. Proceed with caution: the need to raise the publication bar for microplastics research. Sci Total Environ. 2020;748:141426. https://doi.org/10.1016/j.scitotenv.2020.141426.
12. Dehaut A, Hermabessiere L, Duflos G. Microplastics Detection using pyrolysis-GC/MS-based methods. In: Rocha-Santos T, Costa M, Mouneyrac C, editors. Handbook of microplastics in the environment. Cham: Springer International Publishing; 2020. p. 1–35.
13. Picó Y, Barceló D. Pyrolysis gas chromatography-mass spectrometry in environmental analysis: focus on organic matter and microplastics. TrAC Trends Anal Chem. 2020;130:115964. https://doi.org/10.1016/j.trac.2020.115964.
14. Funck M, Yildirim A, Nickel C, Schram J, Schmidt TC, Tuerk J. Identification of microplastics in wastewater after cascade filtration using pyrolysis-GC–MS. MethodsX. 2020;7:100778. https://doi.org/10.1016/j.mex.2019.100778.
15. Steinmetz Z, Kintzi A, Muñoz K, Schaumann GE. A simple method for the selective quantification of polyethylene, polypropylene, and polystyrene plastic debris in soil by pyrolysis-gas chromatography/mass spectrometry. J Anal Appl Pyrolysis. 2020;147:104803. https://doi.org/10.1016/j.jaap.2020.104803.

