Columbia River Basin Restoration, Region 10 QAPP: Crayfish as Indicators for 6PPD-quinone

Project Name: Crayfish as Indicators for 6PPD-quinone

Effective Date of Plan: January, 1, 2023

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ACRYONYMS AND ABBREVIATIONS

6PPD-q	6PPD-quinone, N-(1,3-Dimethylbutyl)-N'-phenyl-p-Phenylenediamine-quinone
BMP	Best Management Practices
CFC	Clark Fork Coalition
CNR-AAL	College of Natural Resources; Aquatic Animal Laboratory
CRB	Columbia River Basin
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
DQI	Data Quality Indicator
EHS	Environmental Health and Safety
EPA	Environmental Protection Agency
GIS	Geographic Information System
GPS	Global Positioning System
IBC	Biosafety
LC-MS	Liquid Chromatography Mass Spectrometry
MFWP	Montana Fish Wildlife and Parks
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QC	Quality Control
RCDS	Research Computing and Data Services
RPD	Relative Percent Difference
SPE	Solid Phase Extraction
TWP	Tire Wear Particles
SPF	Specific Pathogen Free
SSS	Salish School of Spokane
UI	University of Idaho
WSDOT	Washington Department of Transportation

Problem Definition, Background and Project Description

A. Problem Definition

Though ephemeral in nature, stormwater runoff is a significant source of pollution to receiving waters (Muller et al., 2020). The extent of this pollution, and the presence of emerging contaminants in these runoff events, like N-(1,3-Dimethylbutyl)-N'-phenyl-p-Phenylenediamine-quinone (6PPD-q) and tire wear particles (TWP), is not well constrained by science and is poorly understood by the public, in part because these pollutants are difficult to monitor. The main goal of this project is to assess the use of crayfish as an environmental monitoring organism for aquatic exposure to 6PPD-q and TWP, while enhancing public awareness of these pollutants in the Columbia River Basin (CRB).

B. Background

6PPD-q is a highly toxic by-product of an antioxidant ubiquitously used in rubber tire manufacturing which has been shown to have both lethal and sub-lethal effects on a variety of aquatic organisms, including coho salmon (Johannessen et al., 2021; Varshney et al., 2021; Brinkmann et al., 2022; Tian et al., 2022;). Studies indicate bioaccumulation of 6PPD-q in aquatic organisms and biomagnification is also suspected because of its high hydrophobicity (Hiki et al., 2021).

Recent work indicates that 6PPD-q primarily enters aquatic systems through stormwater runoff and is transported by leachates or by direct emplacement of tire wear particles (TWP) in aquatic systems (Capolupo et al., 2020; Halle et al., 2020; Hiki et al., 2021). While the EPA has not yet classified 6PPD-q as a contaminant of emerging concern, TWP are identified as contaminants of concern by the Contaminants of Concern Subgroup of the Colombia River Basin Restoration Working Group and the Puget Sound Stormwater Group (Columbia River COC Framework, 2020; Stormwater Work Group Work Plan, 2021-2022). The recent discovery and limited information available on this toxic chemical create numerous knowledge gaps relative to its impacts on aquatic ecosystems and human health (Hiki et al., 2021; McIntyre et al., 2021; Du et al., 2022).

Research related to 6PPD-q and TWP has primarily focused on fish, creating a critical knowledge gap related to how these contaminants affect other aquatic organisms. The impacts of 6PPD-q on crayfish, a keystone benthic macroinvertebrate, are potentially significant given their ability to accumulate contaminants (Antón et al., 2000; Kuklina et al., 2013; Brittle et al., 2016). Crayfish contaminated with 6PPD-q could directly impact endemic salmonid and trout species as well as human populations with diets reliant on aquatic species, including many native communities in the CRB (Johnson et al., 2014; Noble et al., 2016). Crayfish are also potentially an effective biomonitoring tool for 6PPD-q because they are widely distributed in the CRB, occupy multiple trophic levels, react quickly to environmental changes, and reflect local site conditions (Schilderman et al., 1999; Larson & Olden, 2011; Kuklina et al., 2013).

C. Project Description

The primary goal for this project is to assess the use of crayfish as an indicator species for 6PPD-q and TWP, while building public awareness of these pollutants in aquatic ecosystems throughout the CRB. To achieve this goal, the project consists of three

phases, 1) an ecotoxicological laboratory study that includes field sampling of two different crayfish species followed by laboratory exposure experiments, 2) a field sampling, collection, and monitoring campaign, and 3) a public awareness campaign tied to citizen science collection of crayfish.

Figure 1: Overview of Project Phases

1. Project Objectives:

The primary objectives for each phase of the project are:

- **Phase 1:** Assess the accumulation of 6PPD-q in crayfish tissues under different exposure regimes in project year 1.
- **Phase 2:** Assess the presence of 6PPD-q and TWP in water, sediment, and crayfish and fish tissues in the middle and upper CRB in project years 1 and 2.
- **Phase 3:** Enhance public awareness of 6PPD-q and TWP as aquatic pollutants in the CRB through the project period.

If we are successful in measuring tissue accumulations of 6PPD-q in crayfish through our ecotoxicological laboratory experiments during Phase 1, then we will proceed to Phase 2 and assess tissue concentrations of 6PPD-q from environmental samples throughout the middle and upper CRB. In Phase 2 we will collect crayfish, fish, sediment, and water samples from waterbodies in the middle and upper CRB to determine concentrations of 6PPD-q and TWP in aquatic systems. If the results from Phase 2 indicate that crayfish are effective environmental monitoring organisms for 6PPD-q and TWP, then we will use this information in Phase 3 to inform the public about effective ways to monitor these aquatic pollutants. Additionally, during Phase 3 we will raise public awareness of 6PPD-q, TWP and other aquatic pollutants in the CRB, regardless of experimental outcomes from Phases 1 and 2, by engaging the public through citizen science sampling events and education outreach activities.

If we are unable to assess tissue concentrations of 6PPD-q in crayfish tissue, then we will repeat the experiments with an alternative monitoring species such as bivalves. Bivalves are commonly used in environmental monitoring studies, found ubiquitously in aquatic systems and like crayfish, reflect local site conditions relative to pollutants in aquatic ecosystems (Vaughn and Hoellein, 2018). Bivalves are filter feeders and may sequester higher concentrations of 6PPD-q than crayfish, potentially increasing the likelihood of achieving detectable tissue levels.

