TECHNICAL MEMORANDUM

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Subject: 6PPDQ Roadway Runoff Stormwater Composite Sampling Protocol Recommendations

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Introduction

This technical memorandum presents sampling protocol recommendations for using automated samplers to measure 6PPDQ in stormwater. The recommendations are based on a review of literature on the topic and on the 2023–2024 Field Protocol Sampling Study (field protocol study), which was designed to assess the potential loss of 6PPDQ to various sampling materials used during automated sampling programs. The project is funded by the Washington State Department of Ecology (Ecology). The monitoring project's technical objectives, Ecology's related project requirements, and field protocol study results are described in the 2023–2024 Summary Report (Herrera 2024b).

A common method of characterizing chemical constituents across a storm event is the use of flow-weighted composite sampling. In flow-weighted sampling, flow rate is monitored to trigger the collection of aliquots to represent known volumes of water, which generate a composite sample. A common procedure for flow-weighted sampling is the deployment of an automated peristaltic sampler (autosampler). Autosamplers employ a combination of tubing, a pump, and a carboy. Tubing is placed in the source water, routed through the peristaltic pump, and pumped water is collected in the carboy.

Some stormwater pollutants can chemically adhere (sorb) to different materials, which may result in a decrease of that chemical in the sample analyzed by the laboratory. The loss of 6PPDQ due to sorption to various field equipment is not yet well-characterized nor evaluated for stormwater sampling protocols.

Purpose

The purpose of this technical memorandum is to provide field protocol recommendations for automated composite sampling of 6PPDQ, including sampling methods, sample handling, and selection of sampling equipment. Summary of relevant literature, the results of the field protocol study, limitations of these recommendations, and additional study design considerations are also discussed. Final sampling recommendations are summarized in Table 2 in the Recommendations section.

6PPDQ Properties and Previous Studies

Relevant 6PPDQ properties and previous studies used to inform these recommendations are summarized and discussed in the following sections, followed by a summary of the field protocol study.

Formation and Degradation

6PPD is a rubber antioxidant/antiozonant that is used in automobile tires to extend their lifespan (Tian et al. 2021, 2022; Brinkmann et al. 2022; Hiki et al. 2021). 6PPD is used in tires for its mobility within the tire matrix, reactivity, and ability to neutralize ozone and, to a lesser extent, oxygen (Seiwert et al., 2022). It is therefore expected that 6PPD on a tire or other surface would degrade in the presence of ozone or oxygen. When 6PPD is exposed to air, it reacts with ozone or oxygen to create numerous degradation products, one of which is called 6PPDQ. It is still unclear how long it takes for all the 6PPD to fully migrate out of a tire or its wear particles, but, eventually, the 6PPD will react with oxygen gas or ozone and will be released to the environment in various forms, called transformation products. Recent studies suggest that, upon exposure to the atmosphere and oxidation, about 10 percent of 6PPD in tires transforms to 6PPDQ and that the remaining 90 percent is transformed into other chemical byproducts with unknown fates and toxicities (Hu et al. 2022, Seiwert et al. 2022). Several studies have been conducted related to the transformation of 6PPD to 6PPDQ in the presence of ozone under various conditions (Hu et al. 2022, Zhao et al. 2023, Seiwert et al. 2022); however, more research is needed to understand whether ozone concentration as an environmental variable impacts the transformation of 6PPD to 6PPDQ.

Degradation of 6PPD and 6PPDQ is expected to result from a combination of abiotic and biotic processes (OSPAR Commission, 2006; ECHA, 2021). In the atmosphere, 6PPD undergoes indirect photodegradation via rapid reaction with hydroxyl radicals. Photodegradation is likely a predominant mechanism for 6PPD loss in surficial soils. In water, 6PPD is not “readily biodegradable,” but it is unstable and degrades rapidly by biotic and abiotic processes. Further research is needed to determine 6PPDQ degradation, how long it remains bioavailable and toxic, and how it is transported outside of wet weather events. This is particularly important in Washington State, with a long dry summer season across the state and semi-arid environments to the east of the mountains.