16. Fischer M, Scholz-Böttcher BM. Simultaneous trace identification and quantification of common types of microplastics in environmental samples by pyrolysis-gas chromatography–mass spectrometry. Environ Sci Technol. 2017;51:5052–60. https://doi.org/10.1021/acs.est.6b06362.
17. Unice KM, Kreider ML, Panko JM. Use of a deuterated internal standard with pyrolysis-GC/MS dimeric marker analysis to quantify tire tread particles in the environment. IJERPH. 2012;9:4033–55. https://doi.org/10.3390/ijerph9114033.
18. Käppler A, Fischer M, Scholz-Böttcher BM, Oberbeckmann S, Labrenz M, Fischer D, Eichhorn K-J, Voit B. Comparison of μ-ATR-FTIR spectroscopy and py-GCMS as identification tools for microplastic particles and fibers isolated from river sediments. Anal Bioanal Chem. 2018;410:5313–27. https://doi.org/10.1007/s00216-018-1185-5.
19. Fischer M, Scholz-Böttcher BM. Microplastics analysis in environmental samples – recent pyrolysis-gas chromatography-mass spectrometry method improvements to increase the reliability of mass-related data. Anal Methods. 2019;11:2489–97. https://doi.org/10.1039/C9AY00600A.
20. Fischer M, Goßmann I, Scholz-Böttcher BM. Fleur de Sel—an interregional monitor for microplastics mass load and composition in European coastal waters? J Anal Appl Pyrolysis. 2019;144:104711. https://doi.org/10.1016/j.jaap.2019.104711.
21. Dibke C, Fischer M, Scholz-Böttcher BM. Microplastic mass concentrations and distribution in German bight waters by pyrolysis–gas chromatography–mass spectrometry/thermochemolysis reveal potential impact of marine coatings: do ships leave skid marks? Environ Sci Technol. 2021;55:2285–95. https://doi.org/10.1021/acs.est.0c04522.
22. Primpke S, Fischer M, Lorenz C, Gerdts G, Scholz-Böttcher BM. Comparison of pyrolysis gas chromatography/mass spectrometry and hyperspectral FTIR imaging spectroscopy for the analysis of microplastics. Anal Bioanal Chem. 2020;412:8283–98. https://doi.org/10.1007/s00216-020-02979-w.
23. Dierkes G, Lauschke T, Becher S, Schumacher H, Földi C, Ternes T. Quantification of microplastics in environmental samples via pressurized liquid extraction and pyrolysis-gas chromatography. Anal Bioanal Chem. 2019;411:6959–68. https://doi.org/10.1007/s00216-019-02066-9.
24. Gomiero A, Øysæd KB, Agustsson T, van Hoytema N, van Thiel T, Grati F. First record of characterization, concentration and distribution of microplastics in coastal sediments of an urban fjord in south west Norway using a thermal degradation method. Chemosphere. 2019;227:705–14. https://doi.org/10.1016/j.chemosphere.2019.04.096.
25. Hermabessiere L, Rochman CM. Microwave-assisted extraction for quantification of microplastics using pyrolysis–gas chromatography/mass spectrometry. Environ Toxicol Chem. 2021;40:2733–41. https://doi.org/10.1002/etc.5179.
26. Kirstein IV, Hensel F, Gomiero A, Iordachescu L, Vianello A, Wittgren HB, Vollertsen J. Drinking plastics? – Quantification and qualification of microplastics in drinking water distribution systems by μFTIR and Py-GCMS. Water Res. 2021;188:116519. https://doi.org/10.1016/j.watres.2020.116519.
27. Ribeiro F, Okoffo ED, O'Brien JW, Fraissinet-Tachet S, O'Brien S, Gallen M, Samanipour S, Kaserzon S, Mueller JF, Galloway T, Thomas KV. Quantitative analysis of selected plastics in high-commercial-value Australian seafood by pyrolysis gas chromatography mass spectrometry. Environ Sci Technol. 2020;54:9408–17. https://doi.org/10.1021/acs.est.0c02337.
28. Leslie HA, van Velzen MJM, Brandsma SH, Vethaak AD, Garcia-Vallejo JJ, Lamoree MH. Discovery and quantification of plastic particle pollution in human blood. Environ Int. 2022;163:107199. https://doi.org/10.1016/j.envint.2022.107199.