2. Project Sites or Study Area:

Phase 1 sampling sites will include the North Fork, Middle Fork, and mainstem of the John Day River for rusty crayfish and the Spokane River will be sampled for signal

crayfish. The primary study areas where 6PPD-q and TWP will be monitored in Phase 2 for this project will include four watersheds within the Columbia River Basin: Clark Fork River (ID, MT), Pend Oreille River (ID, WA), Spokane River (ID, WA), and the Snake River (ID, OR, WA). In addition, crayfish, sediment, and water will be sampled from stormwater retention ponds in WA. The UI will collaborate with the Salish School of Spokane to focus sampling efforts within the Spokane and Snake River watersheds. The Clark Fork Coalition and UI will have two sampling events on the Clark Fork River, for a total of four different sampling sites. The UI and MFWP will conduct sampling in additional locations within the Clark Fork River watershed. Crayfish, sediment, and water samples will be collected from each sampling location and analyzed for 6PPD-q. Additionally, trout species will be collocated with MFWP for 6PPD-q fish tissue analysis (See Data Collection Methods). WSDOT and the UI will collaborate for sampling in stormwater retention ponds.

Results from the first year of sampling will determine our approach for establishing project sites in year two. If we are unable to determine tissue, sediment, and water concentrations from some of the 2023 sampling locations, then we will assess potential contributing factors (i.e. traffic density, road proximity) and plan our 2024 project sites accordingly. If we can determine tissue, sediment, and water concentrations from majority of the 2023 sampling locations, then we will expand our geographic sampling plan for 2024 and re-sample specific sites from 2023 by way of comparison.

3. Time Period:

Phase 1 ecotoxicological experiments will be conducted from April 2023 through June 2023. Phase 2 will be ongoing from June 2023 through December 2024. Phase 3 will be ongoing throughout the project duration (April 2023 through December 2024)

4. Data Users:

Data from this project will be utilized by UI personnel, grant partners, regulatory agencies, and the general public. Data will be used by PI and graduate student to publish peer-reviewed papers and for a graduate student dissertation. Data related to geographic distribution of crayfish will contribute to existing crayfish databases housed by state agencies (i.e. IDFG) and organizations like The River Mile.

Data Quality Objectives and Indicators

A. Data Quality Objectives:

Phase 1: Sufficient and accurate water and 6PPD-q tissue concentration data are collected to determine the suitability of crayfish as potential environmental monitoring organisms for 6PPD-q.

Phase 2: Sufficient environmental samples of water, sediment, crayfish, and fish are collected and assessed for 6PPD-q concentration and TWP to demonstrate the suitability of crayfish as an environmental monitoring organism for 6PPD-q.

Phase 3: Crayfish species in the study watershed successfully collected and accurately identified by the volunteers during sampling events with UI.

B. Data Quality Indicators:

Data Quality indicators	Quality control activities and checks	DQI Goal
Precision		
Lab Data	Laboratory replicates of both water and crayfish tissues. Laboratory replicates for 10% or greater of samples.	20% RPD
Field Data	Field replicates of water (x2), sediment (x2), and crayfish (x4) will be analyzed from each site.	20% RPD
Bias		
Lab Data	Blank filters and pre- and post- LC-MS calibration with calibration standards from 0.025µg/L-100µg/L will be used.	10% RPD of known 6PPD-q concentration. Blank filters show no contamination.
Field Data	Pre- and Post- calibration check before each sampling event for YSI temperature, conductivity, DO, pH sensors. In house calibration with commercial standards as needed with return to manufacture for major work as necessary.	10% RPD
Accuracy		
Lab Data	Blank filters and calibration standards from 0.025µg/L-100µg/L will be used.	10% RPD of known 6PPD-q concentration. Blank filters show no contamination.

Field Data	Check calibration and recalibrate before each sampling event if needed for YSI temperature, conductivity, DO, pH sensors. In house calibration with commercial standards as needed with return to manufacture for major work as necessary.	10% RPD
Representativeness		
Lab Data	Base sample exposure design on best available scientific understanding of environmentally relevant concentrations of 6PPD-q	The exposure duration and concentrations will reflect 6PPD-q exposures that crayfish may experience during and following an storm runoff event. The concentrations chosen for the exposures (0, 0.02 µg/L, 0.2 µg/L, 2 µg/L, 20 µg/L) are reflected in reported surface water concentrations (Challis et al., 2022; Tian et al., 2021; Johannessen et al., 2022).
Field Data	Evaluate sample design in terms of spatial and temporal variability.	Sampling sites will be selected to represent sites that receive stormwater runoff. Data are meant to reflect sections of the stream or lake where crayfish naturally inhabit and where evidence of stormwater runoff is observed (close to the banks).
Comparability		
Lab Data	Compare methods to previous or existing studies.	Experimental design and analysis will follow methods in published studies. A single methodology will be used to permit a meaningful analysis and allow for comparability of data.

Field Data	Compare methods to previous or existing studies.	Data collected will follow a singular sampling design to permit a meaningful analysis and allow for comparability of data. (Simmons, 2020).
Completeness		
Lab Data	Evaluate percent of samples collected.	100% of all samples used in these experiments will be analyzed for 6PPD-q concentration (tissue or water)
Field Data	Evaluate percent of samples collected.	100% of field collected samples will be analyzed for 6PPD-q and TWP concentration.

Parameter	**M**ethod	**R**eporting limits or Measurement Range	Precision or Resolution	Accuracy	Completeness
Laboratory Methods					
6PPD-q Concentration	LC-MS	Limit of detection will be 0.025 - 0.1 µg/L for 6PPD-q using standardized LC-MS calibration techniques (Tian et al., 2022).	Field duplicates 20% RPD	10% RPD of known 6PPD-q concentration. Blank filters show no contamination.	100% of all samples used in these experiments will be analyzed for 6PPD-q concentration (tissue or water)
TWP concentration in sediment and gut contents	Visual counts	50 µm is generally considered the limit of visible detection (Knight et al., 2020)	Field duplicates 30% RPD	N/A	100% of collected samples
Field Methods					
Temperature	YSI	-5-70°C	0.1°C	±0.2°C	90%

