Standard Operating Procedures:

6PPD-Quinone Sampling

Version 5

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BRITISH COLUMBIA CONSERVATION FOUNDATION

VANCOUVER ISLAND UNIVERSITY

AERL
Applied Environmental Research Laboratories

OVERVIEW

This standard operating procedure (SOP) is to guide the collection of water samples that are to be analyzed for 6PPD-quinone (6PPDQ) contaminants associated with tire particle leachates and roadway runoff. Samples will be processed by the Vancouver Island University's (VIU) Applied Environmental Research Laboratory (AERL) to determine the presence and concentration of 6PPDQ in aquatic habitat of vulnerable salmonid species, including coho salmon (*Oncorhynchus kisutch*), rainbow trout (*O. mykiss*), and Chinook salmon (*O. tshawytscha*), along the Vancouver Island coastline.

This includes pre-trip preparations and field operations. This SOP is intended for use by staff, contractors, and volunteers with the British Columbia Conservation Foundation (BCCF).

EQUIPMENT LIST

Sample kit should include:

- Cooler
- Ice packs
- Nitrile gloves
- Pre-labelled (QR code) 250-mL amber glass bottles
- Thermometer
- Rain gauge
- Water quality equipment (sonde and turbidity metre) (optional)
- Phone or tablet equipped with the data collection app
- Datasheet (in the event that a phone/tablet fails)
- Pencil
- Sample pole (optional)

PREPARATION

Sample Kit

Amber glass bottles (250 mL) will be used to collect the water samples. **Ensure that the inside of the bottle and lid are <u>not</u> touched during handling.** Sample bottles will be prepped by BCCF/AERL prior to sampling activities; they will each include a label that has a distinct QR code.

Ensure that provided ice packs are frozen before heading to the sample location(s).

Figure 1. 6PPDQ sample collection kit

Cautionary note: Please ensure that sample kits and containers are **not** stored near car and/or bicycle tires before, during, or after sampling efforts. The close proximity could result in contamination of the samples. If there is potential of contamination, please include notes in the 'comments' section of the data collection app and/or notify BCCF via email (tireweartoxins.bccf@gmail.com).

Pre-Trip

Determine the best and safest access location(s) for the sample location(s). Sites can be accessed by parking on the side of highways and roadways and hiking down to the selected waterway(s). Ensure that safety measures are followed, and safety equipment is used when accessing the site(s). If any safety equipment is required for your site, please inform BCCF prior to accessing and equipment can be arranged for drop-off or installation, where applicable.

Prior to leaving your home and a Wi-Fi connection, be sure to re-open the data collection app, allowing it to update/sync any changes, ensuring that your version is the most up to date.

Sample collection app

Prior to heading into the field, ensure that you have downloaded the data collection app onto your phone. The app can be downloaded from the App Store or the Google Play Store by searching for AppSheet.

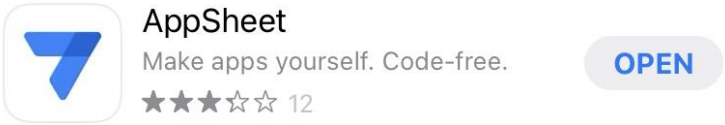

Figure 2. Image of AppSheet icon and name, which can be downloaded from the App Store or the Google Play Store.

Once downloaded, you will need to make an account as this will allow for better offline performance. Once an account has been created, you can click this link or use the camera on your phone/tablet to scan the QR code below (Figure 3) and open the data collection form. When it opens in the browser, it should then redirect you to AppSheet. Once in AppSheet, select "Install", underneath the AERL logo. These steps should only be required once, and the form required for sample collection should stay within AppSheet for all future use.

Figure 3. QR code to access the data collection form on AppSheet.

Prior to every sample date, it is best to re-sync the app to ensure you have the most up-to-date version.

A step-by-step tutorial on how to download the data collection app can be found here.