29. Okoffo ED, Ribeiro F, O'Brien JW, O'Brien S, Tscharke BJ, Gallen M, Samanipour S, Mueller JF, Thomas KV. Identification and quantification of selected plastics in biosolids by pressurized liquid extraction combined with double-shot pyrolysis gas chromatography–mass spectrometry. Sci Total Environ. 2020;715:136924. https://doi.org/10.1016/j.scitotenv.2020.136924.
30. Coralli I, Giorgi V, Vassura I, Rombolà AG, Fabbri D. Secondary reactions in the analysis of microplastics by analytical pyrolysis. J Anal Appl Pyrolysis. 2022;161:105377. https://doi.org/10.1016/j.jaap.2021.105377.
31. Hermabessiere L, Himber C, Boricaud B, Kazour M, Amara R, Cassone A-L, Laurentie M, Paul-Pont I, Soudant P, Dehaut A, Duflos G. Optimization, performance, and application of a pyrolysis-GC/MS method for the identification of microplastics. Anal Bioanal Chem. 2018;410:6663–76. https://doi.org/10.1007/s00216-018-1279-0.
32. Ishimura T, Iwai I, Matsui K, Mattonai M, Watanabe A, Robberson W, Cook A-M, Allen HL, Pipkin W, Teramae N, Ohtani H, Watanabe C. Qualitative and quantitative analysis of mixtures of microplastics in the presence of calcium carbonate by pyrolysis-GC/MS. J Anal Appl Pyrolysis. 2021;157:105188. https://doi.org/10.1016/j.jaap.2021.105188.
33. Matsui K, Ishimura T, Mattonai M, Iwai I, Watanabe A, Teramae N, Ohtani H, Watanabe C. Identification algorithm for polymer mixtures based on Py-GC/MS and its application for microplastic analysis in environmental samples. J Anal Appl Pyrolysis. 2020;149:104834. https://doi.org/10.1016/j.jaap.2020.104834.
34. Matsueda M, Mattonai M, Iwai I, Watanabe A, Teramae N, Robberson W, Ohtani H, Kim Y-M, Watanabe C. Preparation and test of a reference mixture of eleven polymers with deactivated inorganic diluent for microplastics analysis by pyrolysis-GC–MS. J Anal Appl Pyrolysis. 2021;154:104993. https://doi.org/10.1016/j.jaap.2020.104993.
35. Fabbri D, Rombolà AG, Vassura I, Torri C, Franzellitti S, Capolupo M, Fabbri E. Off-line analytical pyrolysis GC–MS to study the accumulation of polystyrene microparticles in exposed mussels. J Anal Appl Pyrolysis. 2020;149:104836. https://doi.org/10.1016/j.jaap.2020.104836.
36. Krauskopf L-M, Hemmerich H, Dsikowitzky L, Schwarzbauer J. Critical aspects on off-line pyrolysis-based quantification of microplastic in environmental samples. J Anal Appl Pyrolysis. 2020;152:104830. https://doi.org/10.1016/j.jaap.2020.104830.
37. Shin T, Hajime O, Chuichi W. Pyrolysis-GC/MS data book of synthetic polymers: pyrograms, thermograms and MS of pyrolyzates. 1st ed. Elsevier; 2011.
38. Harata K, Kitagawa S, Iiguni Y, Ohtani H. Identification of polymer species in a complex mixture by pyrolysis-gas chromatography-atmospheric pressure chemical ionization-high resolution time-of-flight mass spectrometry as a basis for environmental microplastic analysis. J Anal Appl Pyrolysis. 2020;148:104828. https://doi.org/10.1016/j.jaap.2020.104828.
39. Albignac M, Ghiglione JF, Labrune C, ter Halle A. Determination of the microplastic content in Mediterranean benthic macrofauna by pyrolysis-gas chromatography-tandem mass spectrometry. Mar Pollut Bull. 2022;181:113882. https://doi.org/10.1016/j.marpolbul.2022.113882.
40. The Center for Marine Debris Research (2020) Polymer Kit 1.0. https://www.hpu.edu/cncs/cmdr/img/polymerkit1.0_marketingbrochure.pdf
41. Chouchene K, Nacci T, Modugno F, Castelvetro V, Ksibi M. Soil contamination by microplastics in relation to local agricultural development as revealed by FTIR, ICP-MS and pyrolysis-GC/MS. Environ Pollut. 2022;303:119016. https://doi.org/10.1016/j.envpol.2022.119016.
42. Doyen P, Hermabessiere L, Dehaut A, Himber C, Decodts M, Degraeve T, Delord L, Gaboriaud M, Moné P, Sacco J, Tavernier