Conductivity	YSI	0-200 mS/cm	0.001µS (0-500µS); 0.01 mS (0.501-50.00 mS); 0.1 mS (50.01-200 mS)	±0.5% of reading or 0.001 mS/cm, whichever is greater	90%
pH	YSI	0-14 units	0.01 units	±0.2 units	90%
DO - galvanic	YSI	0 to 500% or 0 to 50 mg/L	1% or 0.1% air saturation (user selectable) OR 0.1 or 0.01 mg/L (user selectable)	0 to 200% (± 2% of reading or 2% air saturation, whichever s greater) 200% – 500% (± 6% of reading) OR 0 to 20 mg/L (±2% of the reading or 0.2 mg/L, whichever is greater) 20 to 50 mg/L (±6% of the reading)	90%

Project Schedule

Activity	Person(s) Responsible	Anticipated Date(s) of Initiation	Anticipated Date(s) of Completion
Plan 6PPD-q and TWP Project	PI, graduate student, and grant partners	January 2023	February 2023
Revise QAPP as necessary	PI, graduate student	February 2023	March 2023
Obtain permits for crayfish field collection	PI, graduate student	February 2023	March 2023
Conduct LC-MS training with local water samples (Paradise Creek, ID)	PI, graduate student, Lee Deobald	February 2023	March 2023
Inventory supplies and equipment	PI, graduate student, Jessie Ma, Lee Deobald	February 2023	June 2023
Set up wet lab for experiments	PI, graduate student, Jessie Ma	March 2023	March 2023
Collect native crayfish from Spokane River (Signal crayfish)	PI, graduate student, undergraduate assistant	April 2023	April 2023
Conduct ecotoxicological experiments on native crayfish (phase one)	PI, graduate student, Jessie Ma, Lee Deobald	April 2023	May 2023
Collect invasive crayfish from John Day River (Rusty crayfish)	PI, graduate student, undergraduate assistant	May 2023	May 2023
Conduct ecotoxicological experiments on invasive crayfish (phase one)	PI, graduate student, Jessie Ma, Lee Deobald	May 2023	June 2023
Launch public-facing website	PI, graduate student, undergraduate assistant, RCDS	April 2023	June 2023
Input, review, and report all data from experiments	PI, graduate student, grant partners, EPA	April 2023	June 2023

Site identification for 2023 field sampling	PI, graduate student, SSS, MFWP, WSDOT, CFC	May 2023	July 2023
Field data collection (UI sampling collection and sampling events with grant partners)	PI, graduate student, undergraduate assistant, SSS, MFWP, WSDOT, CFC	June 2023	November 2023
Conduct 6PPD-q and TWP analysis on field-collected samples	PI, graduate student, undergraduate assistant, Lee Deobald	June 2023	November 2023
Input, review, and report all data from field data collection	PI, graduate student, EPA	June 2023	December 2023
Data submission	PI, EPA	November 2023	December 2023
Plan field data collection strategy for 2024	PI, graduate student, grant partners	December 2023	January 2024
Submit peer-reviewed article for publishing	PI, graduate student	December 2023	February 2024
Update public-facing website with project results from 2023 and sampling plan for 2024	PI, graduate student, undergraduate assistant, RCDS	March 2024	May 2024
Prepare for 2024 field sampling season	PI, graduate student, grant partners	May 2024	June 2024
Conduct field sampling for remaining priority sites in the CRB	Graduate student, undergraduate student	June 2024	October 2024
Submit peer-reviewed article for publishing	PI, graduate student	November 2024	December 2024

Training and Specialized Experience

A. Training

Project Function	Personnel/Group to be Trained	Description of Training (Including Trainer(s))	Frequency of Training
6PPD-q Analysis	PI, graduate student	Lee Deobald will train PI and graduate student for proper LC-MS techniques and procedures to analyze 6PPD-q in water and crayfish tissue	Weekly (March 2023)
Stream Monitoring and Crayfish collection	Grant partners and field assistants	Water and macroinvertebrate sample collection procedures and an overview of water safety will be provided by the PI and UI graduate student prior to sampling.	Beginning of each field season
Stream Monitoring and Crayfish collection	Salish School students and CFC volunteers	Water and macroinvertebrate sample collection procedures and an overview of water safety will be provided by UI graduate student and CFC volunteer leader(s) prior to sampling.	Each sampling event

B. Specialized Experience

Person	Specialized Experience	Years of Experience
Mary Engels, PhD	Assistant Professor – Department of Natural Resources and Society, University of Idaho	10+ years of scientific coordination experience
Jessie Ma, PhD	Research Assistant Professor – Fish and Wildlife Sciences, University of Idaho	10+ years of Research experience

Elizabeth Herrmann, Graduate Student	PhD Student – Environmental Science, University of Idaho	6 years of research experience
Lee Deobald, PhD	University of Idaho Mass Spectrometry Core Lab Director with over 30 years of biological research experience and 15 years of LC-MS experience	20+ years of analytical chemistry experience
David Schmetterling	Research Coordinator – Montana Fish, Wildlife, & Parks	20+ years of research experience
Dominique Wiley-Camacho	Lead Teacher/Science Specialist	15+ years of science education and outreach
Julia Crocker	Community Programs Coordinator	10 years of teaching and community outreach
Matthew Cox	Dangerous/Hazardous Waste Compliance Manager	23 hazardous water experience

Documents and Records

A. Document Control:

This QAPP, all paper and electric data sheets (field and lab), project reports, and general project task lists will be stored on UI server. The project management software (currently Asana (https://asana.com)) will also house up-to-date copies of all relevant tasks and project management documents (including the QAPP) for grant partners to access. This will allow us to assign tasks and maintain procedural checklists with our collaborators to meet project objectives. For data reports, QA/QC reviews will be conducted by PI before disseminating to grant partners to ensure representativeness of data.

B. Data Generation:

Laboratory data will be recorded in laboratory specific notebooks and in electronic files. The resulting information will be transcribed to digital files (as needed), uploaded, and archived to UI server. Field notes and data will also be transcribed to digital files, uploaded, and archived to UI server. Hard copy notebooks will be maintained in the CNR-AAL for five years post analysis.

C. Data Report Package:

The specific data records collected will be scanned copies of field and lab notes, data sheets from the field and CNR-AAL, and any other relevant field/laboratory records. The final data report will include our field and laboratory data, details discussing the process of our data analyses, and our conclusions from results. If applicable, audit reports will be included in the final data report. Any existing data from literature searches that may be relevant (data comparison) will also be included.