All data that is submitted via the data collection app that was built specifically for this program, is linked to the sample ID and is recorded in a publicly available database (including any photos taken with the app). Once the sample is analyzed, the concentration of 6PPDQ is stored in a

private Dropbox folder viewable only by project partners at VIU AERL and BCCF ARRC. The data is then vetted by the researchers and released to the publicly available interactive database.

Sampling Conditions & Effort

BCCF will monitor weather conditions and notify sampling teams when a sampling window is approaching. Samples will be collected at pre-selected locations, typically downstream of a potential 6PPDQ point source (i.e., roadway, bridge) before, during, and after the rain event. This method is used to ensure that the lab is ready and available to take in a large number of samples all at once.

If there is a specific rain event that you're interested in sampling because of a specific event (i.e., fish kill, clearly impacted water quality, etc.), please reach out to the BCCF sample coordination team at tireweartoxins.bccf@gmail.com and we can arrange special pick-ups.

FIELD ACTIVITIES

Rain Gauge

Rain gauges will be utilized to capture site specific rainfall and compare those results to nearby weather stations to assess the variability in the region. Not all sample locations will be equip with a rain gauge and it'll be explained to each group which location should have a rain gauge deployed.

For those that are provided a rain gauge for their sample location(s), they will be deployed at the 'before' sample. To deploy the rain gauge, it can be pushed into the ground using the spike on the bottom. It should be placed in an open location with minimal tree cover above it (where possible). If possible, it should be tucked away from footpaths and busy areas to reduce the risk of disturbance/vandalism.

The rain gauge should be checked, and the volume should be recorded (see Data Collection below) when on site for each of the 'during' and 'after' sampling efforts. Following the 'after' sample collection, the rain gauge should be removed and kept with the sampling kit, deployed at the next 'before' sample.

Data Collection

When you open the app, it will be displaying the AERL Map, which shows where all samples have been located to date.

In order to collect data for your sample, select the 'Submit sample' page, located in the bottom right corner of the screen (Figure 4).

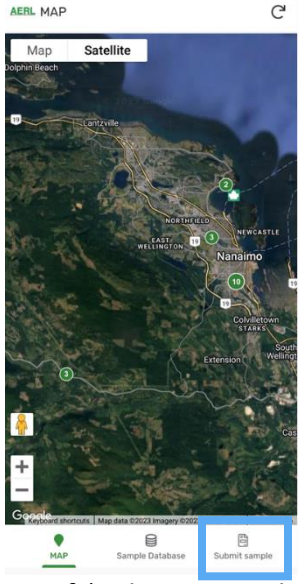

Figure 4. Image depicts location of 'Submit sample' option in the data collection app.

Once on the appropriate screen, fill in the information requested by the data collection app. Each heading below will discuss the data field in more detail and what is required of the data collector. **One submission will be required for each sample collected.**

Samplers: Please record at least the first name or initials of all those that contributed to collecting the sample.

Sample ID: The sample ID refers to the sample label (QR code) on the bottle. Select the icon on the right side of the sample ID field (Figure 5) and 'allow' the app access to your camera (you should only need to do this the first time). Point your camera at the QR code on the bottle. It should recognize the QR code and auto-populate the field. In the event you accidently scan the wrong QR code (i.e., a bottle that has been previously used), you can highlight what populated the field, delete it, and re-scan the correct bottle. In the event you scan the wrong type of QR code (i.e., the location code), the app will notify you that it is incorrect and you will need to then scan the correct QR code on the bottle.

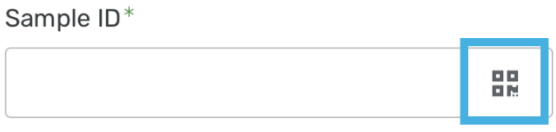

Figure 5. Image depicting the icon to select in the sample ID field to pull up the phone/tablet camera and scan the bottle's QR code.