E, Grard T, Duflos G. Occurrence and identification of microplastics in beach sediments from the Hauts-de-France region. Environ Sci Pollut Res. 2019;26:28010–21. https://doi.org/10.1007/s11356-019-06027-8.
43. Hendrickson E, Minor EC, Schreiner K. Microplastic abundance and composition in Western Lake Superior as determined via microscopy, Pyr-GC/MS, and FTIR. Environ Sci Technol. 2018;52:1787–96. https://doi.org/10.1021/acs.est.7b05829.
44. Becker R, Altmann K, Sommerfeld T, Braun U. Quantification of microplastics in a freshwater suspended organic matter using different thermoanalytical methods – outcome of an interlaboratory comparison. J Anal Appl Pyrolysis. 2020;148:104829. https://doi.org/10.1016/j.jaap.2020.104829.
45. Watteau F, Dignac M-F, Bouchard A, Revallier A, Houot S. Microplastic detection in soil amended with municipal solid waste composts as revealed by transmission electronic microscopy and pyrolysis/GC/MS. Front Sustain Food Syst. 2018;2:81. https://doi.org/10.3389/fsufs.2018.00081.
46. Ainali NM, Bikiaris DN, Lambropoulou DA. Aging effects on low- and high-density polyethylene, polypropylene and polystyrene under UV irradiation: an insight into decomposition mechanism by Py-GC/MS for microplastic analysis. J Anal Appl Pyrolysis. 2021;158:105207. https://doi.org/10.1016/j.jaap.2021.105207.
47. Herrera M, Matuschek G, Kettrup A. Fast identification of polymer additives by pyrolysis-gas chromatography/mass spectrometry. J Anal Appl Pyrol. 2003;70:35–42. https://doi.org/10.1016/S0165-2370(02)00078-5.
48. Ni B-J, Zhu Z-R, Li W-H, Yan X, Wei W, Xu Q, Xia Z, Dai X, Sun J. Microplastics mitigation in sewage sludge through pyrolysis: the role of pyrolysis temperature. Environ Sci Technol Lett. 2020;7:961–7. https://doi.org/10.1021/acs.estlett.0c00740.
49. Nardella F, Bellavia S, Mattonai M, Ribechini E. Co-pyrolysis of biomass and plastic: synergistic effects and estimation of elemental composition of pyrolysis oil by analytical pyrolysis–gas chromatography/mass spectrometry. Bioresource Technol. 2022;354:127170. https://doi.org/10.1016/j.biortech.2022.127170.
50. Nakano R, Gürses RK, Tanaka Y, Ishida Y, Kimoto T, Kitagawa S, Iiguni Y, Ohtani H. Pyrolysis-GC–MS analysis of ingested polystyrene microsphere content in individual Daphnia magna. Sci Total Environ. 2022;817:152981. https://doi.org/10.1016/j.scitotenv.2022.152981.
51. El Hayany B, El Fels L, Quénéa K, Dignac M-F, Rumpel C, Gupta VK, Hafidi M. Microplastics from lagooning sludge to composts as revealed by fluorescent staining- image analysis, Raman spectroscopy and pyrolysis-GC/MS. J Environ Manag. 2020;275:111249. https://doi.org/10.1016/j.jenvman.2020.111249.
52. Fries E, Dekiff JH, Willmeyer J, Nuelle M-T, Ebert M, Remy D. Identification of polymer types and additives in marine microplastic particles using pyrolysis-GC/MS and scanning electron microscopy. Environ Sci: Processes Impacts. 2013;15:1949. https://doi.org/10.1039/c3em00214d.
53. Vilakati B, Sivasankar V, Mamba BB, Omine K, Msagati TAM. Characterization of plastic micro particles in the Atlantic Ocean seashore of Cape Town, South Africa and mass spectrometry analysis of pyrolyzate products. Environ Pollut. 2020;265:114859. https://doi.org/10.1016/j.envpol.2020.114859.
54. Dekiff JH, Remy D, Klasmeier J, Fries E. Occurrence and spatial distribution of microplastics in sediments from Norderney. Environ Pollut. 2014;186:248–56. https://doi.org/10.1016/j.envpol.2013.11.019.
55. La Nasa J, Biale G, Mattonai M, Modugno F. Microwave-assisted solvent extraction and double-shot analytical pyrolysis for the quali-quantitation of plasticizers and microplastics in beach sand samples. J Hazard Mater. 2021;401:123287. https://doi.org/10.1016/j.jhazmat.2020.123287.
56. Lynch JM, Knauer K, Shaw KR. Plastic additives in the ocean. In: Andrady AL, editor. Plastics and the ocean. 1st ed. Wiley; 2022. p. 43–76.
57. Takahahashi T Operating principle of selective sampler. Frontier Lab. Technical report PYT-036E. https://www.frontier-lab.com/assets/file/technical-note/PYT-036E.pdf
58. Seeley ME, Wang Q, Bacosa H, Rosenheim BE, Liu Z. Environmental petroleum pollution analysis using ramped pyrolysis-gas chromatography–mass spectrometry. Org Geochem. 2018;124:180–9. https://doi.org/10.1016/j.orggeochem.2018.07.012.
59. Lin C-C, Yang C-C, Ger J, Deng J-F, Hung D-Z. Tetramethylammonium hydroxide poisoning. Clin Toxicol. 2010;48:213–7. https://doi.org/10.3109/15563651003627777.
60. Rødland ES, Samanipour S, Rauert C, Okoffo ED, Reid MJ, Heier LS, Lind OC, Thomas KV, Meland S. A novel method for the quantification of tire and polymer-modified bitumen particles in environmental samples by pyrolysis gas chromatography mass spectroscopy. J Hazard Mater. 2022;423:127092. https://doi.org/10.1016/j.jhazmat.2021.127092.
61. Rauert C, Rødland ES, Okoffo ED, Reid MJ, Meland S, Thomas KV. Challenges with quantifying tire road wear particles: recognizing the need for further refinement of the ISO technical specification. Environ Sci Technol Lett. 2021;8:231–6. https://doi.org/10.1021/acs.estlett.0c00949.
62. Lauschke T, Dierkes G, Schweyen P, Ternes TA. Evaluation of poly(styrene-d5) and poly(4-fluorostyrene) as internal standards for microplastics quantification by thermoanalytical methods. J Anal Appl Pyrolysis. 2021;159:105310. https://doi.org/10.1016/j.jaap.2021.105310.
63. Cai H, Xu EG, Du F, Li R, Liu J, Shi H. Analysis of environmental nanoplastics: progress and challenges. Chem Eng J. 2021;410:128208. https://doi.org/10.1016/j.cej.2020.128208.
64. Mintenig SM, Int-Veen I, Löder MGJ, Primpke S, Gerdts G. Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging. Water Res. 2017;108:365–72. https://doi.org/10.1016/j.watres.2016.11.015.
65. Wahl A, Le Juge C, Davranche M, El Hadri H, Grassl B, Reynaud S, Gigault J. Nanoplastic occurrence in a soil amended with plastic debris. Chemosphere. 2021;262:127784. https://doi.org/10.1016/j.chemosphere.2020.127784.
66. Ter Halle A, Jeanneau L, Martignac M, Jardé E, Pedrono B, Brach L, Gigault J. Nanoplastic in the North Atlantic subtropical gyre. Environ Sci Technol. 2017;51:13689–97. https://doi.org/10.1021/acs.est.7b03667.
67. Zhou X, Hao L, Wang H, Li Y, Liu J. Cloud-point extraction combined with thermal degradation for nanoplastic analysis using pyrolysis gas chromatography–mass spectrometry. Anal Chem. 2019;91:1785–90. https://doi.org/10.1021/acs.analchem.8b04729.
68. Sullivan GL, Gallardo JD, Jones EW, Hollliman PJ, Watson TM, Sarp S. Detection of trace sub-micron (nano) plastics in water samples using pyrolysis-gas chromatography time of flight mass spectrometry (PY-GCToF). Chemosphere. 2020;249:126179. https://doi.org/10.1016/j.chemosphere.2020.126179.
69. Blancho F, Davranche M, Fumagalli F, Ceccone G, Gigault J. A reliable procedure to obtain environmentally relevant nanoplastic proxies. Environ Sci: Nano. 2021;8:3211–9. https://doi.org/10.1039/D1EN00395J.
70. El Hadri H, Gigault J, Maxit B, Grassl B, Reynaud S. Nanoplastic from mechanically degraded primary and secondary microplastics for environmental assessments. NanoImpact. 2020;17:100206. https://doi.org/10.1016/j.impact.2019.100206.