Storage Study

In 2023, King County Environmental Laboratory (KCEL) performed a study to determine the stability of 6PPDQ over time and under different storage conditions. For the study, stormwater was stored in three conditions:

- 1-L amber glass bottles held at 4.5°C
- 10-L FLPE bottles held at 4.5°C
- 10-L FLPE bottles held at -20°C

Each condition was replicated three times. All samples were measured via LC-MS/MS (Zalusky 2023).

KCEL found no significant change in 6PPDQ concentrations of stormwater over the course of 2 weeks (the longest time for which data at all three storage conditions were measured) in any of the proposed storage methods. Therefore, KCEL intends to collect and store stormwater collected for the purpose of 6PPDQ-specific bioassay testing in FLPE containers. These large volume samples will be stored at 4°C for up to 2 weeks, upon receipt at the lab. Water samples for analytical testing of 6PPDQ concentrations will be stored at 4°C in 250 mL amber wide-mouth glass bottles for up to 4 weeks until analysis (Zalusky 2023).

Sorption Study

A study performed by Hu et al. (2023) evaluated the chemical characteristics and stability of 6PPDQ within the context of analytical measurement and laboratory processing. The study assessed sorption to 12 different materials, including the following:

- Sample tubing, polyethylene housing
- Sample tubing, PTFE (polytetrafluoroethylene) lined
- Silicone sample tubing
- PTFE solid phase extraction tubing
- Parafilm
- 15-mililiter polypropylene centrifuge tube
- 10-mililiter fluorinated ethylene propylene (FEP) centrifuge tube
- 15-mililiter glass centrifuge tube
- Rubber stopper
- 1-liter polypropylene sampling scoop
- Bike tire rubber
- Stainless steel laboratory equipment

For 5-minute exposures, which simulated contact times for transfer between containers or rapid contact with sampling equipment or tubing materials, most materials showed low to moderate sorption (greater than 75 percent recovery of residual 6PPDQ). For 30-minute contact periods, which simulated temporary storage or sample processing, more extensive sorptive losses occurred. Glass, stainless steel, and plastics (PTFE, FEP, polyethylene, and polypropylene) had zero to moderate sorption tendencies (greater than 60 percent recoveries), while rubber (bike tire rubber: 35 ± 8.6 percent recovery; rubber stopper: 8.7 ± 3.2 percent recovery) and silicone (silicone sample tubing 25 ± 0.1 percent recovery) promoted substantial concentration reductions. With 24 hours of contact, even more loss to rubber (bike tire rubber: 0.97 ± 0.13 percent recovery; rubber stopper: 0.7 ± 0.21 percent recovery) and silicone (silicone sample tubing: 0.34 ± 0.22 percent recovery) occurred. No significant 6PPDQ sorption occurred for glass (101 ± 0.3 percent, 100 ± 1.3 percent, and 100 ± 0.3 percent residual concentrations at 5minute, 30minute, and 24hour contact, respectively), suggesting glass is the preferred material for labware and processing (Hu et al., 2023).

Rubber and silicone materials have much higher sorption capacities. This likely results from a combination of more porosity and more surface area, in addition to hydrophobic and electrostatic interactions between 6PPDQ and material surfaces, or compounds that are present on the surfaces of these materials and affect sorbent capacities. The high specific surface area of rubbers is due to a porous structure, which can retain inaccessible or kinetically limited 6PPDQ mass during equilibration compared to smooth surface materials (e.g., glass). Stainless steel and plastics designed to be more chemically inert (e.g., FEP and PTFE) showed moderate sorption potentials, with higher residual 6PPDQ concentrations in solution after 24 hours of contact (Hu et al., 2023).

2023–2024 Field Protocol Sampling Study

Untreated highway runoff samples were collected during two storm events at the Ship Canal Test Facility (SCTF) in Seattle, Washington, using a variety of common field materials and equipment (Herrera 2024b).