Sample Location: Sample locations were pre-determined by BCCF/VIU/project partners. For all sites that a specific group will be sampling, a set of laminated cards will be included in the sample kit. Each card includes the site name, Google Earth birds-eye view of the location, and a site-specific QR code (**Figure 6**). To fill in the sample location, select the site's corresponding card, click the icon on the right side of the sample location field and point your camera at the QR code on the card. It should recognize the QR code and auto-populate the field. In the event you scan the wrong type of QR code (i.e., a sample code), the app will notify you that it is incorrect and you will need to then scan the correct QR code on the location's card.

Figure 6. Sample location card for one of the pre-selected 6PPDQ sample locations. The site card includes the site name, a Google Earth image with approximate location for reference, and a QR code.

Sample Type: Select the type of sample that you are collecting:

- ***Before:*** Stream sample is to be collected prior to the rain event; see 'Stream Sample Collection' below.
- ***During:*** Stream sample(s) are to be collected during the rain event; see 'Stream Sample Collection' below.

- ***After:*** Stream sample is to be collected following the rain event, approximately 24 hours after the first 'during' sample; see 'Stream Sample Collection' below.
- ***Field Blank:*** Sample is to be collected during the rain event; see 'Field Blank Sample Collection' below.
- ***Point Source:*** Sample is to be collected during the rain event; see 'Point Source Sample Collection' below.
- ***Other:*** The sample details will be defined by BCCF/VIU prior to sampling, where necessary; see 'Other Sample Collection' below.

Fish Visible?: Look to see if any fish are present. Select yes or no based on your observations.

Rain Gauge: If a rain gauge was deployed at the site, record the volume present in the rain gauge when on site for the 'during' and 'after' samples. If no rain gauge is present, leave it blank.

Water Temperature (dC): Using the thermometer in the sample collection kit, record the temperature (in degrees Celsius) in this field. You can click directly on "0.00" and update it with the keyboard that pops up.

Date Time: This field should auto-populate anytime the 'Sample ID' field is updated/changed. **Note:** Be sure to double check it to ensure it is correct.

Location: This field should auto-populate anytime the 'Sample ID' field is updated/changed. **Note:** Be sure to double check where it shows you on the map to ensure it is accurate. If your location appears as [0.00000 0.0000], please fully close the app, re-open it and re-scan in the QR codes again. In the event this does not fix the location issue, you may need to go through your settings into the app itself and 'allow' permissions to see your location when using the app.

Site Photo: Select the camera icon and choose 'take a photo'. Take a photo directed upstream of the sample location. A site photo will not be required for field blank samples.

Additional WQ [optional]?: If you have the ability to collect additional water quality data, select yes and fill out the information that the equipment you have will provide. There is space for pH, dissolved oxygen (DO; in percentage), specific conductivity (µS/cm), and turbidity (NTU). This information is only applicable to stream samples, it is not required for the field blank and point source samples.

Comments [optional]: This is the space to add any specific comments or concerns with regards to the specific sample. There is no character limit, so if something went wrong when collecting the sample, please provide as much detail as you can.

Once all data is collected, select the 'Save' option in the bottom right of the screen. Once selected, a 'Submission Receipt' should open (**Figure 7**). **Please take a screen shot.** Once you know that the data has been uploaded into the database, you can delete your screen shot(s).

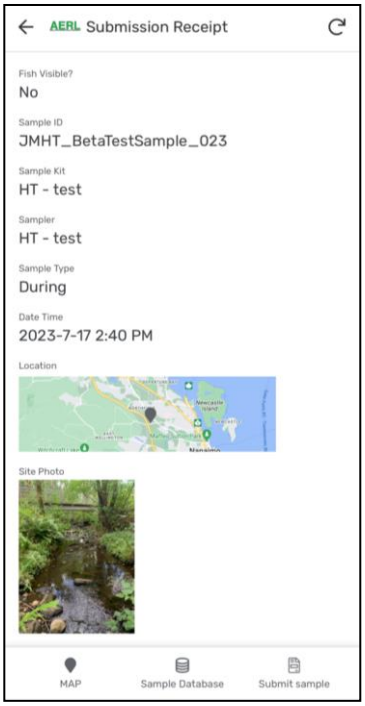

Figure 7. Screenshot of the 'Submission Receipt' page on the data collection app.