71. Mitrano DM, Beltzung A, Frehland S, Schmiedgruber M, Cingolani A, Schmidt F. Synthesis of metal-doped nanoplastics and their utility to investigate fate and behaviour in complex environmental systems. Nat Nanotechnol. 2019;14:362–8. https://doi.org/10.1038/s41565-018-0360-3.
72. Bouzid N, Anquetil C, Dris R, Gasperi J, Tassin B, Derenne S. Quantification of microplastics by pyrolysis coupled with gas chromatography and mass spectrometry in sediments: challenges and implications. Microplastics. 2022;1:229–39. https://doi.org/10.3390/microplastics1020016.
73. Dümichen E, Eisentraut P, Bannick CG, Barthel A-K, Senz R, Braun U. Fast identification of microplastics in complex environmental samples by a thermal degradation method. Chemosphere. 2017;174:572–84. https://doi.org/10.1016/j.chemosphere.2017.02.010.
74. Eisentraut P, Dümichen E, Ruhl AS, Jekel M, Albrecht M, Gehde M, Braun U. Two birds with one stone—fast and simultaneous analysis of microplastics: microparticles derived from thermoplastics and tire wear. Environ Sci Technol Lett. 2018;5:608–13. https://doi.org/10.1021/acs.estlett.8b00446.
75. Kühn S, van Oyen A, Booth AM, Meijboom A, van Franeker JA. Marine microplastic: preparation of relevant test materials for laboratory assessment of ecosystem impacts. Chemosphere. 2018;213:103–13. https://doi.org/10.1016/j.chemosphere.2018.09.032.
76. Cowger W, Steinmetz Z, Gray A, Munno K, Lynch J, Hapich H, Primpke S, De Frond H, Rochman C, Herodotou O. Microplastic spectral classification needs an open source community: open specy to the rescue! Anal Chem. 2021;93:7543–8. https://doi.org/10.1021/acs.analchem.1c00123.
77. Müller YK, Wernicke T, Pittroff M, Witzig CS, Storck FR, Klinger J, Zumbülte N. Microplastic analysis—are we measuring the same? Results on the first global comparative study for microplastic analysis in a water sample. Anal Bioanal Chem. 2020;412:555–60. https://doi.org/10.1007/s00216-019-02311-1.
78. Fabbri D. Use of pyrolysis-gas chromatography/mass spectrometry to study environmental pollution caused by synthetic polymers: a case study: the Ravenna Lagoon. J Anal Appl Pyrol. 2001;58–59:361–70. https://doi.org/10.1016/S0165-2370(00)00170-4.
79. Hermabessiere L, Dehaut A, Paul-Pont I, Lacroix C, Jezequel R, Soudant P, Duflos G. Occurrence and effects of plastic additives on marine environments and organisms: a review. Chemosphere. 2017;182:781–93. https://doi.org/10.1016/j.chemosphere.2017.05.096.
80. Brander SM, Renick VC, Foley MM, Steele C, Woo M, Lusher A, Carr S, Helm P, Box C, Cherniak S, Andrews RC, Rochman CM. Sampling and quality assurance and quality control: a guide for scientists investigating the occurrence of microplastics across matrices. Appl Spectrosc. 2020;74:1099–125. https://doi.org/10.1177/0003702820945713.
81. Cowger W, Booth AM, Hamilton BM, Thaysen C, Primpke S, Munno K, Lusher AL, Dehaut A, Vaz VP, Liboiron M, Devriese LI, Hermabessiere L, Rochman C, Athey SN, Lynch JM, De Frond H, Gray A, Jones OAH, Brander S, Steele C, Moore S, Sanchez A, Nel H. Reporting guidelines to increase the reproducibility and comparability of research on microplastics. Appl Spectrosc. 2020;74:1066–77. https://doi.org/10.1177/0003702820930292.