During each storm event, sample volume was collected at 10 different times (sample splits) and split between nine experimental groups using a Teflon-lined churn splitter. Experimental groups, described in Table 1, included a control sample that was collected first during each sample split and a field duplicate that was collected last during each sample split. The experimental groups were changed between the first and second rounds of the study to further investigate the observed differences (Herrera 2024a). These results are discussed further in the 6PPDQ in Highway Runoff and BMP Effectiveness Summary Report (Herrera 2024b).

All of these experimental groups were selected to represent typical materials and sampling methods used in traditional flow-weighted composite stormwater monitoring. The AUTO_OLD group combines all typical materials and conditions of composite stormwater sampling, including previously used PTFE and silicone tubing, sample storage in a decontaminated used HDPE container with headspace, and laboratory transfer of the sample volume to amber glass containers upon receipt.

The percent recoveries of the experimental groups during the first and second rounds of monitoring, compared to the control group concentrations, are presented in Figures 1 and 2. Recoveries of experimental groups were generally within 90 percent of the control group concentrations, with a few exceptions. During the first round of monitoring, each of the FLPE experimental groups had median recoveries of below 95 percent, with two outliers below 80 percent. Comparing bottle materials, HDPE containers exhibited greater recoveries than FLPE during the first round of sampling and exhibited median recoveries of at least 100 percent during the second round of monitoring—except for the HDEP_24_20L, which had a much greater headspace and surface area to volume ratio than other experimental groups.

Table 1. Field Protocol Study Experimental Groups.

Experimental Group	Description	Round(s)
CONT	Control Group. Amber glass sample bottle filled directly from the churn splitter.	1,2
HDPE_24	HDPE 24 Hour Group. HDPE sample bottle filled directly from the churn splitter, held in the HDPE bottle for 24 hours, then transferred to an amber glass sample bottle.	1,2
HDPE_OLD	HDPE 24 Hour Used Bottle Group. Previously used decontaminated HDPE sample bottle filled directly from the churn splitter, held in the HDPE bottle for 24 hours, then transferred to an amber glass sample bottle.	2
HDPE_24_20L	HDPE 24 Hour Large Carboy Group. Previously used decontaminated 20-liter HDPE carboy filled with 2 liters of sample volume directly from the churn splitter, held in the carboy for 24 hours, then transferred to an amber glass sample bottle.	2
HDPE_FT	HDPE Full Time Group. HDPE sample bottle filled directly from the churn splitter and held in the HDPE bottle until analysis.	1
AUTO_OLD	Previously used decontaminated 1-liter HDPE bottle filled with 250-mililiters of sample volume by pumping from the churn splitter through 10 feet of PTFE tubing and 32.25 inches of silicone tubing that has been previously deployed for stormwater sampling using a peristaltic pump. Sample volume is held in the HDPE bottle for 24 hours then transferred to an amber glass sample bottle.	2
PTFE_TUB	PTFE Tubing Group. Amber glass sample bottle filled by pumping sample volume from the churn splitter through 10 feet of PTFE tubing and 1 foot of silicone tubing using a peristaltic pump.	1,2
PTFE_TUB_OLD	PTFE Used Tubing Group. Amber glass sample bottle filled by pumping sample volume from the churn splitter through 10 feet of PTFE tubing and 32.25 inches of silicone tubing that has been previously deployed for stormwater sampling using a peristaltic pump.	2
SILI_TUB	Silicone Tubing Group. Amber glass sample bottle filled by pumping sample volume from the churn splitter through 2 feet of silicone tubing using a peristaltic pump.	1
FLPE_24	FLPE 24 Hour Group. FLPE sample bottle filled directly from the churn splitter, held in the FLPE bottle for 24 hours, then transferred to an amber glass sample bottle.	1
FLPE_FT	FLPE Full Time Group. FLPE sample bottle filled directly from the churn splitter and held in the FLPE bottle until analysis.	1
FD	Field Duplicate Group. Amber glass sample bottle filled directly from the churn splitter.	1,2

HDPE: High density polyethylene FLPE: Fluorinated high-density polyethylene PTFE: Polytetrafluoroethylene

Figure 1. Field Protocol Round One Experimental Group Recoveries.