D. Reporting Format and Storage:

Data will first be recorded in hard-copy formats and then entered and stored in a digital Master spreadsheet. Lab data sheets for Phase 1 will include information about date and time, exposure concentration and duration, crayfish species, 6PPD-q concentration in crayfish tissue and water, sex and size (mass, length) of crayfish. Lab data sheets will be archived following experiments in a permanent file on UI server. Original LC-MS data will be sent to the P.I. to be archived. Integration results will be copied into spread sheets and sent to the P.I., along with the proprietary integration/quantitation software (TargetLynx) result files to be archived. All results will be stored on UI server in folders that correspond to experimental date(s).

Field data sheets for Phase 2 will first be recorded in hard-copy formats and the entered and stored in the digital Master spreadsheet. Field data sheet will include information about the site location (ID number corresponds with GIS coordinates), which specific equipment was used to make measurements (YSI) the sampling method used for crayfish collection (baited-trap, net, hand), how many crayfish were sampled, how much water and sediment was collected, and equipment calibration results. The field data sheets will be also used to record temperature, DO, and pH. Field data sheets will be archived by sampling season in a permanent digital file.

Lab data sheets from Phase 2 will include information about the sex, species, and size (length, mass) of each crayfish, concentrations of 6PPD-q and TWP in crayfish and sediment. Each lab and field data sheet from Phase 2 will correspond with an ID number. Lab data sheets will be archived by sampling season in a permanent digital file on UI server. LC-MS chromatography data will be integrated with TargetLynx software, and the results will be exported and imported into a spread sheet along corresponding sample identification information to be sent to the P.I. for digital hardcopy archiving. LC and MS analytical conditions will be saved in a digital format to be archived with the resulting data.

1. ***Chain of Custody Forms:***
   Chain of custody forms are completed for each sample. Forms will be standardized and used for each sampling occurrence. Original forms will be stored in PI's office and digital forms will be stored on UI server.

2. ***Sample Labeling:***
   All samples will be labeled with site ID, sampler's initials, date and time of collection, and parameters to be analyzed (6PPD-q and TWP).

3. ***Data Analyses and Reporting:***
   Data will first be recorded as hard-copy in field and lab-designated notebooks. Once data in notebooks is reviewed, validated, and verified by the graduate student and PI, the graduate student will scan and upload all hard-copy and LC-MS data and store in folders on UI server. All verified data will then be entered into a Master spreadsheet onto the UI server. All data will be reviewed by graduate student and PI before data analyses are performed. Once data has been reviewed, verified, and validated the graduate student and PI will prepare for data reporting. Data will be reported to grant partners and EPA before sharing to public-facing website or outside parties.

Existing Data and Data from Other Sources:

Existing Data	Data Source	How Data Will Be Used	Acceptance Criteria	Limitations on Data Use
Crayfish distribution data	National Park Service, The River Mile, Dr. Julian Olden, University of Washington, Dr. Eric Larson, University of Illinois	To determine geographic distribution of crayfish in middle and upper CRB.	Must be located in the middle and upper CRB. Must have potential for receiving stormwater runoff.	Stormwater runoff maps may not correspond to crayfish habitat. Additionally, these maps may not correlate with detectable environmental or tissue levels of 6PPD-q and TWP. Data may be outdated due to changing environmental conditions (invasive species, pollution, etc).
Receiving bodies of stormwater runoff	The Stormwater Heatmap and WSDOT Stormwater Retention Pond (WA), Storm Water IDEQ (ID), City of Missoula Storm Water Management (MT), ArcGIS and ERDAS Imagine.	Determine sampling sites that receive stormwater runoff.	Must be located in the middle and upper CRB. Must inhabited by crayfish.	Existing stormwater data may not be relevant or available for all our study sites.

Sampling Design and Data Collection Methods

A. Sampling Design

1. Phase 1 Experimental Design:

For 6PPD-q exposure experiments, we will collect and expose two species of wild-caught crayfish from the Columbia River Basin. The native signal crayfish (Pacifastacus leniusculus) will be collected from the Spokane River and the invasive rusty crayfish (Faxonius rusticus) will be collected from the John Day River. Samples will be collectedin areas with limited exposure to road runoff, but two crayfish from each sampling site will be analyzed for background levels of 6PPD-q before beginning exposure experiments. The experiments will involve holding crayfish in glass tanks filled with water at various concentrations of 6PPD-q for a variety of times. The exposure concentrations will be determined by reported levels of 6PPD-q measured in surface waters following an runoff event (Challis et al., 2021; Tian et al., 2022, Johannessen et al., 2022). Post-exposure crayfish will be euthanized and crayfish tissue analyzed via LC-MS to measure 6PPD-q concentrations.

2. Phase 1 Methods:

Crayfish will be exposed to five different aqueous concentrations of 6PPD-q (0 µg/L, 0.02 µg/L, 0.2 µg/L, 2 µg/L, 20.00 µg/L) for three different exposure periods (24 hrs, 48 hrs, and 96 hrs) (Figure 1). At the completion of each pre-determined exposure period, duplicate crayfish will be removed from the tanks, measured (length and mass), euthanized by freezing, and stored at -80°C. At the completion of all exposure intervals, individual crayfish will be dissected and specific tissues collected (gills, hepatopancreas, abdominal muscle) for analysis. These tissues will be weighed, homogenized in a glass tube, and 6PPD-q will be extracted from the sample using Solid Phase Extraction (SPE). Finally, tissue extracts will be analyzed for 6PPD-q by LC-MS. Water samples will be collected in duplicate from each tank prior to the exposure experiments and at each exposure interval. These samples will be extracted by SPE and analyzed for 6PPD-q with the LC-MS as described by Brinkmann et al. (2022) and Tian et al. (2022).