Appendix D

Project Forms

HERRERA
2200 Sixth Avenue | Suite 1100
Seattle, Washington | 98121
p 206 441 9080 | f 206 441 9108

Chain of Custody Record

Project Name:	Project Number:	Client:
TWP and 6PPDQ SAM	22-07975-000	City of Tacoma

Report To:	Copy To:
Dylan Ahearn, dahearn@herrerainc.com James Packman, jpackman@herrerainc.com	Stacy Luell, sluell@herrerainc.com Nicholas Harris, nharris@herrerainc.com Nikki VandePutte, nvandeputte@herrerainc.com
Sampled By:	Delivery Method:
Laboratory: Tacoma Environmental Services Laboratory	Requested Completion Date: / Total No. of Containers:

Lab Use:

Sample ID	Date	Time	Sample Type (see codes)	Preser-vative? (Y/N)	Matrix (see codes)	Number of Containers	Analyses Requested: 2. Metals, Total and Dissolved	3. Total Nitrate+nitrite	4. Total Kjeldahl Nitrogen	5. Total Phosphorus	6. Orthophosphate	7. TSS	Petroleum hydrocarbons – NWTPH-Dx	1. 6PPDQ	Microplastics and TWP	PFAS	8. HRMS (1-L for Kolodziej lab)	Lab ID No.
			G	N	SW	7							X	X	X	X	X	
			C	N	SW	1	X	X	X	X	X	X		X			X	

Comments/Special Instructions:

Analyze composite samples in priority order listed (1-8) pending available sample volume.