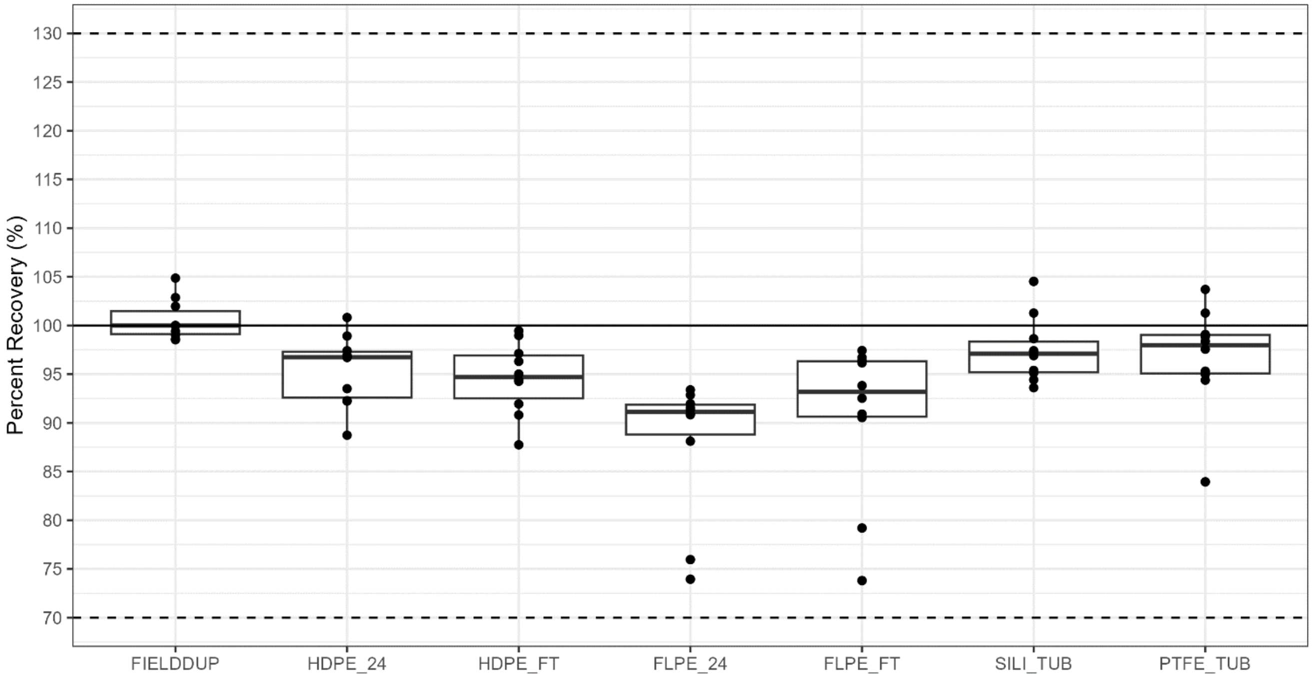
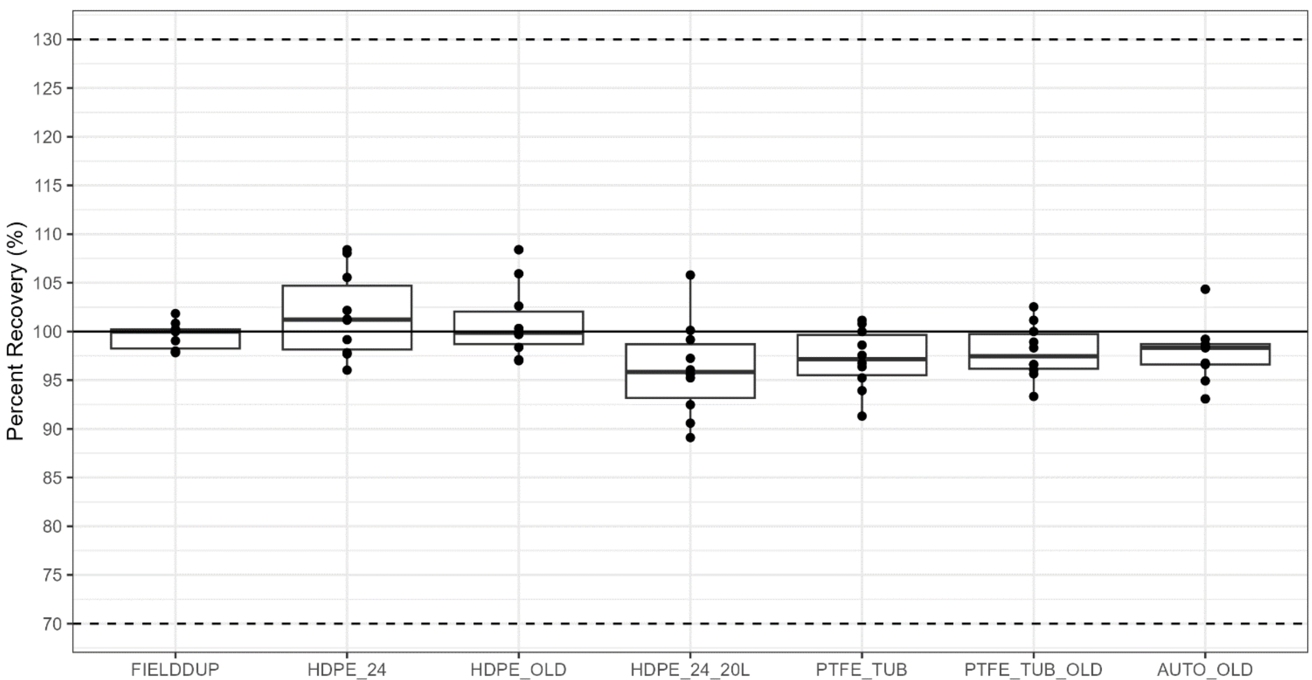