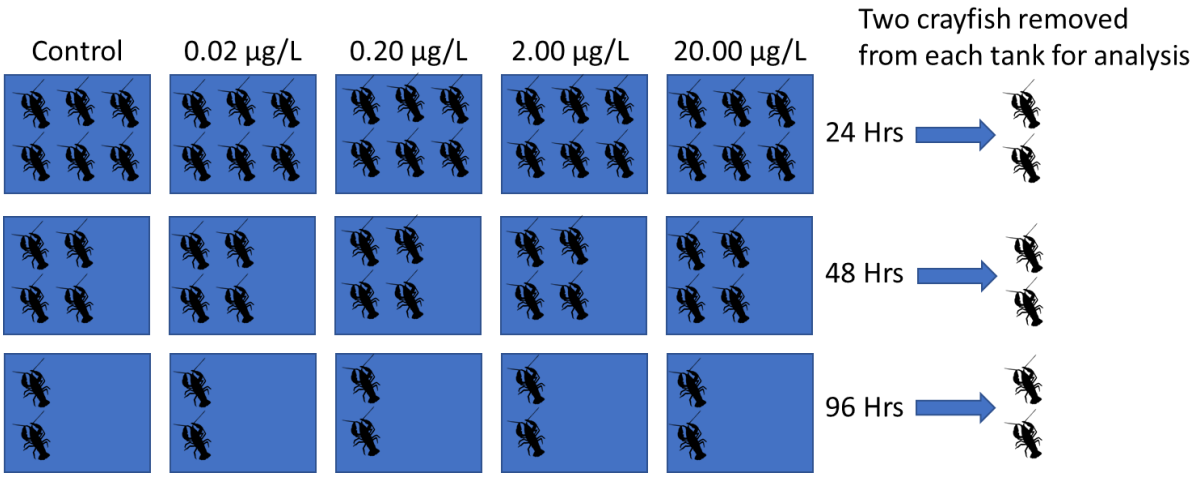

Figure 2: Crayfish Exposure Sample Design

3. Phase 1 Locations:
Signal crayfish will be collected from the Spokane River and rusty crayfish will be collected from the John Day River. Collections will begin following UI acquiring Scientific Collection Permit from WA and OR. All experiments will be conducted in UI's AAL lab. All samples will be analyzed in UI's Mass Spectrometry Core Lab.

4. Phase 1 Schedule:
Experiments will be conducted between April 2023 – May 2023.

5. Phase 1 Quality Assurance and Quality Control:
Lee Deobald will train and assist the graduate student with proper LC-MS techniques and QA/QC will be established before progressing to Phase 2. Jessie Ma will assist in conducting experiments. All equipment will be properly calibrated prior to experiments and analysis. Data will be recorded by the graduate student and checked by Jessie Ma, Lee Deobald, and Mary Engels. All data entered into the digital Master spreadsheet will be checked monthly by Mary Engels (see Templates 14 and 16).

6. Phase 2 Sampling Design:
Sampling sites throughout the middle and upper CRB will be chosen in collaboration with our grant partners using ArcGIS, ERDAS Imagine, and existing crayfish and stormwater data (see Template 8). Sampling sites will be stratified by expected stormwater impact (low, medium, and high). All samples (crayfish, sediment, water, fish) will be stored in pre-labeled glass containers.

7. Phase 2 Methods:
Crayfish will be collected by the graduate student, the undergraduate assistant, Salish School of Spokane students, CFC leaders and volunteers, and MFWP employees. The primary collection method will be with dip nets or hand collections but may also include small baited-minnow traps when wading or snorkeling is not permittable. All crayfish will be identified by species and sex, measured (tip of rostrum to edge of carapace), euthanized by rapid freezing, and placed in a pre-labeled glass containers. Samples will be kept in a cooler until brought to UI lab and stored in 80℃ freezer. Photos will be taken of crayfish samples from each site to avoid confusion regarding species identification. We aim to sample four crayfish specimens from each sampling location. Sediment will be collected by wading into the surface water body and using a spatula to scoop the sediments into a 40 ml glass vial in the upstream direction (Simmons, 2020). Water will be collected from the surface, facing upstream of the current in labeled 40 ml glass vials with no headspace or bubbles (Simmons, 2020). Water temperature, pH, and DO will be measured with YSI. All data will be recorded in field-designated lab book using a standard datasheet template. The graduate student and undergraduate assistant will check recorded data after each sampling event. The PI will check all recorded data each month.

8. Phase 2 Locations:
All sampling sites will be located with the Columbia River Basin. Priority watersheds are the Clark Fork, Pend Oreille, Spokane, and Middle Snake River. Sampling sites will

reflect areas that receive stormwater runoff and have crayfish inhabiting the site. Storm water retentions ponds that are managed by WSDOT will be included in the sampling sites to capture highly contaminated endmember conditions.

9. Phase 2 Schedule:

Phase 2 field sampling will begin in June 2023 and will be ongoing until December 2024. Specific sampling days and times will be determined by UI personnel and grant partners.

10. Phase 2 Quality Control:

Field duplicates and splits, field and lab blanks will be collected routinely. Monthly assessments with graduate student and PI to ensure data collection is meeting Data Quality Indicators and Objectives.

11. Sampling Design QC: Field and Lab

QC Sample Type	**Description**	**Example**
Field Blank	Field: A "clean" sample, produced in the field, used to detect or document contamination during the whole process (sampling, transport, and lab analysis). Clean sampling containers and blank filters.	Water
Equipment or rinse blank	Field and lab: A sample of distilled water is collected in a sample container using and analyzed as a blank sample.	Water
Split Sample	Field and lab: One sample per batch will be divided into two sample containers and analyzed separately.	Water, sediment, crayfish
Co-located samples	Field: Fish will be co-located with crayfish samples with MFWP. Sediment and water samples will be co-located with all crayfish samples.	Crayfish, fish, sediment, water
Replicate Samples	Field and lab: replicate samples of fish and crayfish will be sampled from each site, at the same time, using the same method, and independently analyzed in the same manner. Duplicates will be used as referenced in Template 4.	Crayfish, fish, sediment, water

Spiked Samples	Lab: Known concentration of the analyte (6PPD-q) will be added to water and tissue samples. Percent recovery of the spike material (6PPD-q) will be used to calculate analytical accuracy.	Crayfish, water

B. Data Collection Methods:

1. *Data Collection Methods: Phase 1*

Matrix	Parameter	# of Samples	Sampling Frequency	Total Number of Samples/ Measurements
Crayfish	6PPD-q	68	2 crayfish x 5 concentrations x 3 time steps (24, 48, 96) x 20 species, plus 4 crayfish per species to assess background 6PPD-q tissue concentrations before exposure experiments	68
Water	6PPD-q	~120	2 water samples x 5 conc x 4 time steps (pre-, 24, 48, and 96) x 2 species, plus replicate testing of AAL water sources, crayfish location water, and LC-MS calibration samples.	~120

2. Data Collection Methods: Phase 2

Matrix	Parameter	# of Sampling Locations	Sampling Frequency	# of Samples per location	Total Number of Samples/ Measurements
Crayfish	6PPD-q TWP	40	Typically one time over project duration. Possibly once in 2023 and once in 2024 to re-sample priority site(s).	5 (4 replicates with duplication by halving on 1 sample)	200