Relinquished by (Name/CO)	Signature	Date/Time	Received By (Name/CO)	Signature		Date/Time
Relinquished by (Name/CO)	Signature	Date/Time	Received By (Name/CO)	Signature		Date/Time

Sample Type: G=Grab C=Composite **Matrix Codes:** A=Air GW=Groundwater SE=Sediment SO=Soil SW=Surface Water W=Water (blanks) M=Material O=Other (specify)

HERRERA
2200 Sixth Avenue | Suite 1100
Seattle, Washington | 98121
p 206 441 9080 | f 206 441 9108

Chain of Custody Record

Project Name:	Project Number:	Client:
TWP and 6PPDQ SAM	22-07975-000	City of Tacoma

Report To:	Copy To:
Dylan Ahearn, dahearn@herrerainc.com James Packman, jpackman@herrerainc.com	Stacy Luell, sluell@herrerainc.com Nicholas Harris, nharris@herrerainc.com Nikki VandePutte, nvandeputte@herrerainc.com
Sampled By:	Delivery Method:
Laboratory: Tacoma Environmental Services Laboratory	Requested Completion Date: / Total No. of Containers:

Lab Use:

Sample ID	Date	Time	Sample Type (see codes)	Preser-vative? (Y/N)	Matrix (see codes)	Number of Containers	Analyses Requested: 6PPDQ – 1634	PFAS	Microplastics and TWP	PAHs – 8270E	Phthalates– 8270E	Phenolics –8270E	Bifenthrin and Dichlobenil - 8270E	Grain Size– SM 2450G	PCBs -80270E	Total Organic Carbon – 9060A	Total Solids – PSEP	Total Recoverable Metals– 6020DS	Lab ID No.
			G	N	SE	3	X	X	X	X	X	X	X	X	X	X	X	X	

Comments/Special Instructions:

Please prioritize analyses in the order that they appear. If there isn't enough volume for a given analyte, move to the next one on the list.

Relinquished by (Name/CO)	Signature	Date/Time	Received By (Name/CO)	Signature	Date/Time
Relinquished by (Name/CO)	Signature	Date/Time	Received By (Name/CO)	Signature	Date/Time

Sample Type: G=Grab C=Composite **Matrix Codes:** A=Air GW=Groundwater SE=Sediment SO=Soil SW=Surface Water W=Water (blanks) M=Material O=Other (specify)

SAM CEC Monitoring

Site Name:

Pre-Event Set Up

Personnel: _____ Date: _____ Time: _____

_____ Weather: _____

Checklist:

- ☐ Intake checked
- ☐ Tubing connected
- ☐ Sample line rinsed
- ☐ Sensor Calibrated
- ☐ Clean bottle installed
- ☐ Ice added
- ☐ Modem online
- ☐ Photos taken
- ☐ Sampler armed
- ☐ Check Voltage

Notes:

Grab Sample Collection

Personnel: _____ Date: _____ Time: _____

_____

Weather:

Sample ID: Duplicate ID:

Parameter	Bottle	Sample Method	Parameter	Bottle
☐ TPH[1]	2x 1-L Amber Glass	Bottle Dip	☐ TPH[1]	2x 1-L Amber Glass
☐ 6PPDQ	2x 250-mL Amber Glass		☐ 6PPDQ	2x 250-mL Amber Glass
☐ MP/TWP	22-L Stainless Steel Jug		☐ MP/TWP	22-L Stainless Steel Jug
☐ PFAS	2x 250-mL, 1x 125-mL HDPE		☐ PFAS	2x 250-mL, 1x 125-mL HDPE
☐ HRMS	1-L Amber Glass		☐ HRMS	1-L Amber Glass

Notes: (Note here if blanks collected by parameter. Note the sampling method above, e.g., pumped, bottle dip, etc. Suspected PFAS sources?)