Note: If you are collecting information with your phone's data off, you will need to submit the data when you acquire Wi-Fi. To do so, open the app and select the curled arrow in the top right corner. When there is data to upload, it will show the number of files that need to be uploaded in a small orange circle (Figure 8). Once the data is uploaded, the circle will disappear. **To ensure no data is lost, please be sure to take a screenshot of the 'Submission Receipt' page for reference.**

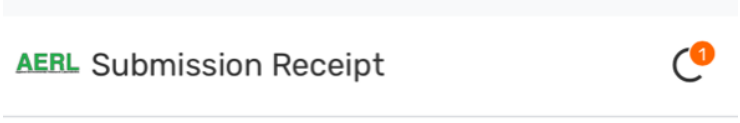

Figure 8. Image depicting the curled arrow and number of files (1) that still require uploading. The icon will appear in the top right corner of the screen following data submission.

To collect another sample, just select the 'Submit sample' tab to begin filling out the next site's specific data.

Stream Sample Collection

Data collection required for stream samples includes: sample ID, sample location, sampler(s) names(s), sample type (before, during, after), fish visible, water temperature, date time, location (GPS), comments [optional], a site photo, rain gauge [site specific], and additional water quality [optional].

Each kit will be equipped with five empty amber glass sample bottles per site for stream samples: before (1), during (1 – 3), and after (1). One sample (the 'before') is collected before the rain starts. Another sample (the 'during') is collected shortly after the runoff beings, when possible (i.e., as close to the start of heavy runoff as possible). Finally, the 'after' sample is collected approximately 24 hours after the 'during' sample was collected.

Depending on the capacity of the sampling individual(s), samples can be collected up to three times while it's raining. The first sample should be collected shortly after runoff begins. The other two samples can be collected based on the individual(s) capacity and schedule within the 24-hour window between the first 'during' sample and the 'after' sample. All samples collected between the 'during' and 'after' samples should be labelled 'during' samples in the data collection app.Stream sample collection methods:

1. Wearing nitrile gloves, open the amber glass bottle and ensure that the inside of the bottle and cap are **not** touched.
2. Use the water on site to rinse the bottle and lid three times.
3. With the cap removed, submerge the bottle upside down.
4. When the bottle is submerged, turn it into the flow and allow it to fill until it is full.
5. When full, secure the lid on the bottle and place in the cooler with the frozen ice packs.

Note: If in a location where safe access is an issue, use a sample pole/scoop to collect the sample. Sample poles are equipped with an end that the sample bottle can be safely placed and secured into. Like when doing it by hand, ensure that the sample bottle is rinsed three times prior to filling it completely and capping it.

Field Blank Sample Collection

Data collection required for field blank samples includes: sample ID, sample location, sampler(s) name(s), sample type (field blank), date time, location (GPS), and comments [optional].

The purpose of collecting field blanks is to detect any onsite contamination that may be occurring during sample handling. Field blanks are simply samples of deionized water that are handled (i.e., collected, filtered, and preserved) in the same conditions that the stream and point source samples are. You will be provided with one 250 ml amber glass bottle full of deionized water for each site and each rain event. Field blanks are to be collected at the same time as the 'during' sample.

2024 Update: Unless directed to, field blanks will not be collected by volunteers. BCCF/VIU will continue collecting a set number of field blanks per rain event.

When opening and handling the amber glass bottles, ensure that the inside of the bottles and caps are **not** touched.