Fish	6PPD-q TWP	4	Typically one time over project duration. Possibly once in 2023 and once in 2024 to re-sample priority site(s).	5 (4 replicates with duplication by halving on 1 sample)	20
Water	6PPD-q	40	Typically one time over project duration. Possibly once in 2023 and once in 2024 to re-sample priority site(s).	2 (2 replicates)	80
Sediment	6PPD-q TWP	40	Typically one time over project duration. Possibly once in 2023 and once in 2024 to re-sample priority site(s).	2 (2 replicates)	80

Sample Handling and Custody

Activity	Name/Organization	Contact Information
SAMPLE COLLECTION, HANDLING		
Sample Collection	University of Idaho graduate student and undergraduate assistant will conduct majority of sampling. For collection events with the Salish School of Spokane and Clark Fork Coalition, community scientists will be supervised by UI graduate, undergraduate assistant, and organization volunteer leaders. Sample collections with MFWP will include MFWP staff, UI graduate, and undergraduate assistant. Collection methods will primarily consist of snorkeling and hand collection/dip nets. Baited minnow traps may be used in locations where overnight soaking is permitted and necessary (i.e. deep water). All samples will be identified by species, sex, and measured with calipers in the field. If native females with eggs are collected, they will be carefully returned to the water where they were originally collected. For sampling events with CFC volunteers and Salish School students, crayfish and other macroinvertebrates will be identified by community scientists and UI personnel. Crayfish samples will be collected for LC-MS analysis but other macroinvertebrates will be returned to stream.	Mary Engels
Sample Handling	For Phase 1, samples will be collected and stored alive in a cooler with water from the site and transported back to UI lab for exposure experiments. All samples for Phase 2 will be euthanized in a glass container on ice and stored in a separate pre-labeled glass container. Immediately following euthanasia, they will be stored in a cooler with ice and graduate student will transport samples to the University of Idaho's AAL. Samples will be put in -80°C freezer for at least 24hrs before graduate student processes for dissection and LC-MS preparation. Crayfish hepatopancreas, gills, and abdominal muscle (tail) will be homogenized in a glass tube and wet weight will be recorded. Samples will then be processed for LC-MS by graduate student.	Mary Engels

A. **Sample Identification Procedures:**
When a crayfish is collected in the field, its species will be immediately identified and recorded. All collected samples of the same species will be kept in the same labeled container (following euthanasia) and a photo will be taken of grouped samples. If concerns about accurate species identification arise, additional photos will be taken, and sample will be placed in a separate labeled container. Photos will be sent to crayfish experts (i.e. Dr. Eric Larson, Rick Reynolds) for species confirmation.

B. **Chain-of-Custody Procedures:**

All pertinent field data will be recorded in a field-designated notebook or on field datasheets. Once graduate student and undergraduate assistant have returned to UI, all lab data will be recorded in a lab-designated notebook which will remain in the AAL. All pertinent information (date, weather, name of samplers, species, sex, and length of crayfish collected, volume of water and sediment collected, and water parameters will be recorded in field-designated notebook. The lab-designated notebook will contain pertinent lab information (date, sample location, mass of tissues). All field and lab data will be scanned to a UI computer and stored in a file with the same folder name as the specific sampling event (i.e. M.Engels_Spokane23). Following LC-MS analysis, all results will be recorded and stored in the designated sampling event folder. Additionally, results will be uploaded to the website after each sampling season.

Data Sheets: Data sheets are preprinted forms on which calibration checks, field and lab measurements, and observations are documented. When used in the field, data sheets are completed and relinquished to the graduate student. Data sheets are archived by sampling event in a permanent digital file for storage. Hardcopy and digital copies of these data are maintained by the graduate student and PI. LC-MS chromatography data will be integrated with TargetLynx software, and the results will be exported and imported into a spread sheet along corresponding sample identification information to be sent to the P.I. for digital hardcopy archiving. LC and MS analytical conditions will be saved in a digital format to be archived with the resulting data. LC-MS data will be stored as digital copies on UI server and entered in the Master spreadsheet by the graduate student.

Equipment/Instrument Maintenance, Testing, Inspection and Calibration

A. Analytical Equipment and/or Instrument: Lab

Analytical Equipment and/or Instrument	Calibration Procedure	Frequency	Acceptance Criteria	Corrective Action	Responsible Person	Analytical SOP Reference
LC-MS	Per manufacture instructions	Daily (or per run)	$r^2 > 0.995$ ICV recovery +/- 15%	1. recalibrate 2. prepare new calib standards 3. instrument maintenance	PI, graduate student, Lee Deobald	Manufacture instructions
Analytical Balance	Per manufacture instructions	Per use	+/- 1 division	1. Recalibrate with known mass 2. Service Replace	PI, graduate student, lab assistants	Manufacture instructions
Micropipettes	Per manufacture instructions	Weekly or as needed	<= 1%	1. Recalibrate 2. Service 3. Replace	PI, graduate student, lab assistants	Manufacture instructions
Dissecting Microscope	Per manufacture instructions	As needed	Improved focus	1. Adjust 2. Service 3. Replace	PI, graduate student	Manufacture instructions

B. Analytical Equipment and/or Instrument: Field

Equipment Type	Inspection Frequency	Type of Inspection	Calibration Frequency	Post Check Criteria	Available Parts	Maintenance	Record Keeping
YSI, Temp	Each sampling occurrence	Battery life, electrical connections,	Annually against triple point of water	N/A	Spare batteries	Annually or as needed	Logbook notation

		membrane condition					
YSI, DO	Each sampling occurrence	Battery life, electrical connections, membrane condition	At beginning of each sampling day, reconfirm after every 5 samples and at the end	Saturated air and zero-DO (<.5 mg/L) checks at beginning of day	Spare batteries, membrane	Annually or as needed	Logbook notation
YSI, pH	Each sampling occurrence	Battery life, electrical connections, membrane condition	At beginning of each sampling day, reconfirm after every 5 samples and at the end	Standard pH 7 solution reads 7.0 +/- .2 SU	Spare batteries	Annually or as needed	Logbook notation