Figure 2. Field Protocol Round Two Experimental Group Recoveries.



The tubing experimental groups (PTFE_TUB, SILI_TUB, PTFE_TUB_OLD, and AUTO_OLD) exhibited median recoveries greater than 95 percent in both rounds of monitoring. Silicone tubing is typically only used as small portions of the overall sample intake line for peristaltic sample pumps, rather than the full length of the sample line. All PTFE tubing and autosampler experimental groups used a small section of silicone tubing, in addition to the main PTFE intake line, to emulate typical field sampling conditions. The AUTO_OLD group, which was only monitored during the second round of sampling, combined elements of multiple different experimental groups to determine if losses from different materials or methods would compound during typical sampling conditions. The AUTO_OLD group had a median recovery of over 95 percent, with a relatively small range mostly within 95 to 105 percent (Figure 2).

Recommendations

The automated sampling protocol recommendations described herein and summarized in Table 2 are based on stormwater sampling best practices, results of the 6PPDQ field sampling at the SCTF, and results of laboratory investigations, which are summarized in the preceding sections. Results of field sampling studies are considered a stronger line of evidence than laboratory studies to support these recommendations, because the field sampling results represent actual field conditions more accurately. To date, limited field protocol sampling has been conducted in receiving waters with lower expected 6PPDQ concentrations, so these recommendations should only be applied for direct stormwater runoff from roadways. Where flow- or time-weighted composite sampling is not necessary, grab sampling—using an amber glass bottle, as described in the EPA Draft Method 1634 (EPA 2023) as well as in this project's Quality Assurance Project Plan (Herrera 2023) and its addendum (Herrera 2024a)—remains the recommended approach to assess instantaneous 6PPDQ concentrations.

Table 2. Summary of 6PPDQ Stormwater Composite Sampling Recommendations.