Field blank sample collection methods:

1. For each location you will be sampling, you will need to bring an empty, pre-labelled (QR coded) 250 ml amber glass bottle (the deionized water will be poured into on site).
2. When at the site, the person collecting the stream 'during' sample will do so first.
3. After the stream sample is collected (with the same nitrile gloves still on), open both field blank bottles (one empty and one full).
4. Pour the full bottle of deionized water into the empty one and recap both.
5. When full, secure the lid on the bottle and place in the cooler with the frozen ice packs. Continue to store field blank samples with the rest of the samples collected until sample pick-up.

Note: A new 'Submit sample' form needs to be filled out for each field blank sample collected at each site. Ensure you **scan the QR code on the empty** field blank bottle (i.e., the one you will pour your sample into and will be submitted to the lab).

Point Source Sample Collection

Data collection required for point source samples includes: sample ID, sample location, sampler(s) names(s), sample type (point source), date time, location (GPS), a site photo, additional water quality [specifically turbidity; optional], and comments [optional].

Point source samples are to be collected for known point sources at the pre-selected sample locations. Each point source will have its own location card in the sample kit; therefore, you will need to use the separate QR code for the point source location.

Point source sample collection methods:

1. Wearing nitrile gloves, open the amber glass bottle and ensure that the inside of the bottle and cap are **not** touched.
2. Use the water flowing from the point source to rinse the bottle and lid three times.
3. After rinsing, fill the bottle until it is full.
4. When full, secure the lid on the bottle and place in the cooler with the frozen ice packs.

Note: A new 'Submit sample' form needs to be filled out for each point source sample collected at each site. Ensure that you **scan the QR code on the point source bottle and the specific point source location card** when submitting data.

"Other" Sample Collection

Data collection required for "other" samples includes: sample ID, sample location, sampler(s) names(s), sample type (other), water temperature, date time, location (GPS), comments [optional], a site photo, rain gauge [site specific], and water quality parameters.

"Other" samples will only be collected when requested/directed by the BCCF/VIU team. These samples may include routine samples (i.e., collected on a set schedule, regardless of rain events), when testing green infrastructure, etc.

"Other" sample collection will follow the same methods as all other sample collection, unless otherwise specified by the BCCF/VIU project team:

1. Wearing nitrile gloves, open the amber glass bottle and ensure that the inside of the bottle and cap are **not** touched.
2. Use the water flowing from the identified "other" sample location to rinse the bottle and lid three times.
3. After rinsing, fill the bottle until it is full.
4. When full, secure the lid on the bottle and place in the cooler with the frozen ice packs.

Note: A new 'Submit sample' form needs to be filled out for each "other" sample collected at each site. Ensure that you scan the QR code on the bottle and the specific "other" location card when submitting data.

SAMPLE COLLECTION PROTOCOL RESOURCES

To allow for viewing anytime, the research team prepared video tutorials on how to collect each different type of sample. Links are below for each type of sample:

- 'Before' sampling
- 'During' sampling
- 'After' sampling

SAMPLE HANDLING & HANDOFF

When returning home from sample collection, store water samples either in a cooler with frozen icepacks or in a fridge until handoff. Ensure that the samples **do not freeze**.

Following a rain event, all samples from each sample site will need to be handed off to BCCF. The BCCF sample coordination team will remain in contact with those sampling and establish the sample pick up locations. Contact information for the BCCF sample coordination team is as follows:

- Email: tireweartoxins.bccf@gmail.com
- Phone: (250) 390-2525 ext. 106

Once samples are collected, BCCF will hand them off to VIU's AERL for 6PPDQ analysis.

DATA RESULTS & UPDATES

The BCCF and AERL project team anticipates that results will be available to volunteers within two to three weeks of sample pick-up. Results will be directly uploaded onto the sampling program's online data dashboard and volunteers will be notified via email when results are updated.

In addition to the online results database, the highlighted results will be shared bi-annually in a digital newsletter that will be sent to the entire group of project volunteers, partners, and funders. Further, there will be a yearly workshop where results will be shared, and up-to-date research will be shared with volunteers, partners, and funders interested in attending.