Analytical Methods

A. Analytical Methods: Lab

Matrix	Parameter	Reporting Limit	Analytical & Preparation Method	Sample Volume	Containers (#, size, type)	Preservation Requirements (chemical, temperature, light protected)	Maximum Holding Time (preparation and analysis)
Tissue	6PPD-q	LOQ: 10 ng/L	Formic acid and methanol	0.5 g	Glass	Store on ice immediately, store in –80 °C freezer	28 d
Water	6PPD-q	LOQ: 10 ng/L	Formic acid and methanol	0.1 L	Glass	4 C	28 d
Sediment	6PPD-q	LOQ: 10 ng/L	Formic acid and methanol	5 g	Glass	4 C	28 d
Gut Content	TWP	N/A	KOH	As found	Glass	20 C	28 d
Sediment	TWP	N/A	KOH	10 g	Glass	20 C	28 d

B. Water Quality Analytical Methods: Field

Matrix	Parameter	Reporting Limit	Analytical Method
Surface Water	Temperature	0.1°C	YSI
Surface Water	Conductivity	0.001µS (0-500µS); 0.01 mS (0.501-50.00 mS); 0.1 mS (50.01-200 mS)	

Surface Water	DO	1% or 0.1% air saturation (user selectable) OR 0.1 or 0.01 mg/L (user selectable)	YSI
Surface Water	pH	0.01 units	YSI

Field and Analytical Laboratory Quality Control (QC) Summary

Matrix	Analytical Group or parameter	Quality Control (QC) Sample Type	Frequency or Number of QC samples	Method or SOP QC Acceptance criteria or DQI goals (See Template #4)	Corrective Actions
Tissue	6PPD-q	Method blank	1 per digestion batch	<RL	New digest if sufficient sample
Water	6PPD-q	Method blank	1 per digestion batch	<RL	New digest if sufficient sample
Sediment	6PPD-q	Method blank	1 per digestion batch	<RL	New digest if sufficient sample

Data Management

A. Data management process and procedures:

Data from Phase 1 experiments will be recorded in laboratory-designated notebooks and the graduate student will create a standardized datasheet to be completed for each experiment. This datasheet will include sections to record water temperature, pH, dissolved oxygen levels, 6PPD-q exposure concentrations and duration, crayfish species, sex, mass, and length, tissue concentrations, and any relevant observations. Data will be recorded as hard copy by the graduate student and checked by Lee Deobald and Mary Engels on a weekly basis. The graduate student will enter the data into the digital Master spreadsheet each week and the Master spreadsheet will be checked by Mary Engels each month. Additionally, a datasheet for QA/QC relative to LC-MS will be kept with the laboratory notebook. These QA/QC checks will be reviewed weekly during the experiment duration (March 2023 – May 2023) by Lee Deobald. Hard copies of data from the datasheets and laboratory-designated notebook will be scanned and uploaded as digital copies. Data will be stored in folders labeled by the day the experiments were performed. The digital Master spreadsheet will be stored on a UI desktop computer. Graduate student will perform weekly QC checks on the digital Master spreadsheet and the PI will perform QC checks each month. LC-MS chromatography data will be integrated with TargetLynx software, and the results will be exported and imported into a spread sheet along corresponding sample identification information to be sent to the P.I. for digital hardcopy archiving. LC and MS analytical conditions will be saved in a digital format to be archived with the resulting data. Digital output of LC-MS runs will be stored on UI server in folders pertaining to experimental date(s).

Data for Phase 2 field sampling will be recorded in field-designated notebooks. Field data will include quantitative data (measurements from the YSI), species identification, photographs of crayfish samples, and photographs of each sampling site. Field data sheet templates will be used for consistent data collection. Data sheet templates will include sections for the data collectors (graduate and undergraduate students) to write the geographical coordinates (from GPS), field parameters (as described in the Quality Assurance Plan), sampling duration, date, time, crayfish sampling details (sex, species, length, amount collection) and any other relevant observations. Volunteers participating in events with the CFC and students from SSS may collect data for their own use, but only data confirmed and recorded by the undergraduate and/or graduate student will be included in the Master spreadsheet. Qualitative data may include written descriptions, illustrations, and photographs of each sampling site. Quantitative datasheets will be created by the graduate student and these datasheets will be kept with field-designated notebooks to ensure consistency in data collection and chain-of-custody procedures. Hard copies of data from the datasheets and field-designated notebook will be scanned and uploaded as digital copies. Undergraduate and graduate student will perform QC checks on field data before, during, and after sampling to ensure data quality objectives are being met. The PI will perform QC checks on field-collected data each month. LC-MS chromatography data will be integrated with TargetLynx software, and the results will be exported and imported into a spread sheet along with corresponding sample identification information to be sent to the P.I. for digital hardcopy archiving. LC and MS analytical conditions will be saved in a digital format to be archived with the resulting data. Digital output of LC-MS runs will be stored on UI server in folders pertaining to field collection date(s).

Field datasheets will be reviewed after each sampling event and verified/validated before being entered into the Master spreadsheet by the graduate student. All field-designated notebooks will be maintained and handled by the graduate student and Mary Engels. The graduate student will enter all data from field datasheets and will flag any unusual, unvalidated, or incomplete data for further review by Mary Engels. The PI (Mary Engels) will determine if data meets the Data Quality Objectives before data analysis process begins.

Lab data from Phase 2 samples will be recorded by the undergraduate student in the laboratory-designated notebook and checked by the graduate student before, during, and after sample processing in the laboratory. A Phase 2 laboratory datasheet template will be created by the graduate student for consistent data collection methods. Laboratory datasheet template will include a section for identifying the sample(s) ID, who is present during lab processing, date, time, crayfish (or fish) species, tissue(s) to be analyzed, what is going to be processed (crayfish, fish, water, sediment), and the type of analysis (TWP, 6PPD-q) to be performed. A processing section will reference data for crayfish (or fish) species, mass, length, tissue mass, sample volume to be analyzed. A results section will include data referencing the sample ID and TWP/6PPD-q concentrations for each sample that was analyzed. Before the graduate student enters data into digital Master spreadsheet, QC checks for any data recorded on the datasheet in the laboratory-designated notebook by the undergraduate assistant will be conducted. The graduate student will flag any unconfirmed or discrepancies in the data for the PI to review. The PI will complete data entry QC checks each month. If the PI deems any data was entered incorrectly, does not relate to data quality objectives, or was subjected to analytical error, the PI reserves the right to remove this data.

PCA analyses will be conducted in R (R Core Team, 2022) by the graduate student to elucidate geographic trends related to 6PPD-q concentrations and occurrences in aquatic systems. Standard statistical tests (ANOVA, T-Tests, Chi-square test, etc.) will be conducted by the graduate student after the PI has verified and validated all data in the Master spreadsheet. The PI and graduate student will collaborate monthly to evaluate the impacts of the data to ensure Data Quality Objectives are being met.