[1] TPH is only collected at the automated sampler locations

Post-Event Sample Pick-up

Personnel: _____ Date: _____ Time: _____

_____ Weather: _____

Sample ID: _____ Duplicate? Y / N Duplicate ID:

Sample Date End: _____ Sample Time End: _____

Sample Count: _____ Estimated Volume (L): _____

Visual Condition:

Checklist:

- ☐ Sampled Without Error
- ☐ Photos Taken
- ☐ Sent to Lab

Notes:

SAM CEC Monitoring

Site Name:

Grab Sample Collection

Personnel: _____ Date: _____ Time: _____

Weather: _____

Sample ID:			Duplicate ID:	
Parameter	**Bottle**	**Sample Method**	**Parameter**	**Bottle**
☐ 6PPDQ	2x 250-mL Amber Glass		☐ 6PPDQ	2x 250-mL Amber Glass
☐ MP/TWP	22-L Stainless Steel Jug		☐ MP/TWP	22-L Stainless Steel Jug
☐ PFAS	2x 250-mL, 1x 125-mL HDPE		☐ PFAS	2x 250-mL, 1x 125-mL HDPE
☐ HRMS	1-L Amber Glass		☐ HRMS	1-L Amber Glass

Notes: (Note here if blanks collected by parameter. Note the sampling method above, e.g., pumped, bottle dip, etc. Suspected PFAS sources?, condition of sediment traps)

SAM CEC Monitoring **Site Name:**

Grab Sample Collection		
Personnel: _____ Date: _____ Time: _____		
Weather: _____		
Sample ID:	Duplicate? Y / N	Duplicate ID:
Time Traps Removed: _____	**Approx. Depth of Sediment (inches)**	**Bottle Style (check one):**
	Bottle 1:	☐ Round Amber Glass
Time Traps Replaced: _____	Bottle 2:	☐ Wide Dish
	Bottle 3:	

Notes:

Appendix E

List of Acronyms and Abbreviations

HERRERA
Science + Planning + Design

Acronyms and Abbreviations

6PPDQ	6PPD-quinone
BMPs	Best management practices
CEC	Contaminant of emerging concern
The City	The City of Tacoma
COC	Chain-of-custody
COM	Commercial
CPR	Cardiopulmonary resuscitation
DQO	Data quality objective
Ecology	Washington State Department of Ecology
EDD	Electronic data deliverable
EIM	Environmental Information Management database
EPA	U.S. Environmental Protection Agency
GIS	Geographic Information System
HDPE	High-density polyethylene
HDR	High-density residential
HUC-12	Hydrologic Unit Code 12 digit
HRMS	High Resolution Mass Spectroscopy
ID	Identification
IND	Industrial
LAU	Laboratory Accreditation Unit
LC/MS	Liquid chromatography-mass spectrometry
LCS	Laboratory control sample
LDR	Low-density residential
MDL	Method detection limit
MP	Microplastics
MRL	Method reporting limit
MQO	Measurement quality objective
MS	Matrix spike

MS4	Municipal Separate Storm Sewer System
NPDES	National Pollutant Discharge Elimination System
NWTPH-Dx	Northwest Total Petroleum Hydrocarbons
OF	Outfall
PAHs	Polycyclic aromatic hydrocarbons
PC	polycarbonate
PCBs	Polychlorinated biphenyls
PCPs	Personal care products
PE	polyethylene
PET	polyethylene terephthalate
PFAS	Per- and polyfluoroalkyl substances
PMMA	poly(methyl methacrylate)
PP	polypropylene
PPE	Personal protective equipment
PS	polystyrene
PTFE	poly(tetrafluoroethylene)
QAPP	Quality assurance project plan
QC	Quality control
RPD	Relative percent difference
S8.D	Section D of Special Condition 8
SAM	Stormwater Action Monitoring
SMAP	Stormwater Management Action Planning
SOP	Standard Operating Procedure
SPU	Seattle Public Utilities
SSPM	Stormwater suspended particulate matter
TAC	Technical Advisory Committee
TBD	To be determined
TESL	Tacoma Environmental Services Laboratory
TPH	Total petroleum hydrocarbons
TSS	Total suspended solids
TWP	Tire wear particles

WQBE	Water Quality Benefits Evaluation model
WSDOT	Washington State Department of Transportation
WY	Water year