Component	Recommendation
Carboy Material	Use amber glass or clear glass with protection from light.
	HDPE can be used with minimal 6PPDQ loss.
	Avoid silicone or rubber gaskets on carboy lids where possible.
Carboy Size	Select the smallest feasible size to minimize headspace and surface area to volume ratio.
Sample Tubing	Use PTFE-lined sample intake tubing.
	Short sections of silicone tubing may be used if necessary to operate peristaltic pumps.
Tubing Installation	Use the minimum necessary length of all tubing to minimize contact with sorptive materials.
	Intake tubing should be installed in conduit or otherwise out of direct sunlight.
Sampler Installation	Automated samplers should be programmed to fully rinse and flush sample intake lines for each aliquot.
	Intake tubing should be rinsed with deionized water prior to target storm events.
	Sample aliquot volume should be calculated to ensure minimal headspace in the final sample volume.
Sample Handling	Keep samples on ice and out of direct sunlight as required by EPA Method 1634[a].
	During field handling and transportation to the laboratory, avoid agitating the carboy to prevent unnecessary contact between sample volume and carboy materials.
Quality Control Samples	Collect equipment rinsate blanks prior to sample collection, upon conclusion of the monitoring period, and in the middle of extended monitoring periods to identify potential contamination
	Collect field duplicate samples for every ten primary samples collected.

[a] EPA Draft Method 1634 (EPA 2023).

HDPE: High density polyethylene

PTFE: Polytetrafluoroethylene (Teflon)

Bottle Selection

Material

Sample carboys should be composed of amber glass or clear glass where possible. If glass carboys are infeasible, HDPE carboys may be used because they pose a minimal risk of 6PPDQ loss due to sorption. Carboys composed of other materials, including FLPE, should be avoided if possible due to potentially higher 6PPDQ loss compared to HDPE. Secondary materials in the carboys, including lids and gaskets materials, should be considered in addition to the main carboy material. Use PTFE-lined lids, where possible, and avoid silicone or rubber gaskets. FLPE bottles are frequently used for bioassay toxicity testing. This memorandum does not make specific recommendations for the appropriateness of FLPE bottles for such testing when 6PPDQ toxicity is a specific concern.

Size

Composite carboys should be sized as small as feasible to minimize headspace. If using HDPE carboys, both the headspace and the surface area to sample volume ratio should be minimized . Accurate calibration of sampler aliquot volume and rating curve (for flow-weighted sampling) is necessary for estimating target composite sample volumes.

Supporting Rationale

Where possible, the sampling recommendations in this memorandum are consistent with the current sampling procedures described in EPA Draft Method 1634, which describes grab sampling for 6PPDQ in amber glass containers free of headspace with PTFE-lined caps. Carboy material should mimic the EPA method, if possible, with clear glass as an acceptable alternative; Hu et al. (2023) found that there was not a statistically significant loss of 6PPDQ due to sorption to glass. Depending on specific field conditions, large glass carboys may not be a safe or feasible option for some stormwater monitoring projects. While some 6PPDQ loss due to sorption appeared to be related to HDPE materials in the field protocol study, HDPE experimental groups exhibited median recoveries of over 90 percent and were typically over 95 percent.

Carboy size should be minimized to prevent excess headspace, as prescribed in the EPA method. The HDPE_24_20L experimental group (1 liter of sample volume stored in a 20-liter carboy for 24 hours before transfer to amber glass) exhibited lower percent recoveries compared to other HDPE groups in the second round of field protocol sampling, albeit with typically over 90 percent recovery.

Silicone and rubber exhibited high sorption potential during the laboratory investigation by Hu et al. (2023). While these materials do not typically form large portions of carboys, they can be present in small quantities in certain carboy lids or gaskets and should be avoided. The EPA method identified PTFE-lined caps, which should be used where available.

Sample Tubing

PTFE-lined sample intake and silicone tubing are appropriate for brief contact with sample volumes for automated sampling. PTFE-lined sample intake tubing should be used as the primary sample collection line, with silicone tubing only used if necessary to operate a peristaltic pump. Only the minimum length required for sample collection should be used, in order to minimize the duration of contact between the sample volume and potentially sorptive materials.

Vacuum samplers may be considered as an alternative, to avoid use of silicone pump tubing. However, vacuum samplers typically have additional materials that come in contact with the sample volume. Therefore, vacuum samplers should be evaluated on a case-by-case basis. Vacuum samplers also may have slightly increased contact times with the field materials and the sample volume, particularly in the dosing chamber of the sampler.