Reporting, Oversight, and Assessments

Type of Report	Frequency (Daily, weekly, monthly, quarterly, annually, etc.)	What is being Assessed	Person(s) Responsible for Report Preparation (Title and Organizational Affiliation)	Report Recipient(s) (Title and Organizational Affiliation)
EPA Quarterly	Quarterly throughout project duration	Field and lab data, project objectives timeline	PI	EPA, Grant Partners
Project Status Assessment	Quarterly throughout project duration	Project objectives, sampling design and locations	Graduate Student, PI	Grant Partners
Assessment of Data Checks	Monthly throughout project duration	Field data entries into Master spreadsheet and UI database	Graduate Student	PI
Lab Inspection	1 week into exposure experiments (March 2023 – May 2023)	Lab procedures and outcomes, assess project objectives	Graduate Student, Lee Deobald, Jessie Ma	PI
Phase 1 Lab Assessment	Once, following completion of experiments (May 2023)	Assessment of crayfish as an indicator species for 6PPD-q	Graduate Student, Lee Deobald, Jessie Ma, PI	Grant Partners, EPA
Field Sampling Inspection	Monthly throughout field sampling season (June 2023 – October 2024)	Assess if field sampling is satisfying project goals	Graduate Student, undergraduate assistant, PI	Grant Partners

Reporting accurate results from Phase 1 experiments will be crucial to the success of the project. For this reason, the graduate student, PI, Jessie Ma, and Lee Deobald will conduct QA/QC checks on all collected and entered data before reporting to grant partners and EPA. Reporting for Phase 1 and Phase 2 will be conducted by the PI and graduate student who will present the report to grant partners before preparing report for the EPA. Quarterly reports for the EPA will include an assessment of how the project is meeting deliverables stated in the QAPP.

Deviations from project objectives will be addressed immediately by the PI to ensure the goals stated in this QAPP are achieved. The Quality Assurance Plan will be referenced frequently to

ensure the project goals and Data Quality Objectives are met. Our collaborative approach will help to identify any potential problems associated with the collection or processing of data for this project. All data will be reviewed, validated, and verified by several personnel frequently throughout each phase of the project. This will provide ample opportunity to identify any potential data quality issues and address the need for solutions quickly. If problems are identified, or if collected data does not appear to be meeting the project goals, Mary Engels and experienced personnel on this project will define and implement effective actionable responses. If necessary, changes to this Quality Assurance Plan will be made with approval from the EPA to ensure project goals and Data Quality Objectives are met, as agreed upon by the grant recipients and EPA.

Data Review and Usability

A. Data Review:

As part of the data review and validation, all field and lab data will be reviewed and discussed by the PI to determine if the data meet the objectives as outlined in the QAPP. The graduate student will flag any data in the digital Master spreadsheet to highlight for PI review. PI will review all data quarterly throughout the duration of the project. Decisions will be made to accept or reject the data before presenting the information in any presentations or reports. Errors in data entry will be corrected and any outliers will be flagged for further review. Any data deemed to be not acceptable will be noted in the comments fields of the program database (Master spreadsheet) and will be removed from any statistical calculations.

B. Data Verification and Validation:

All data reported by the graduate student will be subject to checks by the PI for errors in transcription, calculation, or computer input. Additionally, all datasheets will be reviewed to ensure that they are completed and signed by the field and lab data collector(s). Any changes made to the datasheets must be initialed and dated, and any action taken because of the data review must be recorded on the data form below the reviewers signature. Only data that meets the following conditions will be accepted and entered into the digital Master spreadsheet:

- LC-MS analysis must have been conducted by Lee Deobald, Mary Engels, or graduate student (or properly trained UI personnel).
- Data entries are signed, legible, and have the appropriate information with accordance to the QAPP.
- Equipment has been calibrated correctly and checked prior to data collection.
- Data was collected by appropriate levels of training for the data being conducted (Jessie Ma, Lee Deobald, Mary Engels, graduate student, undergraduate assistant).

C. Data Usability:

All data will be validated by Mary Engels to ensure Data Quality Objectives are being met. If the PI determines that Data Quality Objectives are being compromised or that data is not meeting QC acceptance criteria, data will be flagged for consideration of rejection from the dataset. All data must be reviewed, verified, and validated by Mary Engels before being used for reporting.

D. Data Presentation:

Data related to Phase One experiments will be presented in a written report that will also include photographs of the experiments (taken by graduate student) and visual representations of the data analysis performed using R (R Core Team, 2022). PowerPoint will be used when presenting the data to the EPA and other interested groups (as permitted by the EPA). Phase Two data will be presented in a written report and will include photographs of participants, sampling sites, and crayfish. Visual representations of the data will be performed using R (R Core Team, 2022) and the ArcGIS interface will be used for the geographical representation of 6PPD-q concentrations in the CRB. Additionally, the

public-facing website will visualize our project results through a storyboard with the ArcGIS interface.

Project Organization

Name	Title	Organization Affiliation	Responsibilities
Mary Engels	Assistant Professor, PhD	University of Idaho	Primary Investigator (PI) is responsible for management of this project. At minimum, the PI is responsible for obtaining adequate equipment and supplies, managing sampling process, scheduling, reporting, and taking constructive corrective actions when required.
Jessie Ma	Research Assistant Professor, PhD	University of Idaho	CNR-AAL
Elizabeth Herrmann	Research Assistant	University of Idaho	Field and lab technician, sample coordinator, data recorder, reporting
Lee DeoBald	Director, Mass Spectrometry Core Lab, PhD	University of Idaho	LC-MS Training
David Schmetterling	Research Coordinator	Montana Fish, Wildlife & Parks	MT Sampling Coordinator
Dominique Wiley-Camacho	Lead Teacher/Science Specialist	Salish School of Spokane	Developing Salish School Curriculum
Julia Crocker	Community Programs Coordinator	Clark Fork Coalition	MT Community Sampling Coordinator
Matthew Cox	Dangerous/Hazardous Waste Compliance Manager	Washington Department of Transportation	WA Sampling Coordinator
Lucas Sheneman	Director of Research Computing Data Services	University of Idaho	Website Development

Project Distribution List

Name	Title	Address	E-mail
Lauren Mcdaid	EPA Project Officer		Mcdaid.Lauren@epa.gov
	EPA QA Chemist		
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