Supporting Rationale

PTFE materials are referenced in the EPA method and identified in the Hu et al. (2023) study as appropriate material for brief contact with sample volume. PTFE-lined sample intake tubing was used with a short section of silicone tubing in several experimental groups during the field protocol study, with all groups exhibiting median recoveries of greater than 95 percent (Figures 1 and 2). Because both PTFE and silicone materials saw increased 6PPDQ sorption with longer exposure time (Hu et al. 2023), it is important to ensure that automated samplers are fully purging the sample intake line prior to aliquot collection.

Sampler Installation and Operation

Automated sampler components and sample intake lines should be installed within a conduit or out of direct sunlight when possible. Sample tubing should be flushed with deionized water prior to targeting a sampling event, to minimize risk of contamination from previous sampling events. In addition, automated samplers should be programmed to fully pre-rinse and purge the intake lines prior to collection of individual aliquots, to prevent prolonged contact of sample volume with silicone and PTFE tubing.

Supporting Rationale

Both 6PPD and 6PPDQ can degrade in sunlight, which can (depending on proportions of these parameters) either increase or decrease the 6PPDQ concentrations in the sample volume (OSPAR Commission, 2006). While automated samplers should fully purge sample volume from the intake tubing between each aliquot, protecting the intake tubing from direct sunlight is recommended to prevent photodegradation. Exposure of PTFE-lined sample intake lines to sunlight can also cause structural degradation and premature cracking, so protection of sample lines from sunlight has the added benefit of increased longevity. 6PPDQ has an affinity for organic matter and fine sediment—and may sorb to settleable solids, which can become trapped in sample intake lines. Flushing of intake lines with deionized water between sampling events is recommended to prevent sample contamination in this manner. During the field protocol study, experimental groups with previously used sample intake tubing (PTFE_TUB_OLD and AUTO_OLD) were rinsed in a similar manner prior to sample collection; 6PPDQ concentrations in all rinsate blanks collected on these groups were either undetected or below the analytical reporting limit.

Sample Handling and Storage

Automated samplers should be either refrigerated or insulated to allow placement of ice, to keep the composite sample cold during sample collection. During post-storm sample retrieving, carboys should be immediately placed in ice and out of direct sunlight. If using a translucent or clear glass or HDPE carboy, extra precautions should be taken to protect the sample volume from direct sunlight, including lining the exterior of the sample carboy with an opaque cover. While handling the sample carboy, minimize unnecessary contact between the sample volume and additional carboy or lid gasket materials by keeping the carboy upright at all times and avoiding agitating the container during transportation.

Supporting Rationale

Composite sample carboys should be handled in a manner consistent with EPA method preservation and storage requirements, which includes keeping the bottles away from sunlight and on ice at or below 6°C. Because the field protocol HDPE experimental groups typically exhibited some degree of 6PPDQ losses, unnecessary contact between sample volume and the HDPE container should be avoided in order to minimize losses due to sorption.

Field Quality Control Samples

Consistent with stormwater composite sampling best practices, at least one field duplicate sample should be collected for every 10 primary field samples collected. Rinsate blanks should be collected by pumping laboratory provided deionized water through the sample tubing and collected in a decontaminated sample carboy. Rinsate blanks should be collected, at minimum, once before the start of the monitoring period and upon conclusion of the monitoring period. For extended monitoring programs, additional rinsate blanks should be collected mid-project (approximately quarterly).

Supporting Rationale

Standard stormwater composite sampling best practices include collection of at least one field duplicate for each 10 primary field samples and collection of rinsate blanks before and after the monitoring period. These practices should be sufficient for 6PPDQ field sampling.

Additional Considerations

The field protocol study referenced in this document was conducted with typical stormwater sampling materials, but other bottle materials, such as stainless steel, were not considered and may perform better (or worse). Additional studies on materials to replace potentially sorptive components, such as platinum-cured silicone tubing to replace traditional silicone tubing, would help refine best composite sampling practices. Conducting additional rounds of sampling at lower 6PPDQ concentrations would be helpful in determining whether the field sampling materials recommended herein are still appropriate for sampling the lower 6PPDQ concentrations typical of receiving waters.

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